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Royce Paddock,

March 20th, 1911.

College of Physicians & Surgeons,
Columbia University.

TEXT-BOOK

OF

PHYSIOLOGICAL CHEMISTRY

IN THIRTY LECTURES

BY

EMIL ABDERHALDEN

O. PROFESSOR FÜR PHYSIOLOGIE DES PHYSIOLOGISCHEN INSTITUTS
DER TIERÄRZTLICHEN HOCHSCHULE BERLIN UND
UNIVERSITÄTS-PROFESSOR

TRANSLATED BY

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AUTHOR'S PREFACE.

THE following lectures are not intended to embrace all the results of physiological-chemical investigation. On the contrary, the aim has been to discuss only those discoveries which are known to be of general interest and importance. All isolated facts, of however great significance for the individual investigator in the field, of which the value is not fully established and where the connection to other observations is not clearly known, have been intentionally omitted. The lectures should lead to individual thought and serve to incite further investigation and at the same time give one a general survey of the field covered by physiological chemistry. Corresponding to this purpose, great care has been used with regard to selecting the literature upon which the lectures are based. It is evident that within the space allotted only a limited number of researches could be cited. The "Zentralblätter" with their abstracts and especially Asho and Spiro's "Ergebnisse der Physiologie" must serve to fill in the gaps. Methods and descriptions of individual compounds are not discussed in detail. It is not possible for any one to work well from brief descriptions. Practical laboratory experience is necessary, and cannot be replaced by anything else. The reader is referred to Felix Hoppe-Seyler's "Handbuch der physiologisch-chemischen Analyse" for all such particulars.

E. ABDERHALDEN.

TRANSLATOR'S PREFACE.

One of the chief difficulties which arose in the preparation of this translation was with regard to the proper spelling of the compounds mentioned. Many of them have not been described much in English, so that English-speaking scientists are often better acquainted with the German orthography. Some of the chemical compounds have been spelled in three different ways by writers of good English. This would almost lead one to believe that there is no good authority in English for the spelling of chemical names. Many writers have followed the German spelling as nearly as possible in describing compounds which have hitherto been mentioned only in German literature. This must necessarily lead to confusion, particularly because the ending *e* in German usually signifies the plural, whereas it does not in English. The Chemical Society of London in its *Abstracts* has mentioned nearly every substance touched upon in this book and has adopted certain rules for spelling which its abstractors are required to follow. These rules have, in the main, been adopted by the American Chemical Society and the American Chemical Journal. According to these rules, — (1) All hydroxyl derivatives of hydrocarbons should end in *ol*, thus glycerol, resorcinol and mannitol rather than glycerine, resorcin and mannite. (2) Compounds which are not alcohols and have names ending in *ol* should be written *ole*, as anisole, indole. (3) When a substituent is one of the groups NH_2, NHR, NR_2, NH or NR its name should end in *ine*, thus aminopropionic acid and not amidopropionic acid. (4) The ending *ine* should be reserved for these basic substances, as aniline instead of anilin, and the termination *in* should be reserved for glycerides, glucosides, bitter principles and proteins, such as palmitin, amygdalin and albumin. It seems to us that these are the best rules for English-speaking chemists to follow at present. The rules cited are those which pertain particularly to the substances described in these lectures.

In two cases we have intentionally deviated from the practice of the above-mentioned chemical journals. We have used the word *ferment* to designate "that which is capable of causing fermentation" (Century Dictionary) and have not attempted to distinguish between *ferments* and *enzymes*. This distinction was based upon an error, as Buchner has so positively shown, and has led to much confusion. Again, we have followed the author rather than the chemical journals with regard to the

use of the words *protein* and *proteid*. While this book was in press the "Joint Recommendations of the Committees on Protein Nomenclature" was published in *Science*, in which the first recommendation was that the word *proteid* should be abolished. They would call what Dr. Abderhalden designates as *proteids* the *conjugated proteins*. The confusion with regard to the word *proteid* has arisen from the fact that some writers have designated as proteids the whole protein group, while others have used the word only for these compound proteins. It was too late to adopt the recommended nomenclature, as many of the plates were already cast. It seems probable, however, in view of the rapid progress which is now being made in this branch of chemistry that before long we shall be able to adopt a chemical classification of the proteins which shall be better than any yet proposed.

It has not seemed best to give all of the titles to the papers cited in the footnotes. Most of these titles which appear in the original lectures are in German, and in some cases they were evidently taken from the *Centralblatt* and are German translations of English titles. It seemed sufficient to give merely the abbreviated titles of the journals where the references could be found with the volume and page. The abbreviations used are, in the main, those adopted by the American Chemical Society in their Chemical Abstracts, the principal ones being given in the front of this book.

Dr. Abderhalden has kindly looked over all of the "page proof" and has suggested numerous changes bringing the literature in some cases up to 1908. Professor F. Jewett Moore of the Massachusetts Institute of Technology as well as Dr. Percy G. Stiles of Simmons College and the Institute of Technology have also read all of the proof and have rendered invaluable aid by their many suggestions and criticisms. If this translation meets with the same friendly reception that has been accorded to the original, credit is due fully as much to each of these two gentlemen as to either one of the translators.

WILLIAM T. HALL.

Massachusetts Institute of Technology,
July 11, 1908.

ABBREVIATIONS USED IN FOOTNOTES.

ABBREVIATED TITLE.	FULL TITLE.
Allgem. med. Zentr.	Allgemeine medizinische Zentralzeitung.
Am. Chem. J.	American Chemical Journal.
Am. J. Physiol.	American Journal of Physiology.
Ann. Phil.	Liebig's Annalen der Chemie.
Ann. chim. phys.	Annales de chimie et de physique.
Ann. de chim.	Annales de chimie.
Ann. inst. Pasteur	Annales de l'institute Pasteur.
Ann. Phil	Annals of Philosophy.
Arch. Anat. Physiol.	Archiv für Anatomie und Physiologie.
Arch. exper. Path. Pharm.	Archiv für experimentelle Pathologie und Pharmakologie.
Arch. exper. Path. Therapie	Archiv für experimentelle Pathologie und Therapie.
Arch. Gynäk.	Archiv für Gynäkologie.
Arch. Hyg	Archiv für Hygiene.
Arch. Kinderheilk.	Archiv für Kinderheilkunde.
Arch. klin. Med.	Archiv für klinische Medizin.
Arch. path. Anat.	Archiv für pathologische Anatomie und Physiologie und für klinische Medizin.
Arch. Pharm.	Archiv der Pharmacie.
Arch. physiol. Ges.	Archiv der physiologischen Gesellschaft.
Beitr.	Beiträge. *See* Hofmeister.
Ber.	Berichte der deutschen chemischen Gesellschaft.
Ber. Berl. Akad. Wissensch.	Berichte der Berliner Akademie der Wissenschaften.
Ber. deut. bot. Ger.	Berichte der deutschen botanischen Gesellschaft.
Berl. klin. Wochschr.	Berliner klinischer Wochenschrift.
Biochem. Zentr.	Biochemischer Zentralblatt.
Bot. Ztg.	Botanische Zeitung.
Brit. Med. J.	British Medical Journal.
Bull. soc. chim.	Bulletin de la société chimique de Paris.
Centralbl.	Chemischer Centralblatt.
Compt. rend.	Comptes-rendus hebdomadaires des séances de l'académie des sciences.
Compt. rend. soc. biol.	Comptes-rendus hebdomadaires des séances et mémoirs de l'académie des sciences.
Deut. Arch. klin. Med.	Deutsche Archiv für klinische Medizin.
Deut. med. Wochschr.	Deutsche medizinische Wochenschrift.
Ergeb. Physiol. (Asher and Spiro)	Asher and Spiro's Ergebnisse der Physiologie.
Hofmeister's Beitr.	Beiträge zur chemischen Physiologie und Pathologie.

ABBREVIATED TITLE.	FULL TITLE.
J. Anat. Physiol.	Journal of Anatomy and Physiology.
J. Exper. Med.	Journal of Experimental Medicine.
J. Chem. Soc.	Journal of the Chemical Society, London.
J. pharm. chim.	Journal de pharmacie et de chimie.
J. Physiol.	The Journal of Physiology.
J. pr. Chem.	Journal für praktische Chemie.
Med. Klinik.	Medizinische Klinik.
Mém. couronn. acad. roy. Belg.	Mémoires couronnés de l'académie royale de Belgique.
Monatsh.	Monatshefte für Chemie und verwandte Teile der Wissenschaften.
Münch. med. Wochschr.	Münchener medizinische Wochenschrift.
Pflüger's Arch.	Pflüger's Archiv für die gesammte Physiologie des Menschen und der Tiere.
Pharm. Zentr.	Pharmazeutische Zentralblatt.
Phil. Trans. Roy. Soc.	Philosophical Transactions of the Royal Society of London.
Pr. Chem. Soc.	Proceedings of the London Chemical Society.
Pr. Physiol. Soc.	Proceedings of the Physiological Society.
Pr. Roy. Soc.	Proceedings of the London Royal Society.
Pr. Roy. Soc. Edinburgh.	Proceedings of the Edinburgh Royal Society.
Sitzber. Akad. Wiss. Berl.	Sitzungsberichte der königliche Preussischen Akademie der Wissenschaften zu Berlin.
Sitzber. Akad. Wiss. Wien.	Sitzungsberichte der königliche Akademie der Wissenschaften zu Wien.
Sitzber. Gesel. Morph. u. Physiol. München.	Sitzungsberichte der Gesellschaft für Morphologie und Physiologie in München.
Sitzber. kgl. Gesel. Wiss. Upsala.	Sitzungsberichte der königlichen Gesellschaft der Wissenschaften zu Upsala.
Sitzber. physikal. med. Gesel. Wurzburg.	Sitzungsberichte der physikalisch-medische Gesellschaft zu Wurzburg.
Sitzber. Münchener Akad.	Sitzungsberichte der königlichen bayerischen Akademie der Wissenschaften zu München.
Skand. Arch. Physiol.	Skandinavisches Archiv für Physiologie.
Trans. Chem. Soc.	Transactions of the Chemical Society.
Verh. Ges. Naturforsch. Aerzte.	Verhandlung der Gesellschaft deutscher Naturforscher und Aerzte.
Virchow's Arch.	Archiv für pathologische Anatomie und Physiologie und für klinische Medizin.
Z. allg. Biol.	Zeitschrift für allgemeine Biologie.
Z. allg. Physiol.	Zeitschrift für allgemeine Physiologie.
Z. Biol.	Zeitschrift für Biologie.
Z. Elektrochem.	Zeitschrift für Elektrochemie.
Z. exper. Path. Therap.	Zeitschrift für experimentelle Pathologie und Therapie.

ABBREVIATED TITLE.	FULL TITLE.
Z. Hyg.	Zeitschrift für Hygiene und Infektionskrankheiten.
Z. innere Med.	Zeitschrift für innere Medizin.
Z. klin. Med.	Zeitschrift für klinische Medizin.
Z. physikal. Chem.	Zeitschrift für physikalische Chemie.
Z. physiol. Chem.	Hoppe-Seyler's Zeitschrift für physiologischen Chemie.
Z. rat. Med.	Zeitschrift für rationelle Medizin.
Zentr. Bakt. u. Parasitienkunde.	Zentralblatt für Bakteriologie und Parasitienkunde.
Zentr. med. Wissensch.	Zentralblatt für die medizinische Wissenschaften.
Zentr. Physiol.	Zentralblatt für Physiologie.
Zentr. Stoffwechs. Verdauungskrankheit.	Zentralblatt für Stoffwechsel und Verdauungskrankheiten.

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PHYSIOLOGICAL CHEMISTRY.

LECTURE I.

INTRODUCTION.

Physiological chemistry forms an important branch of the large field of investigation included under physiology. It has, for one thing, the task of determining the chemical composition of the material from which the separate tissues in the living organism are formed. A knowledge of the chemical construction of the different organs gives us the answer to certain questions, and forms the basis for further inquiry. It is perfectly clear that the organism must receive in its nourishment all those elements of which its tissues are composed. With the knowledge of the composition of the separate organs, we obtain, by comparing their functions, certain interesting views regarding the significance of the different substances which take part in their formation. Closely related to such problems stands the investigation of metabolism. This has become almost exclusively the domain of the physiological chemist. We desire, first of all, to know as much as possible concerning the nutriment that the organism receives, and especially as regards its utilization in metabolism. We obtain an insight into such processes by carefully studying the excretions from the organism. The physiological chemist has long since passed beyond these boundaries. His foremost task is now to ascertain what becomes of each separate group of food-stuffs in the organism, in what way it reaches the tissues, and how the cells utilize the different substances in their metabolism and are built up by them. For a long time we have not been content with merely contrasting the income with the outgo of the organism. A final goal of physiological-chemical research will be attained, when we are able to follow, in every separate phase, each and every food-stuff from the time of its introduction into the alimentary canal throughout its entire stay in the tissues until it is finally eliminated; so that a whole chain, without any missing links, of all the different transformations and complicated processes will lie exposed to our view. We are still very far from the solution of this problem. To be sure, in recent years certain progress has been made in our knowledge of metabolism, and indeed here and there the advance of pure chemistry has placed certain

interesting biological discoveries upon a firm foundation; yet, nevertheless, the investigations of the physiological chemist are obliged to halt for the present before the individual cell. Here for a time is our boundary, but that it will not prove insurmountable is shown by recent investigations.

Physiological chemistry seeks to accomplish still another result. We desire to know not merely the manner in which each separate substance passes through the body, and how it is broken down, but we also wish to know its relations to the other compounds which are likewise introduced into the body. This is true especially for our organic food-stuffs. We wish to know whether they can mutually replace and supplement one another, and whether it is possible for a representative of one class of foods to exercise a function usually assumed by another.

Beyond these limits, physiological chemistry sets itself a number of other tasks which are now only just beginning to be attacked. As little as we are content with the discovery of the anatomical structure of a definite organism, but seek to understand clearly its ontogenesis and phylogenesis, just so little should we be satisfied to trace in the case of a single individual, or in only one species, all of the processes which may be referred back to chemical decompositions. Comparative physiological-chemical research is called upon to explain clearly certain important processes which now appear enigmatical to us. On the other hand, comparative chemical investigation concerning the nature of the bodies and the metabolism of individuals belonging to different animal species, will give a new support for purely morphological research. The gradual evolution in the entire animal kingdom takes place step by step parallel to an ever more delicate and ever more marked specialization of the single organs. In many cases where the histology of an organ permits some doubt to arise as to where it belongs, the determination of its function often gives a clear decision.

More and more the investigation of physiological-chemical processes in the animal organism extends beyond its more narrow field. For a long time it has been evident that there is no sharp boundary between the animal and vegetable kingdoms. More and more it becomes recognized that the line once drawn was an entirely artificial one. We now know that countless processes take place in plant and animal organisms which are common to both. The more this uniformity has found expression, the more sharply defined have become the differences in the metabolism of the different members of each kingdom. Everywhere there are transition stages, and nowhere do we meet with sudden changes. To-day there is no longer any doubt but that a complete understanding of the processes transpiring within the animal organism is only possible when attention is paid as well to those changes taking place in plant organisms. We desire to know, moreover, the source of each article of food, how it has been

formed, and to what extent the animal cell is able to build it up for itself. In such problems as these we meet with deep-seated differences in the chemism of plant and animal cells.

Physiology and physiological chemistry were at one time a single field of investigation. The latter, owing to the remarkable progress of the exact sciences chemistry and physics, has now developed to such an important branch of natural science that it is altogether impossible to-day for any one scientist to master thoroughly in all its details the whole field of physiology. Little by little, there has developed a sharp distinction between pure physiology and physiological chemistry. It is clear that an artificial separation of two such closely related fields of investigation must work to disadvantage for the development of each, so that it is fortunate to find it recognized more and more that the whole science of physiology can only develop satisfactorily when there is an intimate exchange of ideas between investigators in the two fields. The chemical decompositions in the tissues do not take place *beside* the "physiological processes," but are bound up with them. In no single case can we carry out any distinction in such a sense; and with those organs in which the relations between the functions and the metabolism are not known, this is chiefly because of our limited knowledge concerning the latter. In this connection we need only recall the whole nervous system.

Finally we must mention the fact that physiological chemistry is constantly approaching new fields of investigation which a few years ago apparently had almost no connection with it. We have in mind here the great field of infectious diseases and the changes in the metabolism of the cells which they occasion. The apparently great gap existing between the methods of protecting the organism against the substances produced by micro-organisms and the products given off by the cells under normal conditions is at last bridged over by means of certain analogies and numerous transitions. Pathology also, in a broad sense, seeks to unite itself more and more with the field of physiological chemistry. Many a pathological process has afforded us a desired physiological experiment, and conversely it is possible to obtain by comparing physiological and pathological processes a clearer conception of the latter. It becomes more and more evident that pathological changes in the tissues and cells should not be judged entirely from a morphological standpoint. We can easily understand that a disturbance in the metabolism of the cell, if continued long enough, can make itself evident in the outer appearance. On the other hand, it is perfectly conceivable that a definite functional disorder may exist without the anatomist being able to localize it by the means at his disposal. In no case does it follow necessarily that the limit of discernible degeneration is a measure for any given functional disturbance. Again, an abnormal morphological change may cause but a slight dis-

turbance in the metabolism of the cell. At present we are very far from gaining insight into the physiological processes concerned in cell-metabolism, and still less should we expect to obtain already a clear picture of pathological disturbances. This opens up for us a particularly enticing field for further investigation, the foundations of which must rest upon physiological chemistry. It is apparent from this cursory glance that the tasks of physiological chemistry are extremely varied.

In order to be able to form a correct judgment of results brought forward in a field of investigation, it is necessary to decide in the first place whether the methods employed are satisfactory and rest upon a firm basis. The methods used by the physiological chemist are for the most part those of pure chemistry. Such experience forms the basis of his work. We shall soon see that in spite of the fact that in many cases perfectly similar methods are employed, there is a marked difference in the two fields of investigation. Chemistry, we know, is an exact science. This designation means, more than anything else, that the chemist arrives at his conclusion by carrying out from case to case a direct proof, in a perfectly objective manner. If he discovers a new substance, he is able by various processes to so purify it that by its crystalline appearance, the results of analysis, its melting-point, molecular weight, etc., and by a study of the substances he can form from it, he can decide whether it is a simple substance or a mixture. Finally, he can decompose the substance, it may be by hydrolysis, or perhaps by oxidation, or by reduction, thus transforming it into constituents of known composition, or into some other well-known compound, and in this way eventually establish its composition in all its details. But even after all this work the chemist is not satisfied. He is not absolutely positive that he has a substance of definite constitution until he has succeeded in effecting its synthesis. To be sure, metaphysical speculations also play an important part in the domain of pure chemistry, and rightly. This has long since been shown to be justifiable on account of the fruitfulness of such speculations. The chemist must never forget, however, where the facts end and where the hypothesis begins.

Let us see now how the physiological chemist conducts his proofs. One might be tempted to believe that an insight into the chemical processes which take place in the organism would be obtained soonest if we chose for our study the simplest form of organized being, the single cell, i.e., a unicellular individual. A little thought, however, shows us that the morphological unity of the cell corresponds to an uncommonly complicated cell-mechanism. Each cell-unit here takes up nutriment, decomposes and assimilates it, and eventually breaks it down to deposit at last the final products of metabolism. All of these processes take place in a single minute cell. From this point of view, every morphological differentiation must in a

certain sense be regarded as a simplification of the functional processes. The more localized the separate functions are, and the more they pertain to certain organs only, the greater becomes the possibility of our succeeding in studying them by themselves and explaining them. In this sense the most suitable objects for our study are those highly complicated beings, the vertebrates.

In order to show the value of the results obtained by physiological-chemical investigation, a few examples may be cited briefly. We will disregard here that part of the field which concerns itself with the knowledge of the separate components of our food and the substances which go to form our tissues. In such cases it is evident that the physiological chemist will make use of the same methods for determining the value of his work as does the pure chemist. He will, furthermore, proceed in precisely the same way as we have described for a chemical investigation, and will thus establish the proof for the constitution of a definite compound. Here physiological chemistry is constantly receiving much aid from the field of pure chemistry, and in fact this part of the work has been developed by trained chemists.

We will choose here, as an example, a question which has been asked repeatedly, namely this: What becomes of a definite compound after it is introduced into the animal organism, in what way is it broken down, and in what form is it finally excreted? We will use for our illustration a very important discovery, rich in results, which we owe to Wöhler. This scientist was interested to learn what became of benzoic acid after it was introduced into the intestine. He was unable to detect it in the urine, nor could he find there any substance which he recognized as one of its lower derivatives. On the other hand, he did find in the urine another acid, hippuric acid, which is closely related to benzoic acid. This is formed, as we shall see later on, by the combination of benzoic acid with glycocoll, the latter substance being formed by the hydrolysis of albumin. Hippuric acid on being boiled with strong mineral acids or alkalies is decomposed into these two constituents. Wöhler, by establishing the fact that the benzoic acid which he introduced into the organism left it in the form of hippuric acid, proved for the first time that syntheses may take place in animal organisms. In this way the path was broken and the obstruction to the development of physiological chemistry which had existed for years was removed. Up to that time it was regarded as an established fact that only plant cells were capable of accomplishing synthetical work, while those of the animal organism could only effect decomposition. This first observation of Wöhler was particularly fruitful, and inspired a great deal of similar work, so that to-day we are perfectly justified in ascribing complicated syntheses to the action of the animal cells. If we attempt to subject Wöhler's method of proof to close critical analysis, we

find, first of all, that it is indirect in character. Here we meet with the most remarkable point in all physiological-chemical investigation. A very large part of it is based upon indirect proofs. Its inadequacy is clearly shown if we choose for illustration, instead of the above very transparent example, something more complicated, such as, for example, the question now in the foreground of general interest with regard to the formation of sugar from other sources than carbohydrates. By extirpating the pancreatic gland of a dog, its carbohydrate metabolism may be so disturbed that sugar is constantly eliminated in the urine, and one would naturally think *a priori* that in such a case it would be possible to determine without difficulty whether, for example, albumin or fat can cause secretion of sugar in the urine. As a matter of fact, this secretion of sugar continues even after all carbohydrates are completely eliminated from the nourishment. We may assume this to prove that albumin and fat can cause secretion of sugar in the urine. Although we are justified in drawing such a conclusion, it is not necessarily a correct one. A result from a given experiment can lead to different conclusions according to the standpoint assumed by the individual investigator. In this case it is possible to explain the continued secretion of sugar in another way. The animal organism possesses constant reserves. Their extent has only recently been realized. From them, and especially from carbohydrate stores, the sugar may have its source. The conclusion that the animal cell is capable of forming sugar from other sources than the carbohydrates can only be drawn with certainty after it has been established that the organism has no more carbohydrates at its disposal. Not till this has been clearly shown will the above conclusion rest upon a firm basis. It remains still undecided, even if the carbohydrates as sugar-formers are fortunately excluded, as to whether fats and albumins belong to this class of compounds. Now it has been often observed that in feeding albumin to a dog with no pancreas the elimination of nitrogen runs practically parallel to that of sugar. This repeatedly established relation between the breaking down of albumin and the formation of sugar has been given as a direct proof for the formation of sugar from albumin, and in fact one might be tempted to assume that this is actually a direct demonstration. E. Pflüger, whom we have to thank for a detailed critical review of all the work in this field, is of an altogether different opinion. If we assume as correct that the elimination of sugar and of nitrogen increases at an equal rate, we are still far from being justified in assuming that the increase in sugar is directly due to the breaking down of the albumin which is taking place. The cells of a dog with no pancreas, and those of a diabetic, have not lost entirely the power of consuming sugar, and in all cases a part of the sugar formed is burned up. If now albumin be fed, it will also be burned; in other words, there will be set free in the tissues of the organism

a definite number of heat units which it can use in performing its functions. By means of the calories of heat coming from the albumin the organism is spared a corresponding amount which were otherwise taken from non-nitrogenous material. We may assume that the cells, which moreover are capable of consuming sugar only with great difficulty, now do not use so much of it. More and more unchanged sugar circulates in the tissues and in the blood, and since the kidneys, as we shall see later, are sensitive to the slightest increase of sugar in the blood over the normal and serve to remove all such excess, it follows that there must necessarily be an increase in the elimination of sugar. According to this hypothesis the action of albumin is an indirect one as regards the sugar. Naturally this explanation is not necessarily the correct one. We have mentioned these experiments and the two explanations of the results obtained briefly in order to show by a somewhat complicated example how varied the conclusions may be that are drawn with regard to an apparently simple problem. It would not be difficult to cite numerous other examples to illustrate this point. Later on we shall repeatedly come back to these indirect proofs and mention again and again the fact that it is of fundamental importance for the further development of all physiological-chemical investigation that it should always be clearly and sharply recognized as to what extent we are justified in speaking of *facts*, and at what place the indirect conclusions, corresponding to the still unsettled part of our field of investigation, begin. When such a gap is discovered, it is our duty not to rest satisfied until all of the conclusions have been subjected here also to direct proof.

Before taking up the discussion of ways and means to accomplish this end, we will turn back once more to the synthesis of hippuric acid in the animal organism. This was established indirectly, and its assumption rests solely upon probability. After introducing benzoic acid into the organism of a mammal we find a corresponding increase in the amount of hippuric acid in the urine. It is a fact that hippuric acid can be formed from benzoic acid and glycocoll. The chemist is able to make hippuric acid in the laboratory from these two components, but under conditions which it is impossible to realize in our tissues. It requires a high temperature, considerable pressure, and the exclusion of water. We have, however, long since been forced to the conclusion that the cells have the power of causing chemical reactions to take place which require entirely different conditions when carried out in a test tube. We are satisfied if an observed chemical process does not outwardly contradict our general experience. We base our explanations of the chemical decompositions taking place in the animal organism upon the results of chemical research, and seek to go farther and bridge over all the large gaps which we meet with everywhere on account of our insufficient knowledge of metabolic processes. Here also we must be conscious that we are only speaking of

probabilities, and in no case should it be credited as if resting upon facts established experimentally. Analogies in many cases are without doubt very valuable, and often form the skeleton upon which we can build further.

We receive a new impulse and gain a new point of view with every advance made by pure chemistry concerning substances of physiological interest. Our task is to utilize each discovery thus made and to give it a strictly objective test with regard to its application to the processes taking place in the tissues. Here again we meet all too frequently with hypotheses which are stated as facts. We hardly need to mention how extraordinarily restraining the direct amalgamation of these entirely different elements is for a healthy progress in the knowledge of chemical processes in the animal organism. For these reasons Wöhler's experiment proves positively merely that when benzoic acid is introduced into the system it causes an increased elimination of hippuric acid. It must remain an open question as to whether the benzoic acid introduced stands in direct relation to the other acid or merely indirectly causes its formation. In this particular case, however, the latter case seems scarcely probable, although we must make such a limitation unless we propose to draw our conclusions beyond the realms of fact.

We must now mention an important aid which the chemist makes use of constantly in his experiments, which are often indirect in nature. We refer to the control experiment. It is clear that there is nothing to be gained by merely feeding an animal with benzoic acid and determining subsequently the amount of hippuric acid eliminated. We must first learn how much hippuric acid the animal in question eliminates under normal conditions. If the experiment is to be made convincing, the amount of hippuric acid contained in the urine of one and the same animal fed uniformly must first be determined and this continued for several days. Then for a time a little benzoic acid should be added to the food, which otherwise must remain qualitatively and quantitatively the same as before, and again the hippuric acid be determined in the urine. If now the experiment be continued for another period of several days in which no benzoic acid is fed to the animal, then, if the whole experiment is consistent, it will be possible to determine whether the benzoic acid stands in any relation to the elimination of hippuric acid.

The uncertainty of the significance of experiments made with animals is in many cases greatly increased by the fact that individual variations often play an important part. Many contradictions to be found in the literature are due solely to the fact that the experiments were not carried out long enough. We must not only require that such experiments should be carried out in a single individual for quite a length of time, but in different individuals of the same animal species as well. It is, furthermore, of great value to make experiments with different species of animals, for

frequently they behave altogether differently physiologically. It is apparent already from these brief remarks what great demands are laid upon the experimentation of the physiological chemist. He always has to deal with complicated processes. He is acquainted usually only with the initial and the final products of the metabolism, and is compelled to clear up theoretically the whole chain of transformations which are necessary for the formation of the latter from the former. Here and there it is possible to get hold of intermediate steps, and thus we encroach more and more upon the great domain of the unknown.

A considerable advance in the subject was made when experimentation was begun upon surviving organs rather than upon the whole organism. Here the initial and end products are more closely related, or at least apparently so, although here also, as soon as the change in cell substance begins really to take place, the complication is naturally practically as great as in following one substance through the whole body. Experimentation with surviving organs has in itself quite a number of advantages. In many cases we are able to change an indirect proof into a direct one. We are able to work out accurately the composition of a definite organ. We can definitely decide the question as to whether it has stored up in it sufficient amounts of definite substances to cause the formation of certain compounds, and thus determine positively whether the organ makes use of a substance introduced into it in a definite process.

Let us return again to our hippuric acid hypothesis. It is possible to establish which organ is capable of carrying it out. G. Bunge and O. Schmiedeberg have shown that the kidneys of mammals, or more accurately those of a dog, are capable of forming hippuric acid from glycocoll and benzoic acid. They caused a dog to bleed to death, cut out its kidneys, and introduced defibrinated blood through the arteries of the kidneys and allowed it to flow out through the renal veins. On introducing glycocoll and benzoic acid, there appeared hippuric acid in the blood and in the liquid emptying out through the ureter. A control experiment with the second kidney showed that it as well as the blood from the first kidney was free from hippuric acid. If benzoic acid but no glycocoll was introduced into the blood, the amount of hippuric acid formed was extremely small. The conclusion to be drawn from this experiment is that benzoic acid effects the formation of hippuric acid because it is itself used in the synthesis. However, this fact is not yet absolutely proved. The objection may still be raised that hippuric acid may arise from another source. The formation of this acid is, at best, a very complicated process. We have, on the one hand, the kidney containing a very complex tissue, and, on the other hand, the blood with its constituents. As a matter of fact, it was not possible to effect the above synthesis after the red corpuscles were removed from the blood.

Another significant advance in the knowledge of chemical processes which take place in the animal organism was caused by the discovery that it was possible to work out certain processes by means of extracts of tissue. We are furthermore fortunately able in many cases to isolate the active principle. The great advantage of such experiments is clear from the fact that an extremely small amount of these products, called ferments, is capable of causing considerable change without itself appearing in the end products of the reaction. It has been attempted to effect the hippuric acid synthesis in this way by means of a ferment which has been isolated from the kidneys. This has not yet been done satisfactorily. An instructive example of great significance as regards the use of such tissue extracts and of the ferments obtained from them is found in the recent experiments to form uric acid from the purine bases, investigations made by Horbaczewski, Wiener, Spitzer, Schittenhelm, and Burian. The first-mentioned has shown that purine bases added in the presence of oxygen to an animal organ which has been macerated to a paste causes an increase of uric acid. Against this experiment the objection may be raised that it is not conclusive. The paste itself contains some purine bodies and perhaps substances of unknown nature which stand in close relation to uric acid. The purine bases added may in some way have an indirect action upon the given synthesis. The proof becomes much more satisfactory if instead of using the whole organ, we make use of a ferment extracted from it. To be sure, the nature of this ferment is not known, but we know its action. We can free it completely from purine substances, and furthermore we require but a small amount of it. It is of great significance with regard to our conclusions, that it is possible here to follow the experiment quantitatively. We can weigh accurately the amount of purine bases added, and similarly the amount of uric acid formed, so that we are now able to establish sharply the relation between the purine bases and the uric acid. This method has still further advantages. It has been possible to identify certain intermediate products formed in the transformation of certain purine bases into uric acid, and to establish the fact that certain organs possess ferments which are capable of breaking down the uric acid formed. In this way it is at once possible to establish clearly the complete metabolism of purine substances. It would, of course, be unsafe to apply the results of such experiments without further investigation to the processes taking place in the living organism. It is perfectly conceivable that the conditions prevailing in the tissues may be entirely different from those prevailing in the fermentation experiments carried out artificially. Such a limitation holds for all investigations carried out with ferments, and especially those with digestive ferments. In such experiments the ferments develop their action under entirely changed conditions. We can merely imitate the temperature; further than this we are practically helpless. In the alimentary canal, for example, absorption

takes place immediately hand in hand with the hydrolysis of food by the ferments of the digestive juices. The decomposition products are immediately taken away. We are still entirely ignorant of the manner in which each individual ferment does its work, how the different ferments assist one another, and how their work is influenced by other factors. At all events, it is evident that the decomposition of food takes place much more rapidly than in a test tube. All the products of the decomposition remain in the latter case, and serve to hinder the further action of the ferment, or perhaps even cause it to act in a different direction. On the other hand, in the test tube we are often able to identify products which otherwise escape our observation owing to the rapid absorption in the bowels. It is possible here also to draw conclusions only by combining experiments; that is, on the one hand we will study digestion as accurately as possible in the test tube, following it up in its separate phases, and, on the other hand, we must attempt to identify the products of digestion in the bowels themselves; in this way we gradually draw a picture of the entire process of digestion. In an entirely similar manner the metabolism of purine substances must be studied in the whole organism in order to find out how far the facts thus ascertained agree with the results of experiments with ferments.

We must consider still another important condition, namely, the concept of quantity. In physiological-chemical experiments this is too frequently neglected. Its importance is perfectly obvious. We must always require that every chemical process taking place in the organism be followed quantitatively. Qualitative experiments are prone to lead to great errors, and it is never possible to recognize clearly by means of them the relation between individual products. We must always know how much of this or that substance has been changed over into a definite product. Oftentimes a minor process will otherwise be considered the essential one simply because it was easy of discovery, whereas the main change may be entirely overlooked.

It is almost superfluous to mention the fact that the methods employed must be suitable for the problem to be investigated. Every investigation in the field of physiological chemistry must start out with a critical examination of the value of available methods. We must clearly recognize the sources of error and take them into consideration, especially when definite conclusions are to be drawn from any discovery. The methods are the foundation pillars in every experimental investigation. Every advance is closely dependent upon them, so that we must lay great stress upon their final development. The great importance in the improvement of methods too often falls into the background, especially in physiological-chemical investigation, and apparently more weight is laid upon the more or less fruitful hypotheses. It must not be forgotten, however, that essentially new facts are usually closely connected with the discovery of new methods.

The latter alone cause the science to progress upon a solid foundation. They assure an objective investigation, and above all else one that is free from prejudgment. Certainly hypotheses and speculations are of great value, and their importance should not be underestimated. They form the framework upon which we can build further. The facts, however, should never be adjusted in accordance with them. The facts, and never the hypotheses must always be decisive. This warning is not unnecessary, for in contrast to the exact sciences such as chemistry and physics, here in physiological-chemical investigation the hypotheses step boldly into the foreground, especially in questions concerning metabolism, and in particular that of the cell substance.

We make these few preliminary remarks in order to show at the start the nature of our lectures and the principles which are authoritative. It will be our aim to define as sharply as possible what discoveries are to be regarded as well-established facts and at what place the probability proofs are justifiable. Above all else we shall strive to follow every separate food-stuff from its introduction into the organism to its complete breaking down and the elimination of the end-products in order thus to obtain a comprehensive view of its behavior in the organism and its participation in metabolism. We shall intentionally consider the building materials and composition of the separate organs only in special cases. This knowledge we acquire in studying metabolism. A consideration of the quantitative relations in which the different substances are present would be of use to us only when the separate values are based upon a broad foundation and upon a great many observations. For the present our methods are not adequate to give us a satisfactory picture of the building up of the separate tissues. Neither is our knowledge sufficient to permit the valuation of the results for comparative studies, nor are we in general in a position to draw conclusions with regard to the functions of certain organs from a knowledge of their composition. In the special cases where this is possible we shall speak of it.

LECTURE II.

CARBOHYDRATES.[1]

I.

In General — Monosaccharides — Glucosamine — Glucuronic Acid.

The carbohydrates are extremely abundant in nature. They take a prominent share in the building up of the vegetable kingdom, and play an important part as food in animal economy; while on the other hand, compared with the protein bodies, they scarcely come into consideration at all as building materials for the animal tissues and cells. The most important representatives of this class of bodies have been known for a long time, especially cane sugar, which before the beginning of the Christian era was obtained in India in a solid form by boiling down the juice of the sugar-cane. To-day, besides the sugar-cane, the sugar-beet[2] forms an important raw material. Again, grape-sugar has been known for a long time, and was first discovered in honey although prepared pure for the first time by Marggraf in the middle of the eighteenth century. In the year 1615 Bartolleti[3] isolated a third member of this group from milk, namely milk-sugar. If we add to these cellulose and starch we have named all of the members of the carbohydrate group which were known up to the time that the study of organic chemistry as we know it to-day began as a result of the experiments of Lavoisier and Scheele. If we disregard a few isolated although very important observations — e.g., Kirchhoff's discovery that starch was changed into grape-sugar by boiling with dilute acids,[4] and that the same process could be brought about by a substance found in grain or malt[5] — very little was known in chemistry, and consequently in physiology, concerning carbohydrates up to within very recent times, and this period of darkness disappeared only with the important investigations of Kiliani and of Emil Fischer especially.

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[1] The following references cover this field:— Emil Fischer: Ber. **23**, 2114 (1890). E. O. V. Lippmann: Die Chemie der Zuckerarten (1904). B. Tollens: Kurzes Handbuch der Kohlehydrate (1898).

[2] Discovered by Marggraf (1747); Ber. Berlin. Akad. Wissensch. **79**, 1749.

[3] Encyclopædia dogmatica, 1615.

[4] J. d. Pharm. **74**, 199 (1811).

[5] Schweigger's J. **14**, 389 (1814).

In the course of the following discussion we shall see how closely the development of the chemistry of carbohydrates follows the general development of chemistry, and especially that of stereochemistry and the theory of structure, and what a comprehensive outlook dawned all at once for the whole field of biology.

The carbohydrates are all composed of the elements carbon, hydrogen, and oxygen, and these are the same elements that are found in fats. The two classes of compounds, however, contain these elements in different relative amounts. Oxygen and hydrogen in the former are present in the ratio 1:2, which is the same as in water. This is the reason that the name carbohydrates has been given to the group. Many other compounds which do not belong to the sugar group, for example acetic and lactic acids, are, however, also composed of the same elements and in the same ratio. Formerly, the carbohydrates were defined as containing six, or a multiple of six, carbon atoms. This limitation was shown to be incorrect by the discovery of sugars containing less than six atoms of carbon, and by the synthesis of sugars with seven, eight, and nine carbon atoms. It is in fact impossible to give a sharply-defined, satisfactory definition of a carbohydrate, for to some extent the individual members of the group have very different properties from one another. In general, *the carbohydrates are aldehyde or ketone derivatives of polyatomic alcohols.*

As is the case with almost all branches of physiological chemistry, so here, as has already been indicated, it was only possible to obtain a clear idea of the formation and transformations of carbohydrates in the animal and vegetable organisms after the compounds in question had been prepared synthetically. It was Emil Fischer who first succeeded in this effort, by preparing from glycerol — the same glycerol which we shall meet with again in the discussion of fats — by gentle oxidation, a substance with the typical properties of a sugar. This compound, called *glycerose*, contains, to be sure, only half as much carbon as grape-sugar. As it was found possible to prepare by the action of dilute alkali upon two molecules of glycerose a true sugar with six atoms of carbon, there was no longer any doubt that glycerose was to be regarded as a member of the carbohydrate group. This synthesis is of especial value to us, as it establishes a relation between the fats and the sugars. Finally, it was even possible to effect the synthesis from the elements; for starting with formaldehyde, CH_2O, Emil Fischer succeeded by polymerization ($6 \times CH_2O = C_6H_{12}O_6$) in obtaining the same sugar as that made from the glycerose prepared from glycerol.[1]

This complete synthesis is particularly interesting to us, because some time

[1] Ber. **23**, 2114 (1890); Die Chemie der Kohlehydrate und ihre Bedeutung für die Physiologie, Berlin, 1894; Synthesen in der Purin-und Zuckergruppe, Braunschweig, 1903.

before this Adolf v. Baeyer [1] had explained in exactly the same way the formation of carbohydrates in plants. According to this conception, which up to the present time has not been proved absolutely, the leaves containing chlorophyll reduce the carbon dioxide of the air to formaldehyde, and the latter is transformed into sugar by condensation. The formation of sugars containing a different number of carbon atoms can be similarly explained with the help of the same hypothesis. It is, indeed, perfectly possible that the building up of the higher sugars by nature takes place through the same intermediate stages as have been observed in the artificial synthesis.

Now, an accurate examination showed that the sugar obtained from glycerose, or from formaldehyde, containing six atoms of carbon was not identical in all its properties with grape-sugar. It was, therefore, given a special name, *acrose*. Biot [2] made the important discovery that cane-sugar rotates the plane of polarized light. This property, which other sugars found in nature likewise show, was quickly utilized technically for the quantitative determination of cane-sugar in cane-juice, etc.[3] It proved to be also of considerable aid in distinguishing the different kinds of sugar from one another. Now acrose does not have this property: it does not rotate the plane of polarized light. The reason for this is that acrose is composed of components each having the opposite effect upon polarized light, and as a matter of fact it is possible to decompose acrose into these unlike individuals. According to the conditions of the experiment, it may be changed into fruit-sugar or mannose or grape-sugar. Herewith the final step in the artificial synthesis of sugars such as occur in nature was accomplished.

The *optical activity* of almost all natural products — a property which for a long time served to distinguish natural products sharply from artificial ones, and gave support to the theory that a special force peculiar to a living organism was necessary for the production of such compounds, until at last here also successful synthetical chemistry made a breach in the wall which had been considered as impregnable — was first explained by the well-known fruitful hypothesis of Le Bel and van 't Hoff [4] (1874). These two scientists independently traced the *asymmetry of the molecule*, which Pasteur [5]

[1] Ber. **3**, 63 (1870).

[2] Compt. rend. **10**, 264; **16**, 619 (1843).

[3] Clerget: Compt. rend. **16**, 1000 (1843); **22**, 1138 (1846); **23**, 256 (1846); **26**, 240 (1848).

[4] Cf. van't Hoff: Die Lagerung der Atome im Raume. Dix année dans l'histoire d'une théorie. La chimie dans l'espace (1875). K. Auwers: Die Entwicklung der Stereochemie (1890).

[5] Leçons de chimie professées en 1860, Paris, 1861. See also H. Landolt: Das optische Drehungsvermögen organischer Substanzen und dessen praktische Anwendungen, Braunschweig, 1898. A. Werner: Lehrbuch der Stereochemie, Jena, 1904.

had ingeniously brought forward in order to explain the optical difference between dextro-tartaric and lævo-tartaric acids, to the individual carbon atom. This atom is in combination with four different masses. Every asymmetric carbon atom in a compound causes the possibility of two optical isomers, one rotating the plane of polarized light to the right, and the other to the left. We can illustrate this best, according to van 't Hoff, by imagining the valences or affinities of the carbon atom extending towards the apexes of a tetrahedron in the center of which the carbon atom itself is placed.

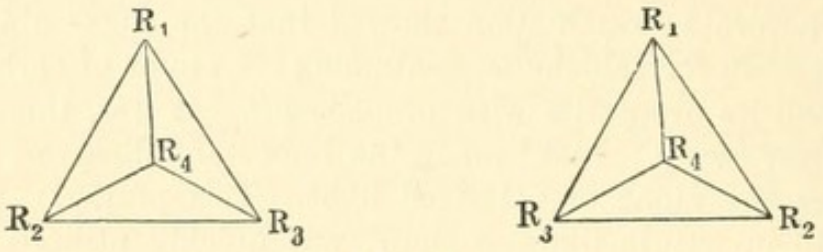


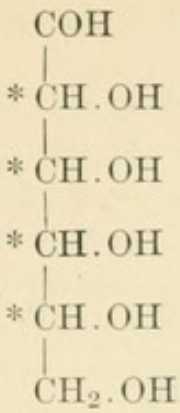
Fig. 1.

(R_1, R_2, R_3, R_4, are the four different masses with which the carbon atom is combined.)

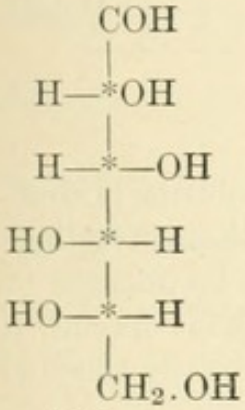
The above drawing represents this kind of isomerism. The two forms are in the same relation to one another as an object and its reflected image, or as a right and left glove; i.e., they cannot be superposed one upon the other, so that the corresponding parts will all coincide. There are, then, three possible modifications in the case of every carbon compound containing an asymmetric carbon atom, namely, two optically active forms, and one which is inactive, being composed of an equal number of molecules of each of the other two forms. In the last case the two asymmetric carbons, although both active, have an equal and exactly opposite effect upon polarized light, so that they neutralize one another.

If these assumptions are correct, then if there are two or more asymmetric carbon atoms in the molecule, the number of possible optical isomers must increase regularly and amounts to 2^n where n is the number of asymmetric carbon atoms. This theory has been confirmed empirically to a most remarkable degree, and, indeed, in no part of chemistry has the work of Le Bel and van 't Hoff been so strongly supported as in the development of carbohydrate chemistry according to this point of view by Emil Fischer.

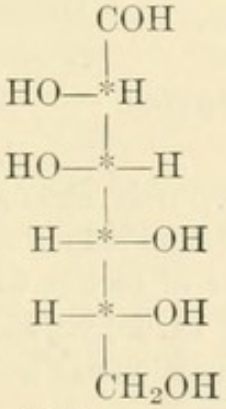
In advance, it may be mentioned, for example, that there are several different sugars having the empirical formula $C_6H_{12}O_6$. Of these we need mention only *d*-glucose, mannose, and galactose. Now all of these sugars contain, as shown by the following general structural formula, no less than four asymmetric carbon atoms:



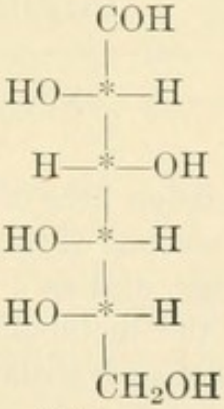
According to the rule given above, $2^4 = 16$ different compounds should exist having such a structure. There is really no doubt concerning this, for already no less than twelve isomers representing six optical pairs have been isolated. For each one of these the geometric constitution is explained by the theory, and the configuration of each single molecule represented by a definite structural formula. The following is a summary of the configuration formulæ given by Fischer to the hexoses,[1] in which the asymmetric carbon atoms are marked with an asterisk.



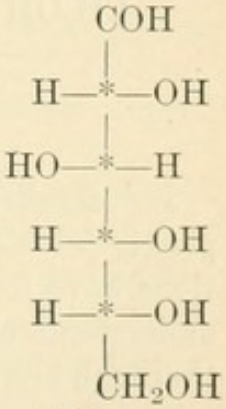
l-Mannose



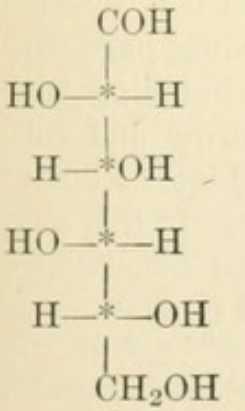
d-Mannose



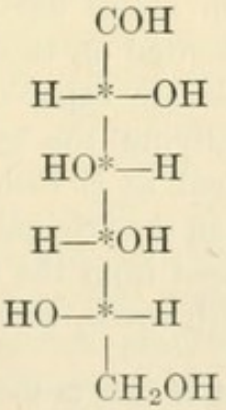
l-Glucose



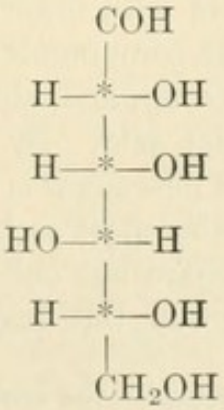
d-Glucose



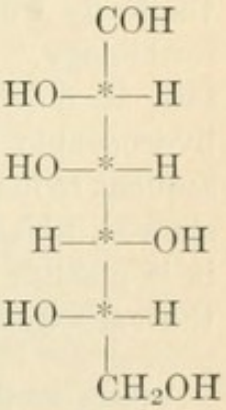
l-Idose



d-Idose

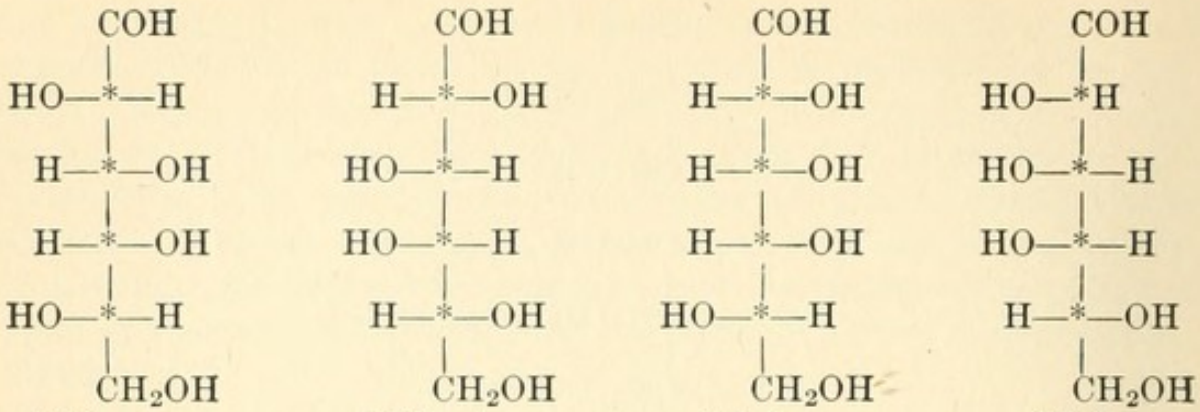


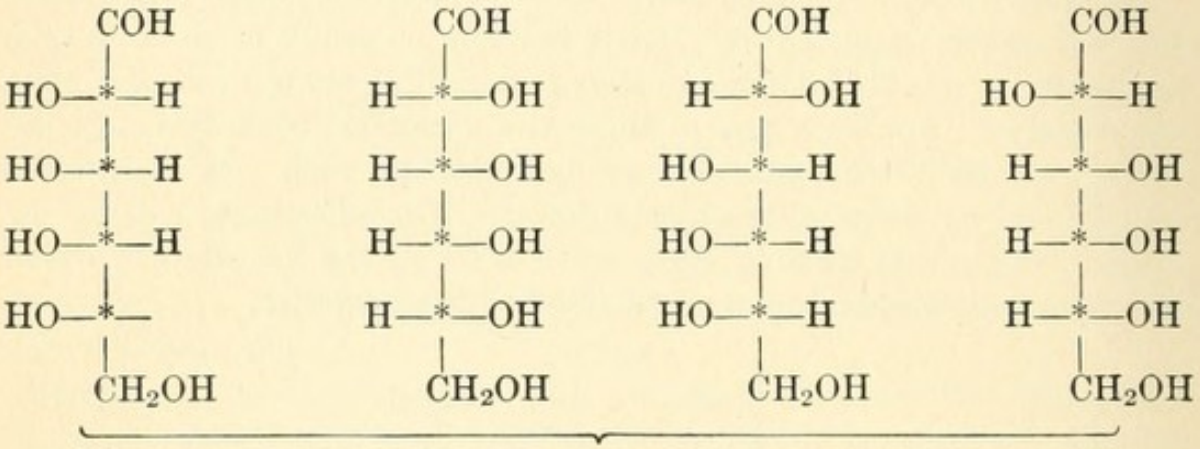
l-Gulose



d-Gulose

[1] The configuration formulæ for sugars with less carbon have also been worked out.





Not yet prepared.

The detailed discussion here of these purely chemical problems is completely justified on account of the great significance which these investigations have for biology, and as a matter of fact it will not be possible to understand clearly the metabolism of carbohydrates without having a thorough knowledge of such structure questions as we have briefly touched upon.[1]

The ways and means by which sugars rich in carbon are prepared synthetically from those with fewer carbon atoms are not without interest for biology. All the compounds represented on the preceding page contain an aldehyde group, and aldehydes are capable of combining with hydrocyanic (prussic) acid. By saponifying the cyanhydrins and subsequent reduction, a new sugar is obtained, as E. Fisher and later Kiliani [2] showed, which contains more carbon than the original sugar. In this way, it is possible to prepare not only hexoses from the simplest members of the carbohydrate group, but sugars with seven, eight, and nine atoms of

[1] Since we shall meet with the same point of view in the case of other classes of substances, especially the proteins, the student is advised to refer to some of the books on the subject.

[2] Ber. **18**, 3066 (1885); **19**, 221, 767, and 1128 (1886).

carbon as well.[1] There appears really to be no limit to the applicability of the synthesis.

Perhaps this synthesis throws some light upon the formation of the different sugars in plant organisms, for it must be often necessary for them to build up from sugars containing a small number of carbon atoms those with more carbon. The possibility of such transformations shows that there is no sharp distinction between the separate sugar groups containing different numbers of carbon atoms, and unites, both chemically as well as biologically, the different classes to a large unit which becomes even more closely related by reason of the fact that the artificial representatives of the same group such as grape-sugar, mannose, and fruit-sugar, can be changed into one another by successive oxidation and reduction.

The large number of sugars prepared synthetically, some of which have not yet been found in nature, together with the natural sugars are subdivided into groups. We distinguish, in the first place, between the more simple sugars called *monosaccharides* and compound sugars called *polysaccharides*. The latter may be regarded as formed from two or more molecules of the former with elimination of water, and, as a matter of fact, the simpler sugars may be formed from them by hydrolysis.

The monosaccharides again are divided into subclasses governed by the number of carbon atoms in the molecule. Thus we have a *diose* (glycol aldehyde, or glycolose, $\begin{vmatrix} HCO \\ CH_2OH \end{vmatrix}$) which is the simplest possible sugar, and *trioses*, *tetroses*, *pentoses*, *hexoses*, *heptoses*, etc. We have seen, furthermore, that, in general, a sugar is a polyatomic alcohol containing either a ketone or aldehyde group. Corresponding to this, from the trioses on, we distinguish between aldoses (aldehyde alcohols) and ketoses (ketone alcohols). Again, an important type which we frequently meet with in nature is that of the methyl derivatives of these sugars; thus we have fucose (from rockweed, known botanically as fucus), rhodeose (from jalap) and rhamnose (prepared from numerous plants), which are all designated as *methyl pentoses*.

Of all the numerous sugars, but few are found in the animal organism, and only a few play any considerable part as forms of nourishment, although we must admit that our present knowledge concerning the physiological significance of numerous sugars found distributed throughout the vegetable kingdom, partly free and partly combined with other substances, is still far from being complete. Members of the last-mentioned class of substances, the number of which is extremely large are known as glucosides, and as such we designate all substances which are more or less readily decomposed into a sugar on the one hand and a compound either aromatic or aliphatic in nature on the other. Such decom-

[1] Emil Fischer: Ann. **270**, 64 (1892).

positions may be effected by ferments (emulsin, myrosin, betulase, etc.) as well as by chemical reagents. Thus, for example, amygdalin breaks down by the action of emulsin into two molecules of grape-sugar, one molecule of benzaldehyde, and one of hydrocyanic acid:

$$C_{20}H_{27}NO_{11} + 2\,H_2O = 2\,C_6H_{12}O_6 + C_6H_5CHO + HCN.$$

The structure of these compounds has been cleared up perfectly by Emil Fischer,[1] who succeeded in making sugar combine with alcohol and similar substances by the mere action of dilute hydrochloric acid.[2] The glucosides are in fact compounds perfectly analogous to the polysaccharides, both being formed by the combination of two molecules of simpler compounds with loss of water, although in the former case the molecules reacting are unlike, whereas in the case of polysaccharides only sugar molecules are concerned. Not only monosaccharides, but polysaccharides take part in the formation of true glucosides, as Emil Fischer showed in the case of amygdalin.[3] Such observations are of great value for biology, as they permit us to consider the formation of such large classes of substances from a single point of view.

The significance of the glucosides in the narrower sense of the economy of the animal organism has up to the present time been but slightly investigated. Without doubt a part of the sugar contained in the organism is in such a form, and perhaps this enables such sugar to escape combustion. It is only recently that such substances have been carefully studied. The investigations of Thierfelder[4] upon *cerebron*, a substance isolated by means of indifferent solvents from the human brain, may be mentioned in this connection. On being subjected to hydrolysis this substance took up two molecules of water and formed one molecule of *cerebronic acid*, one of *sphingosine* and one of *galactose*:

$$C_{48}H_{93}NO_9 + 2\,H_2O = C_{25}H_{50}O_3 + C_{17}H_{35}NO_2 + C_6H_{12}O_6.$$

A similar glucoside is the glycoproteid prepared by Schulz and Ditthorn[5] from the albuminous glands of the frog, which on being subjected to hydrolysis yields among other products an amido-sugar, *galactosamine*, a result which is closely analogous to the finding of glucosamine by Friedrich Müller[6] in the mucin substances of the human respiratory organs.

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[1] Ber. **26**, 2400 (1893).

[2] For example: $C_6H_{12}O_6 + CH_3OH = C_6H_{11}O_6 \cdot CH_3 + H_2O$.

[3] Emil Fischer: Ber. **28**, 1508 (1895). From amygdalin the yeast-enzyme splits off one molecule of sugar, and forms a new glucoside called mandelonitrile-glucoside, which emulsin decomposes completely into sugar, benzaldehyde, and hydrocyanic acid.

[4] Z. physiol. Ch. **43**, 21 (1904) and **44**, 366 (1905).

[5] *Ibid.*, **29**, 373 (1900); **32**, 428 (1901).

[6] Sitzungsber. Gesellsch. Förderung gesamt. Naturwissensch. zu Marburg. **1896**, 6; **1898**, 6.

Later on, when we come to consider the proteins, we shall have to take up these substances in detail.

From the group of glucosides, furthermore, there are derived a great many compounds, some of which have strong toxic properties and are very important drugs, their pharmaceutical value having been discovered purely empirically.[1] Of this large group of such compounds we shall mention only the saponin substances, phloridzin, salicin, helleborin, and the digitalis glucosides (digitalin, digitonin, digitoxin)[2]. Finally, it may be stated that alizarin, the well-known red dye, likewise occurs in nature as a glucoside (ruberythric acid) in madder root (*Rubia tinctorum*). This glucoside has lost most of its practical importance on account of the famous synthesis of alizarin by Graebe and Liebermann (1868). This synthesis was considered a great triumph of chemical investigation, and awakened many bright dreams for the future. Even then it was suggested that the time was near at hand when foods could be produced practically by synthetic methods. Although this hope has not yet been fully realized, nevertheless, such syntheses as that of alizarin have an indirect effect upon the production of foods because whenever a natural substance is replaced by an artificial one a considerable amount of acreage is released.

As far as the animal organism is concerned, the hexoses are the most important representatives of the monosaccharides, and for a long time they, and the corresponding polysaccharides, were the only carbohydrates to be considered at all. It was not until 1892 that a sugar with five molecules of carbon corresponding to the formula $C_5H_{10}O_5$ was discovered. In that year Jastrowitz[3] found a specimen of human urine showing strong reducing properties but which fermented little if any, and was moreover optically inactive. Salkowski[3] then showed that a pentose was present. In the same year Kossel,[4] by the hydrolysis of yeast-nucleic-acid with hydrochloric acid, obtained furfurol, and soon afterwards Hammarsten[5] made similar observations in studying the nucleoproteid obtained from the pancreas; later on Salkowski[6] followed the matter still further, and proved finally that pentoses are present in the above-mentioned products. Sugars, then, of the five carbon series, have been detected one after another in various products found in the body,[7] especially in the nucleoproteids. The

[1] See text-books on pharmacology for the physiological and pharmacological action of these substances.

[2] For other special cases, see van Rijn: Die Glykoside, Berlin, 1900.

[3] Zent. med. Wissensch. **19** and **35**, pp. 337 and 593 (1892).

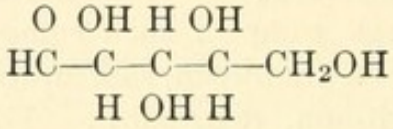
[4] "Ueber die Nucleinsäure," Verhandl. physiol. Gesellsch, Berlin, and in Arch. Physiol. Anat. **1893**, 157.

[5] Z. physiol. Chem. **19**, 19 (1894).

[6] *Ibid.* **27**, 507 (1899).

[7] Z. klin. Med. **34**, 160 (1898).

pentoses accordingly take part in the construction of animal tissue. The only pentose which has been isolated from the organs and closely studied is that from the pancreas-proteids, which C. Neuberg [1] has identified as *l*-xylose with the following configuration:



It belongs, therefore, to the aldoses.

Recently Wohlgemuth [2] has isolated the same pentose from liver-proteid.[3] According to other investigations, it appears that the pentoses in the animal organism are limited to the class of nucleoproteids, and in fact that the carrier of the pentoses is not the albumin, but rather the other component of the compound protein. This was shown, namely, by the experiments of Ivar Bang,[4] who succeeded by warming pancreas-nucleoproteid with dilute caustic potash in decomposing it into albumin and into a product free from albumin, the so-called guanylic acid. This latter contains the *l*-xylose. Guanylic acid is decomposed by boiling with mineral acids into guanine, glycerol, and pentose; it may therefore be considered as glycerophosphoric acid in which the hydroxyl groups are partly replaced by guanine and partly by pentose. As to whether the pentose is similarly combined in the other nucleoproteids has not yet been established.

The quantity of pentoses contained in the separate organs varies greatly and depends directly upon the amount of nucleic substances present. Grund [5] estimates the percentage of pentoses (calculated as xylose) present in certain organs as follows:

Pancreas	2.48
Liver	0.56
Thymus	0.56
Submaxillary Gland	0.53
Thyroid Gland	0.50
Kidneys	0.49
Spleen	0.46
Brain	0.22
Muscle	0.11

The values represent the percentage of pentose (calculated as xylose) in the dry substance.

As has already been mentioned, the first pentose was discovered in urine and formed by metabolism in the human organism. The disease

[1] Ber. **35,** 1467 (1902).

[2] Z. physiol. Chem. **37,** 475 (1903).

[3] For further particulars and other literature, see Neuberg in Ergeb. Physiol. (Asher and Spiro), **3,** Abt. I, p. 373.

[4] Z. physiol. Chem. **31,** 411 (1900–1901).

[5] *Ibid.* **35,** 111 (1902). See also Bendix and Ebstein: Z. allg. Physiol. **2,** Heft 1 (1902).

causing the elimination of this substance is called, if we adopt the suggestion of Salkowski, *pentosuria*. Up to the present time about a dozen cases are known. Pentosuria is distinctly different from true diabetes. It exists for years without showing the clinical indications of the latter metabolic disturbance. In rare cases the elimination of pentose has been detected in diabetes also,[1] but it is not known that there is any connection between the two diseases. The amount of pentose eliminated varies in individual cases between 0.2 and 1 per cent.

It is a striking fact that the pentoses eliminated in urine are, as a rule, optically inactive. Luzzato,[2] alone, has described an optically active pentose. The question next arises as to the source of pentose in urine. First of all, Bial and Blumenthal [3] have proved that pentosuria is independent of the composition of the diet and especially as regards the amount of pentoses in it. They have also shown that with persons afflicted with the disease the combustion of carbohydrates, and thus also of *l*-arabinose, is perfect. Pentoses in urine, therefore, do not find their source in the food. The next possibility which would suggest itself is that perhaps they arise from the breaking down of tissue and especially of the nuclei. If this supposition were correct, we should expect that the pentose found in urine would be the same as that found in the organs, namely, inactive xylose. This now is not the case, as Neuberg [4] has shown, for the pentose in urine is arabinose, and curiously enough almost always the inactive, racemic form. Furthermore, this pentose for the most part does not exist free in the urine, but is combined with urea. For the present, we can only formulate hypotheses concerning its formation. Above all, we are ignorant as to why the pentose should nearly always be eliminated in an inactive form.

The five-carbon sugars are much more widely distributed in the vegetable kingdom than in the animal. Up to the present time they have not been identified with certainty in a free state, but on the other hand they appear to be deposited in the nucleoproteids of certain plants in the same manner as in animals, for Osborne and Harris [5] have isolated triticonucleic acid from wheat flour. This manner of occurrence is inappreciable as compared with that of high molecular *pentosans*, i.e., polysaccharides of the pentoses. These substances are extremely widely distributed, and take part in various ways in the building up of plants. By their hydrolytic cleavage the simpler members of this series are obtained. Not only the pentoses are found as pentosans, but we have methyl-pentosans as well

[1] Külz and Vogel: Z. Biol. **1895**, 185.
[2] Hofmeister's Beiträge, **6**, 87 (1904).
[3] Deut. med. Wochenschr. **22** (1901).
[4] Ber. **33**, 2243 (1900).
[5] Z. physiol. Chem. **36**, 85 (1902).

(especially the polysaccharides of fucose), which are found everywhere in sea-moss. The fucose pentosans are furthermore found in gum-arabic, cerasin, and gum-tragacanth, in the leaves of plane and linden trees, in pine and beechwood, etc. The *rhamnoses*, first found in *quercitrin*, the coloring principle contained in the bark of dyer's oak (*Quercus tintoria*), are also widely distributed. Most pentosans, however, are derived from the simple pentoses. The most important of these are *l*-arabinose, which is obtained from different gums; and *l*-xylose, also called wood sugar because it is the most important mother substance of lignin (xylogen). Xylogen is also found in oat-, rye- and wheat-straw, etc. The pentosans in general are by no means simple compounds, and yield on being subjected to hydrolysis all sorts of different sugars of the five and six carbon series. Doubtless there are a great many intermediary products lying between the simpler and more involved complexes. As regards their physiological function in the plant organism, our knowledge is still very limited. We shall see later on that they are of importance for the nourishment of animal organisms, particularly the herbivora. The following table will give some idea concerning the occurrence of pentosans, the values being given in terms of pentose:

	Per cent.		Per cent.
Meadow hay	21.64	Bruised barley	7.96
Molasses fodder	15.98	Sesame cake	3.87
Rape cake	11.50	Table turnip	1.13
Turnip	2.26	Spinach	1.02
Oil-seed cake	9.07	Sauerkraut	0.96
Acrospires	14.12	Coffee beans	6.5
Rice flour	5.73		

As regards the formation of pentoses in plant organisms, we have no experimental data. Chemically, we can, as has already been mentioned, easily account for it in three ways. The simplest explanation is that of the formation from formaldehyde, which is hypothetically the first assimilation product of the carbonic acid in the air by the green leaves. Five molecules of the aldehyde will condense to form one molecule of pentose ($5 \times CH_2O = C_5H_{10}O_5$). It is also conceivable, that glycerose obtained by the oxidation of glycerol is the starting-point, and from thence by the third method of building up a sugar, namely, the addition of carbon atoms, the pentoses may be formed. Finally it is possible that the pentoses are formed by the breaking down of higher sugars. At all events this relatively simple class of chemical compounds shows to what extremely diverse purposes the vegetable organism is capable of building itself up.

We know absolutely nothing with regard to the formation of sugars of the five carbon series in the animal organism and concerning their significance in the organism. It would seem most likely that the source of their presence is to be sought in the diet, although such a relationship has not yet been definitely traced.

We now come to that group of monosaccharides which is most important for the animal organism, namely, the hexoses of the general formula $C_6H_{12}O_6$. We have already seen that, according to the rule of Le Bel and van 't Hoff, there are sixteen possible isomeric aldehydes having this general formula. We need consider here only the *mannoses*, the *glucoses*, the *galactoses*, and the *fructoses*. Before taking up these sugars in detail we will mention some of the more important general reactions of sugars to the extent necessary for us to understand the physiological behavior of different kinds of sugars.

The simple sugars, in accordance with their aldehyde or ketone nature, are very readily oxidized. They reduce, therefore, metallic oxides on warming their alkaline solutions. Some of the qualitative and quantitative methods of analysis, such as those of Fehling, Trommer, and Böttcher, are based upon this property.

On heating a solution of sugar in caustic soda, or potash, a decomposition takes place (Moore's test). The liquid turns brown, and among other substances, lactic acid, catechol, and formic acid are formed.

If half a cubic centimeter of a dilute, aqueous solution of *d*-glucose is treated with a few drops of a ten per cent solution of α-naphthol in alcohol and then one cubic centimeter of concentrated sulphuric acid cautiously added, the zone of contact becomes reddish violet in color. On shaking, the mixture assumes this color (Molisch). This test is often used for detecting the presence of sugar in proteins, etc., and depends upon the formation of furfurol by the action of the concentrated sulphuric acid upon the sugar.

If a sugar solution is evaporated to dryness and the residue heated somewhat, or if the sugar itself is at once exposed to direct heat, carbonization takes place with a characteristic odor. The mass is called *caramel*.

An important reaction, and one which has become of great consequence in the investigation of the different kinds of sugars, is the combining of many sugars with hydrazines in acetic acid solution, water being eliminated and hydrazones formed. The most important of these compounds are those with phenyl-hydrazine.[1] If an approximately ten per cent aqueous solution of glucose, for example, is treated with a solution of phenyl-hydrazine in acetic acid and then heated on the water-bath for ten or fifteen minutes, fine yellow needles are deposited whose composition

[1] Emil Fischer: "Verbindungen des Phenylhydrazins mit den Zuckerarten," Ber. **17**, 579 (1884), and **20**, 821 (1887).

corresponds to the formula $C_{18}H_{22}N_4O_4$. This compound is known as *glucosazone*.[1] It is formed by the action of two molecules of phenyl-hydrazine upon one molecule of sugar, and in fact the reaction takes place in two stages. First the sugar combines with one molecule of the base, forming a hydrozone as follows:

$$CH_2(OH)\,[CH(OH)]_3CHOH\,.\,CHO + C_6H_5NH\,.\,NH_2 =$$
$$CH_2(OH)\,[CH(OH)]_3\,.\,\underbrace{CHOH}_{*}\,:N\,.\,NH\,.\,C_6H_5 + H_2O.$$

As this compound is very readily soluble in water, this phase of the reaction is not noticeable in the reaction with glucose.[2] Then on warming with an excess of phenyl-hydrazine, the alcoholic group marked with an * undergoes a peculiar oxidation. The group is changed into carbonyl, CO, and is thus capable of combining with a second molecule of phenyl-hydrazine, to form an ozazone of the formula:

$$CH_2OH[CH(OH)]_3\,.\,C - CH$$
$$\quad\quad\quad

Natural and artificial sugars which reduce Fehling's solution (including milk sugar and maltose) give the above reaction. The products obtained are very characteristic, and are the most valuable means that we possess for recognizing and separating the sugars.

An important property of the natural sugars which has been mentioned already is their optical activity. Quantitative methods for the analysis of sugars are based upon this property, and it serves for classifying the sugars as well. Thus the dextro-rotary dextrose was first designated as *d*-glucose, and lævo-rotary lævulose as *l*-fructose. Emil Fischer then proposed a different method of nomenclature which shows the relation of the compounds to one another. The monoses derived from a *d*-, *l*-, or *i*-monose are also designated by the letters *d*-, *l*-, and *i*-, even when they possess a rotary power opposite in sign to that indicated by these letters. In this way lævulose is now designated as *d*-fructose, because of its close relation to *d*-glucose, or dextrose.

The most interesting reaction of sugar from a biological standpoint is its ability to undergo fermentation. Thus different yeasts cause dextrose to break down into alcohol and carbon dioxide (alcoholic fermentation):

$$C_6H_{12}O_6 = 2\,C_2H_5OH + 2\,CO_2.$$

On the other hand, *bacterium lactis* converts it into ordinary lactic acid (lactic acid fermentation):

$$C_6H_{12}O_6 = 2\,C_3H_6O_3.$$

Finally dextrose may be converted into butyric acid, carbon dioxide, and hydrogen by the action of certain microbes (butyric acid fermentation):

$$C_6H_{12}O_6 = C_4H_8O_2 + 2\,CO_2 + 2\,H_2.$$

At this place we shall not take up these processes in further detail. We shall later on find opportunity for showing how great an influence the stereo-configuration of the different sugars has upon their fermentability, and how Emil Fischer by the aid of fermentation studies was able to formulate hypotheses and arrive at conclusions which are of great biological importance and form the foundation of our whole knowledge concerning fermentation reactions.

It remains still to show certain relations between the sugars and two other classes of compounds, namely, their reduction products (the corresponding alcohols) and their oxidation products (the acids). The former relation is at once apparent from the following summary: glucose is the aldehyde of sorbite, mannose of mannite, and galactose of dulcite, while fructose is the ketone of mannite. By oxidation we obtain:

From *glucose*, first the monobasic *gluconic acid*, then by further oxidation the dibasic *saccharic acid*.

From *mannose*, the monobasic *mannonic acid* and then the dibasic *manno-saccharic acid*.

From *galactose* the monobasic *galactonic acid* and then the dibasic *mucic acid*.

Fructose behaves quite differently on oxidation. In the case of the above-mentioned sugars, which are all aldoses, acids are obtained by oxidation having the same number of carbon atoms as the original sugars. Fructose, on the other hand, is a ketone, and on being oxidized breaks down into compounds containing a smaller number of carbon atoms.

These reactions are naturally not peculiar to hexoses, and for the simpler or higher monosaccharides there are corresponding alcohols as well as monobasic and dibasic acids. Thus we have for example:

	Alcohol	Sugar	Monobasic Acid	Dibasic Acid
Bioses:	CH_2OH	CHO	CO . OH	CO . OH
	|	|	|	|
	CH_2OH	CH_2OH	CH_2OH	CO . OH
	Glycol	Glycolose (Glycolaldehyde)	Glycollic Acid	Oxalic Acid
Trioses:				
	CH_2OH	CHO	CO . OH	CO . OH
	|	|	|	|
	CHOH	CHOH	CHOH	CHOH
	|	|	|	|
	CH_2OH	CH_2OH	CH_2OH	COOH
	Glycerol	Glycerose (Glyceraldehyde)	Glyceric Acids	Tartronic Acid
Tetroses:				
	CH_2OH	CHO	CO . OH	COOH
	|	|	|	|
	CHOH	CHOH	CHOH	CHOH
	|	|	|	|
	CHOH	CHOH	CHOH	CHOH
	|	|	|	|
	CH_2OH	CH_2OH	CH_2OH	COOH
	d- and *l*- Erythritol	*d*- and *l*- Erythrose	Erythric Acids	Tartaric Acids (4)

Erythritol was discovered by Lamy (1852) in the algæ *Protococcus vulgaris*. It is optically inactive.

In the group of the pentoses, it has already been stated that arabinose corresponds to the alcohol arabitol and the two acids, arabonic and l-trioxyglutaric, while with xylose we have the alcohol xylitol and two corresponding acids. Although these last alcohols and acids have up to the present

time not been found in nature, yet a native alcohol adonitol, obtained in an optically inactive form from *Adonis vernalis*, corresponds to rhibose, a sugar of the pentose series and which has only been prepared synthetically.

In the case of the sugars containing more than six atoms of carbon and which up to the present time have only been obtained artificially, the seven-carbon alcohols perseitol and volemitol are found in nature. The former is present in the unripe seeds, leaves, and pericarp of *Persea gratissima*, while the latter is contained in *Lactarius volemus*, and has recently been prepared from the rhizomes of several species of *Primula*.

Of the four above-mentioned hexoses, glucose, galactose, fructose and mannose, only the first three are found in the animal organism. *d*-Mannose is found only in the vegetable kingdom partly as such (for example, in the sap of Japanese *Amorphophallus Konjako*), to some extent as glucoside-like compounds (thus, strophantobiose decomposes into *d*-mannose and rhamnose), and finally very extensively in anhydride-like, condensation products known as mannans.

Fructose occurs similarly in the vegetable kingdom, and likewise either free or combined. In the former state it is seldom found pure, but usually is mixed with other sugars as a component of many fruits. Fructose is formed, furthermore, by the hydrolysis of many vegetable substances; thus, of inulin, the reserve-substance in the tubers of dahlia, helianthus, sweet potato, elecampane, etc.

Its most important occurrence is in cane sugar, by the hydrolysis of which one molecule of *d*-glucose and one molecule of *d*-fructose are obtained. This mixture is known as invert sugar.

In the products of the animal kingdom, fructose is not often found. In honey it occurs together with glucose. It is sometimes to be found in the urine after one has eaten considerable fruit. In rare cases it is found in larger amounts in the urine of a diabetic. That fructose occurs normally in animal tissues is extremely doubtful.[1]

Grape sugar, *d*-glucose, glucose or dextrose, as it is variously called, plays without question the most important *rôle* of all the monosaccharides in the animal system. It is this form which carbohydrates in general assume before absorption and assimilation. As *d*-glucose the greater part of the carbohydrate is conducted from organ to organ, from the place of storage to the place of consumption. Glucose is always present in the blood, and the amount varies only within narrow limits, averaging from 0.05 to 0.1 per cent in different animals.[2] These figures, however, are not accurate, because glucose is not the only sugar that is found in blood. On

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[1] Cf. Adler and Adler: Pflüger's Arch. **110**, 99 (1905); Neuberg and Strauss: Z. physiol. Chem. **36**, 233 (1902); Rudolf Ofner: Monatsh. **25**, 1153 (1904); **26**, 1165 (1905); and Z. physiol. Chem. **45**, 359 (1905).

[2] See Lecture XXIII.

the other hand, it is not true, as Asher and Rosenfeld[1] have recently shown, that the greater part of the glucose, or perhaps all of it, is present in a combined state. Glucose from blood diffuses through a parchment membrane, even when the outer liquid is a blood deficient in sugar, made so by the action of yeast. Glucose is also present in certain organs (muscles). It is often hard to decide whether the glucose found was pre-formed, or whether it has been produced secondarily from a carbohydrate of higher molecular weight by hydrolytic fermentation. Normal human urine frequently contains glucose, but always in very small quantities. It may appear in larger amounts after a diet rich in carbohydrates, especially after large amounts of grape sugar have been taken into the system. This is spoken of as *alimentary glucosuria*.[2]

The elimination of considerable quantities of sugar in the urine has been observed after the introduction of numerous chemical substances into the system, as, for example, strychnin, curari, phosphorus, etc.

The most interesting form of glycosuria is that produced by phloridzin and known as *phloridzin-diabetes*. Phloridzin[3] is a glucoside obtained from the root-bark of apple, pear, cherry, and plum trees, and yields by hydrolysis glucose and phloretin:

$$C_{21}H_{24}O_{10} + H_2O = C_6H_{12}O_6 + \underset{\text{phloretin}}{C_{15}H_{14}O_5}$$

The phloretin is decomposed further into phloroglucinol and phloretic acid:

$$C_{15}H_{14}O_5 + H_2O = \underset{\text{phloroglucinol}}{C_6H_3(OH)_3} + \underset{\text{phloretic acid}}{C_6H_{10}O_3}$$

We shall consider these artificially-produced glucosurias at another place.

Glucosuria has been observed, furthermore, by Hofmeister[4] when he fed starch to starved dogs. Böhm and Hoffmann[5] have described the appearance of large amounts of sugar in the urine of cats which were confined and protected from cooling off by coverings. Glucosuria can also be produced by a cold.

Glucose is very widely distributed in the vegetable kingdom partly as such, partly in large storage deposits in the form of starch, and partly as

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[1] Asher and Rosenfeld: Zentr Physiol. **19**, 449 (1905).

[2] Concerning sugar in urine, see Pflüger, Schöndorf, and Wenzel; Pflüger's Arch. **105**, 121 (1904). It is to be remembered that chloroform, for example, when boiled in alkaline solution shows a strong reducing power.

[3] J. S. Stass: Ann. **30**, 192 (1840).

[4] Arch. exp. Path. Pharm. **26**, 355 (1890).

[5] *Ibid.* **8**, 271 and 375 (1878).

the framework-substance in all varieties of cellulose. Finally it takes part in the formation of a great number of glucosides.

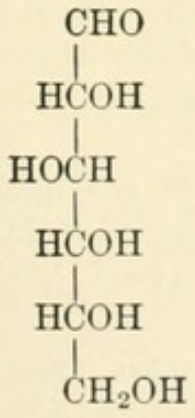
The last member of this series which we have to consider is *galactose*. Up to the present time it has never been detected with certainty in the free state. It is found as a constituent of certain vegetable glucosides, for example, digitonin and sapotoxin.

On the other hand, numerous polymers of galactose, the so-called *galactanes*, are known, some of which yield galactose alone on hydrolysis, while some give other sugars as well.

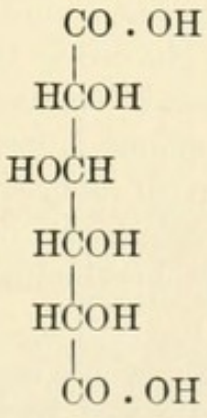
In the animal organism, galactose is present chiefly in milk-sugar, but similarly it has been obtained by the hydrolysis of cerebron (see page 20). We know very little concerning the formation of galactose or of milk-sugar. There are, as has been mentioned, certain well-known higher sugars in the vegetable kingdom which yield galactose, but it is very questionable that there is any direct connection between the galactose in the nourishment and that of milk-sugar. We shall come back to this point in the discussion of the latter compound.

At this place we will consider two compounds which are very closely related to glucose, namely, *glucuronic acid* and *glucosamine* (chitosamine).

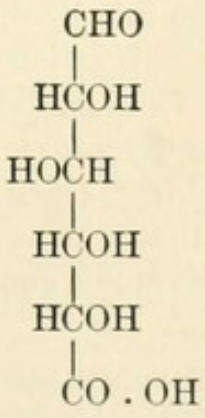
Glucuronic acid (also written glycuronic) is a derivative of glucose. Schmiedeberg and Meyer [1] suspected this, for they realized that the compound combined the properties of an acid, an aldehyde, and a polyvalent alcohol. It was not proved, however, until Thierfelder [2] succeeded in changing glucuronic acid into *d*-saccharic acid, and Fischer and Piloty [3] effected its synthesis from *d*-saccharic acid. By the work of the last named, the configuration was established, as shown by the following summary:



d-Glucose



d-Saccharic acid



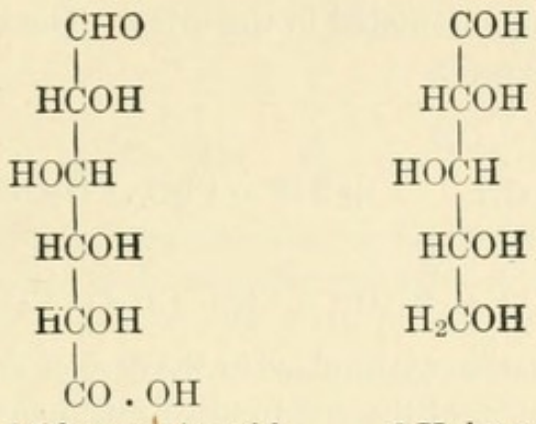
d-Glucuronic acid

[1] Z. physiol. Chem. **3**, 422 (1879).
[2] *Ibid.* **11**, 389 (1887).
[3] Ber. **24**, 521 (1891).

Glucuronic acid itself is not crystalline, but on boiling its solution, *glucurone lactone*, $C_6H_8O_6$, crystallizes out:

$$\begin{array}{l} CHO\,.\,CHOH\,.\,CH\,.\,CHOH\,.\,CHOH\,.\,CO. \\ \qquad\qquad\qquad\qquad\quad \llcorner\!\!\!-\!\!\!-\!\!\!-

seemed probable that the glucuronic acid was formed from decomposed albumin. According to recent investigations concerning the reserve stores of sugar in the starved organism, especially of glycogen, all such conclusions have become doubtful. Furthermore, the whole question of the formation of *glucuronic* acid from albumin coincides with that of carbohydrates from proteins. At another place we shall discuss this problem in detail. On the other hand, an observation made by Salkowski and Neuberg[1] is worthy of mention here. They found that glucuronic acid when exposed to intense putrefaction goes over into the aldopentose, *l-xylose*, with the splitting off of carbonic acid.



d-Glucuronic acid *l*-Xylose

We have met with *l*-xylose before. It is found in the nucleoproteids of the pancreas and liver, and is perhaps the sole pentose occurring in the animal organism. This observed transformation connects the aldohexose, glucose, with the aldopentose, xylose. It is indeed conceivable that the formation of xylose in the animal system may take place in a similar way. For the present we have no precise knowledge about this process, and we know just as little regarding the place of formation of glucuronic acid, i.e., in what part of the body the compound is synthesized. It is extremely probable that it is not limited to a single organ.[2]

Glucuronic acid is to be considered as a substance which protects the organism against the action of various kinds of substances some of which are formed in the body while some are brought into contact with the cells of the body from the outside. It combines with these substances and makes them harmless. We shall subsequently meet with other compounds (glycocoll, sulphuric acid) which perform the same task. The poisons thus neutralized are almost always substances which cannot be destroyed in the organism by direct oxidation. Glucuronic acid may combine directly with these poisons, that is, without the latter undergoing any

[1] Z. physiol Chem. **36**, 261 (1902).

[2] Julius Pohl: Arch. exp. Path. Pharm. **41**, 97 (1898).

other change. This is, for example, the case with substances containing an hydroxyl group. Thus, trimethyl carbinol introduced into the system is eliminated in the urine as a conjugated glucuronic acid:[1]

$$(CH_3)_3 . COH + C_6H_{10}O_7 = (CH_3)_3 . CO.C_6H_9O_6 + H_2O$$

Many compounds, however, are not chemically suitable for coupling with glucuronic acid. They are prepared for combination by the action of the animal body either by reduction, by oxidation, by hydration, or by the two last-named processes acting together. Thus, for example, chloral hydrate[2] and butylchloral hydrate[3] are reduced. The former is changed into trichlorethyl alcohol, and this combines with glucuronic acid, the conjugated acid being eliminated in the urine. The compound formed is known as *urochloralic acid:*

$$Cl_3C . CH\begin{matrix}\diagup OH \\ \diagdown OH\end{matrix} + 2\,H = Cl_3C . CH_2OH + H_2O,$$

$$Cl_3C . CH_2OH + C_6H_{10}O_7 = Cl_3C . CH_2O . C_6H_9O_6 + H_2O.$$

A preliminary oxidation takes place in the case of *o*-nitrotoluol[4] it being changed in the organism of the dog to nitrobenzyl alcohol:

$$NO_2 . C_6H_4 . CH_3 + O = NO_2 . C_6H_4 . CH_2OH,$$
$$NO_2 . C_6H_4CH_2OH + C_6H_{10}O_7 = NO_2 . C_6H_4 . CH_2O . C_6H_9O_6 + H_2O.$$

Many camphor varieties undergo a similar preliminary oxidation.

In other cases the animal organism causes the poisonous substance to take on water, and oxidation may take place simultaneously. An example of this is found in the transformation of thujone into thujone hydrate,[5] which then unites with glucuronic acid:

$$OC_{10}H_{17}OH + C_6H_{10}O_7 = OC_{10}H_{17}O . C_6H_9O_6 + H_2O.$$

Camphene[6] is changed into camphene glycol:

$$C_{10}H_{16} + O + H_2O = HO . C_{10}H_{16} . OH.$$

As the above equations show, the fundamental principle of the coupling is in all cases the same, except that sometimes the poison can unite directly with the glucuronic acid (or glucose — see above), while otherwise this

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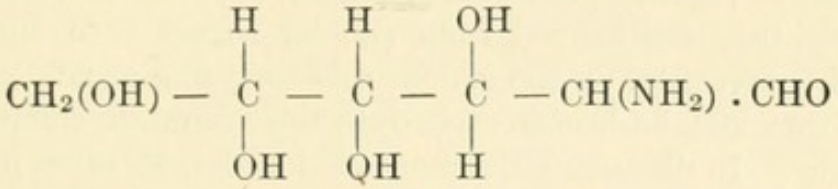
[1] Thierfelder and von Mering: Z. physiol. Chem. **9**, 511 (1885).
[2] von Mering: *ibid.* **6**, 480 (1882). von Mering and Musculus: Ber. **8**, 662 (1875).
[3] Külz: Pflüger's Arch. **28**, 506 (1882); **33**, 221 (1883).
[4] Jaffé: Z. physiol. Chem. **2**, 47 (1878–79), and Ber. **12**, 1092 (1878).
[5] Emil Fromm and Hermann Hildebrandt: Z physiol. Chem. **33**, 579 (1901).
[6] Fromm, Hildebrandt, and Clemens: *ibid.* **37**, 189 (1902–03).

union is possible only after the animal organism itself has produced some change in the objectionable substance.[1]

Quite different from glucuronic acid in its properties is the compound *d*-glucosamine, also known as chitosamine. It was first prepared in large amounts from the chitin of lobster shells,[2] and later, as has been mentioned, from mucin substances and as a cleavage product of proteins. The constitution of glucosamine has been recently cleared up by Fischer and Leuchs.[3] It is to be regarded as a derivative of either *d*-glucose or *d*-mannose, in which the hydroxyl of the α position is replaced by the amido group, NH_2. Its configuration is the following:



Glucosamine is a very interesting compound. It forms an intermediate step between the hexoses and the hydroxy-α-amino acids, which we will soon meet with as cleavage products of the proteins, so that glucosamine, in a sense, forms a bridge between the proteins and the carbohydrates. At present we know nothing concerning the physiological significance of glucosamine. It does not occur free in the above-mentioned substances, but in a polymeric form either alone or with other sugars.

Finally there remains one other amino-sugar to mention which we have already touched upon, namely, galactosamine. It was discovered by Schulz and Ditthorn and represents a component of the glucoproteids in the albuminous gland of the frog. Its constitution is not known at present.

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[1] Cf. Fromm's Die chemischen Schutzmittel des Tierkörpers bei Vergiftungen, Strassburg, 1903.

[2] Ledderhose: Z. physiol. Chem. **2**, 213 (1878–79); **4**, 139 (1880). H. Steudel: *ibid.* **34**, 353 (1902).

[3] Ber. **35**, 3787 (1902); **36**, 24 (1903).

LECTURE III.

CARBOHYDRATES.

II.

Polysaccharides.

The polysaccharides, or compound sugars, which we now have to consider, can be regarded, as we have seen, as glucosides of sugar itself; in other words, they are formed from simpler sugars with elimination of water; and, conversely, by the action of hydrolyzing agents (chemicals or ferments), they may be broken down into their separate components, i.e., simple sugars. In discussing the monosaccharides, we often found opportunity to point out how widely distributed these complicated sugars are, for the simple sugars themselves in some cases only occur in nature in this state. Biologically this group assumes a distinctive position. The animal and vegetable organisms store their reserves of carbohydrates in this form. On the other hand, many representatives of the class may be looked upon as the intermediary products between the more complicated and simpler sugars, and owe their origin to a progressive, spontaneous hydrolysis. In the center of all these processes of transformation taking place in the animal organism, we find the hexoses, especially glucose; whether a complicated sugar molecule such as starch breaks down, or whether such a one as glycogen is formed, for example. In the plant organism the relations are to some extent similar, except that here, as has been previously mentioned, the sugars of the five-carbon series are more common. It is, however, still an open question as to whether the simple pentoses here take such a central position in the metabolism of carbohydrates as the hexoses in the animal system, for up to the present time the pentoses are known almost exclusively in the form of polysaccharides (pentosans, etc.), concerning the formation of which our knowledge is still very limited.

The group of polysaccharides is subdivided, according to the number of sugar molecules which enter into their composition, into di-, tri-, tetrasaccharides, etc., and the true polysaccharides.

The disaccharides [1] consist of two molecules of the simple sugar minus

[1] Here only the *hexobioses* are considered, i.e., those composed of two molecules of hexose. There are also bioses built up of sugars containing fewer carbon atoms, for example, gluco-apiose, which is built up from β-oxymethyl-tetrose (apiose) and glucose, and is obtained from the glucoside in *Petroselinum apiin*. Again, we have the manorhamnoses prepared from strophanthin.

one molecule of water. This conception corresponds to the empirical formulæ:

$$C_{12}H_{22}O_{11} = (C_6H_{12}O_6)_2 - H_2O \quad \text{or} \quad C_{12}H_{22}O_{11} + H_2O = 2\,(C_6H_{12}O_6).$$

They correspond in their behavior to the monosaccharides. Cane-sugar is an exception to this rule, as its alkaline solution is not capable of reducing metallic oxides, whereas the other disaccharides retain entirely the properties of the aldehyde alcohols. The exceptional behavior of cane-sugar has been ascribed to a protected position of its aldehyde and ketone groups.

To this group belong sugars which are found in nature as such, besides other sugars which are formed by cleavage from sugars of higher molecular weight. Cane-sugar, milk-sugar, and maltose are of especial importance, whereas the remaining members of the group are, according to their occurrence and importance, of only limited interest. Worthy of mention are trehalose, first found in ergot of rye; gentiobiose, obtained from gentianose by partial hydrolysis produced by invertin or very dilute sulphuric acid; and cellose (cellobiose), which is believed to stand in the same relation to cellulose as maltose to starch, but has not yet been found in the vegetable kingdom. Melibiose is another hexobiose, and is formed by partial inversion of either melitriose or of raffinose, either by the action of dilute acids or by certain varieties of yeast.

Before discussing the above-mentioned, more important disaccharides, we must mention two other hexobioses which have been obtained in a peculiar manner, namely, isomaltose and isolactose. The former was synthesized by Emil Fischer from grape-sugar by the action of cold, fuming hydrochloric acid, and is said to be formed, on the other hand, together with maltose by the breaking down of starch. It must be mentioned, however, that the identification of isomaltose in most cases has not been entirely satisfactory, so that we are still unable to tell much about the building up and breaking down of carbohydrates in plant and animal organisms, and especially because it seems probable that quite a number of different products have been designated as maltose by various investigators. Isomaltose excited the interest of biologists in particular when, in 1898, A. C. Hill [1] succeeded in obtaining it from grape-sugar by the aid of the maltoglucase of yeast, and likewise by the so-called taka-diastase from *Aspergillus oryzae*. Hill added the ferment to concentrated solutions of grape-sugar, and showed that the fermentation reaction was to some extent a reversible process. Hill himself regarded the product formed

[1] J. Chem. Soc. **73**, 634 (1898); Ber. **34**, 1380 (1901); Proc. Chem. Soc. **19**, 99 (1901); **17**, 184 (1901).

as maltose. Emmerling[1] showed, however, that isomaltose was chiefly present in this case. Later on we shall take up the syntheses produced by the action of ferments more in detail, as well as their biological importance.[2]

Isolactose occupies a quite similar position, and has been obtained by Fischer and Armstrong[3] from a mixture of *d*-glucose and *d*-galactose under the influence of lactoglucase.

Cane-sugar, also known as sucrose, saccharose, and saccharobiose, is of great importance for plant and animal organisms.[4] It plays an important part in the reserve-stores of all *Phanerogamia*, and is found chiefly in tissues containing no chlorophyll, although it is present in smaller quantities in all parts of the plant. It occurs to the greatest extent in the stalk of the sugar-millet (sorghum) and sugar-cane, in the sap of certain kinds of palm, that of the sugar-maple, the birch and the carob tree (St. John's bread). Considerable amounts are found in the ripe fruits and leaves of various growths. At present the sugar-beet is cultivated extensively on account of the cane-sugar it contains, and, together with the sugar-cane, forms the source of practically all commercial sugar.

This important food and condiment has never been positively identified in the animal organism. It is certain that it takes no part in intermediary metabolism. This follows from the fact that cane-sugar introduced into the veins is not utilized, but passes off unchanged in the urine. In order for this sugar to be of value to the animal organism, it must first be subjected to hydrolysis in the digestive tract.[5]

Cane-sugar, as proved by Liebig in the year 1834, corresponds in its composition to the formula $C_{12}H_{22}O_{11}$. It decomposes under the action of hydrolytic agents into one molecule of *d*-fructose and one of *d*-glucose. Since the *d*-fructose in this mixture rotates the plane of polarized light more to the left than *d*-glucose does to the right, the product is lævorotary, that is to say, in the opposite direction as compared with cane-sugar, which is strongly dextrorotary. For this reason this mixture of equal parts of the two hexoses obtained by the cleavage of cane-sugar is called *invert-sugar*, and the process is spoken of as *inversion*.[6] Its formation was first studied by Dubrunfaut[7] in 1830. Mixtures of fruit- and grape-sugars, moreover, occur very extensively in nature (honey, fruit, etc.).

[1] Ber. **34**, 600 and 2206 (1901).

[2] See lecture on Ferments.

[3] Ber. **35**, 3144 (1902).

[4] E. Schulze and S. Frankfurt: Z. physiol. Chem. **20**, 511 (1895).

[5] Claude Bernard: "Leçons sur le Diabète," p. 249 (1877). Fritz Voit: Deut. Arch. klin. Med. **58**, 523 (1897).

[6] This term is also used in general to denote the hydrolytic decomposition of compound carbohydrates into simple sugars. The opposite change is called reversion.

[7] Compt. rend. **25**, 308 (1847); **29**, 51 (1849); **42**, 901 (1856).

Milk-sugar, also called lactose or lactobiose, occurs similarly in nature, and was described as long ago as 1615 by Fabricio Bartoletti in the "Encyclopædia dogmatica," and described in 1700 by Testi and in 1715 by Vallisneri as a newly-discovered medicine. Milk-sugar is found in varying amounts in the milk of all mammals. During confinement it is often found in small quantities in the urine.[1] Similarly in calving it has been detected in the urine for several days before and after the birth. Again after weaning, sugar is wont to pass off through the kidneys. Recently, Porcher [2] has carefully studied the origin of the lactose in milk. He found that extirpation of the breast-glands of milch-goats and cows soon caused a marked increase in the amount of sugar in the blood, while, at the same time, glucose appeared in the urine. These experiments make it seem very probable that the milk-sugar is first formed in the breast, and apparently from glucose alone, and not out of the glucose and galactose in the food

Milk-sugar has never been found in the vegetable kingdom.[3] On being subjected to hydrolysis it breaks up into one molecule of glucose and one of galactose. By oxidizing it with nitric acid, mucic acid, $COOH \cdot (CHOH)_4 \cdot COOH$, is formed.

Maltose, also called malt-sugar, maltobiose, ptyalose, and cerealose, occupies a quite different position from the above two disaccharides. It is a cleavage product of starch, and in fact an intermediary product which usually is immediately hydrolyzed further as fast as it is formed. It is true that small amounts of maltose are met with now and then in plant organisms, and it is quite possible that it is here also a transitory product in the metabolism of carbohydrates. Recent investigations make it seem probable that maltose is also found as a glucoside in the vegetable kingdom.

In animal organs (liver, blood, etc.) maltose has been repeatedly found, although always in small amounts; and furthermore, in many cases the methods of identification have not been entirely satisfactory. The most important manner of formation is by the action of a ferment upon starch.

As long ago as 1785 Irvine, and in 1815 Kirchhoff,[4] observed that extract of malt was capable of breaking down starch. The sugar formed was recognized first by Dubrunfaut [5] in 1822. The active principle in malt, the so-called diastase, was first isolated by Payen and Persoz.[6] Starch

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[1] Cf. Franz Hofmeister: Z. physiol. Chem. **1**, 101 (1877–78), P. Kaltenbach: *ibid.* **2**, 360 (1878–79). F. A. Lemaire: *ibid.* **21**, 442 (1895–96).

[2] Ch. Porcher: Compt. rend. **141**, 73 and 467 (1905).

[3] Bouchardat [Compt. rend. **73**, 462 (1871)] claimed to have found milk-sugar in the ripe fruit of *achras sapota*, but this has not been confirmed.

[4] Schweigger's J. **15**, 389.

[5] Ann. chim. phys. **3**, 21 and 178.

[6] *Ibid.* **2**, 53, 56, 73, and 337.

does not decompose into maltose alone, but quite a number of other products are formed at the same time. The whole process of dissolving the starch by the action of diastase has been made the subject of countless studies. A large number of intermediate products have been isolated and provided with special names, but it would be out of place to discuss here all the transformation products that have been described, for their manner of formation and their chemical characteristics are not yet accurately known. The reason for this is mainly that we are in doubt concerning the homogeneity of the starting material, the starch itself, and know still less concerning diastase.

Ferments, corresponding in their action to this malt-diastase, are widely distributed in nature, and take an important part in the metabolism of carbohydrates in plants. They give back to the metabolism of the plant its reserve-stores, the insoluble starch.

The animal organism, as well, is known to contain ferments which dissolve starch and convert it into sugars, and in this process maltose is formed as an intermediate product, which then breaks down into two molecules of grape-sugar. Later on we shall have to consider such transformations in detail. It may be mentioned here, however, that in the breaking down of glycogen, the stored carbohydrate of the animal system, maltose has also been observed.

Polysaccharides which are anhydrides of three and four sugar molecules are also known and have been accurately described, while in the case of the more complicated compound sugars we know nothing at present concerning the number of sugar molecules which take part in their formation. We know of a trisaccharide, rhamninose, which is composed of two pentoses and one hexose; this is obtained in the decomposition of a glucoside obtained in the fruit of *Rhamnus infectoria*, the xanthorhamnin. Rhamninose breaks down into two molecules of rhamnose and one molecule of *d*-glucose. Trisaccharides composed of three molecules of hexose are more widely distributed in nature. Of these we will mention raffinose (also known as melitriose or gossypose) which is found in different plants and in the sugar-beet. A tetrasaccharide, stachyose, (manna-tetrasaccharide), is known, and was first obtained from the manna of ash. On being treated with dilute mineral acids it takes on water, and is decomposed into one molecule of *d*-fructose, one of *d*-glucose, and two of *d*-galactose.

The higher polysaccharides have been studied but little. We know merely that the complete hydrolysis of these compounds gives monosaccharides as final products. We know nothing, however, concerning the number of molecules of simple sugar which take part in their formation. To the widely different substances of this large group the general formula $(C_6H_{10}O_5)_x$ is given, which signifies that the compound is com-

posed of x-molecules of sugar anhydrides. The attempt has frequently been made to determine the molecular weight of many of these compounds. Thus with starch, the formula derived in this way has been given as $C_{18}H_{30}O_{15}$ on the one hand, and as $C_{360}H_{600}O_{300}$ on the other. We will meet with the same difficulty when we come to study the proteins.

The following substances belong to this group: starch, inulin, cellulose, gums, vegetable mucilages, and glycogen. They are, with the possible exception of glycogen and inulin, all known only in the amorphous state. Water dissolves some of them completely, others merely swell, while the remainder are unaffected. The solutions do not taste sweet, but are optically active. In general they will not diffuse through a parchment membrane, and for this reason they are also called saccharo-colloids. Chemically they are indifferent compounds, and will not combine, for example, with phenyl-hydrazine. With the exception of dextrin, they will not reduce metallic oxides in alkaline solution.

These various higher polysaccharides differ widely in biological significance. Thus starch and glycogen, which on account of their similar nature may be designated as vegetable and animal glycogens, are found to be the most important reserve-substances of the carbohydrate group that occur in the vegetable and animal kingdoms respectively. Inulin has a similar nature. The gums and vegetable mucilages, on the other hand, fulfill an entirely different purpose. They serve, at least to some extent, to close up injuries, and correspond to the wound-secretions of animals. Then again, those substances classed together under the name of cellulose have a still different significance. They are found extensively in the vegetable world, and form in general the chief constituents of the walls of plant cells; or at least this is true from the mosses and ferns up through the whole order of phanerogams, while in the studies concerning bacteria, fungi, and algæ, the conclusions drawn have not been uniform.[1] A peculiar position is occupied by the dextrins, which it is now certain are not individual substances, but very complicated mixtures. As we have already seen, they are to be looked upon as the decomposition products of starch.

Now, after this brief introduction, we shall turn our attention to the individual representatives of this class. Sharply distinct in its entire behavior from all the other higher polysaccharides, is cellulose. It is perfectly insoluble in the ordinary solvents, water, alcohol, ether, etc. There is, in fact, only one good solvent known for cellulose, and this is an ammoniacal solution of copper oxide (Schweitzer's reagent). If cellulose is treated with concentrated sulphuric acid at ordinary temperatures, first of all the sulphuric acid ester of cellulose is formed. If this sulphuric acid solution

[1] For the chemical composition of the cell membranes of different cryptogams, see Karl Müller: Z. physiol. Chem. **45**, 265 (1905).

is diluted with water and boiled, glucose is formed. Cellulose is found almost exclusively in the vegetable kingdom. In the animal world it has only been identified with certainty in the shells of the tunicata.[1] In the cell walls of plants there are found not only sugars of the cellulose group, but other complicated carbohydrates as well, which, on being subjected to hydrolysis, sometimes yield glucose, sometimes no glucose at all, besides other sugars (arabinose, xylose, etc.). These substances have been designated by Schulze as *hemicelluloses*.[2] In building up the cell walls, furthermore, the pentosans, which yield only pentoses on hydrolysis, also take an active part.

As is well known, the cell walls undergo changes with age which at first are manifest externally only by greater rigidity. We speak of *lignification*. This process has been made the subject of much careful investigation without ever being clearly explained. Erdmann [3] assumes that "wood" is formed from cellulose by its combination with other substances which are perhaps of an aromatic character.[4]

Exuding from the various tissue-complexes (medullary-, wood-, and bark-parenchyma) of the cell membranes come the different gums. They are very widely distributed in nature, and, by breaking them down with dilute acids, usually galactose and arabinose are formed. Naturally this group cannot be regarded as homogeneous. Especially well known are gum-arabic and cherry-gum. To this class belongs agar-agar (obtained from East-Asiatic algæ), which has become important as a culture medium for bacteria. Again, the common vegetable mucilages are included, being different from the gums only by their insolubility, or difficult solubility, in water.

We now come to those members of the carbohydrate group which the animal and vegetable organisms temporarily withdraw from the general metabolism in order to be able to make use of them at any time by transforming them back into simple sugars. We have, in cane-sugar, already met with such a reserve-substance for plants. At least an equally important part is taken by the starches [5] (amylum) which are found in the seeds, roots, bulbs, tubers, pith of trees in winter (especially in vegetation robbed of their leaves at this season of the year), etc. The amount of starch present in some of these stores may amount to even eighty per cent of the dry substance. Amylum occurs in the form of stratified granules,

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[1] C. Schmidt: J. pr. Chem. **38,** 433 (1846). Franchimont: Ber. **12,** 1938 (1879). Winterstein: Ber. **26,** 362 (1893).

[2] Schulze, Steiger, and Maxwell: Z. physiol. Chem. **14,** 227 (1890). Schulze: *ibid.* **16,** 387 (1892); **19,** 38 (1894); Ber. **22,** 1192 (1889), and **24,** 2271 (1891).

[3] J. Erdmann: Ann. Suppl. **5,** 223 (1867).

[4] Cf. Viktor Grafe: Monatsh. **25,** 987 (1904).

[5] Cf. Brown and Heron, Ann. **199,** 165 (1879).

differing in form and size with different plants. The concentric rings represent its gradual growth. Starch is scarcely changed at all by cold water, but warm water makes the grains swell up and finally burst, forming starch-paste. Starch-paste does not reduce metallic oxides in alkaline solutions. A very rapid swelling is brought about at ordinary temperatures by means of concentrated solutions of metallic salts. Even with dilute alkalies a starch-paste may be prepared in a short time. A well-known test for starch is the indigo-blue coloration produced by iodine solutions in the presence of hydriodic acid or an iodide. The coloration is not permanent on boiling, but reappears on cooling. The presence of substances capable of being oxidized by iodine (caustic alkali, sulphurous acid, arsenious acid, etc.) will prevent the appearance of this test, the blue color only being obtained when such impurities have been oxidized. All varieties of starch do not give a blue color with iodine; some of them give a reddish-brown color, and with others the color is that of red wine.

At present we do not know a great deal concerning the significance of these different colorations; it is positively certain, however, that starch cannot be regarded as a chemical individual. The conception " starch " comprises a large group of substances of similar physical and chemical properties, which form a unit on account of their common biological significance. An attempt has been made to get an idea concerning the structure of starches by studying their decomposition products. On boiling them with dilute acids, glucose is obtained. If the acid is allowed to act in the cold, or with only gentle heating, a hydration product is produced which is known as " soluble starch." By the action of cold, dilute mineral acids for several weeks, or by an hour's treatment with 4 per cent. sulphuric acid at 80° C., the so-called " amylodextrin " is obtained, and, on further hydrolysis of the latter, dextrins are formed, while, as just mentioned, the final product is grape-sugar. We stated in connection with maltose that a similar breaking up of the starch molecule could be effected by ferments, in this case *diastatic ferments*. It was also mentioned then, that at present we are not able to deduce a picture of the starch formation from a study of the great number of intermediate products obtained by partial hydrolysis and designated in the literature with particular names. We must be satisfied, for the time being, with the knowledge that amylum contains a large number of anhydride-like grape-sugar molecules, and by taking on water it is decomposed, step by step, into smaller molecules, and finally into the basal component glucose. We shall find, later on, that the proteins are quite similarly constituted. Soluble starch, amylodextrin, dextrin, etc., correspond to the albumoses and peptones, while glucose, the elementary building material, corresponds to the amino-acids. A similar analogy is found with the fats, although here the relations are much simpler.

In discussing fruit-sugar we met with a reserve-carbohydrate, inulin, which in its biological relations completely corresponds to starch. It is found in the roots of *Inula Helenium*, the bulbs of dahlias, etc., and is different from starch in so far as it yields on hydrolysis fructose instead of glucose. Furthermore, inulin dissolves in warm water without forming a paste; iodine colors it yellow, and diastatic ferments do not attack it.

Finally, in many lichens, especially in Iceland-moss, another kind of starch is recognized which likewise is colored yellow by iodine; it dissolves in hot water, and yields glucose upon complete hydrolysis. This is lichenin, and, like inulin, it is not effected by diastatic ferments.

Besides these carbohydrates which are found in the reserve-stores of plants, others, such as amylin, lavosin, cerosin, and secalin, are found in grain-seeds. These substances yield, as a result of hydrolytic decomposition, sometimes glucose and sometimes fructose. In the constructive-tissue of *Lupinus luteus* is found galactin, a carbohydrate of this group yielding only galactose upon complete hydrolysis. Again, in the class of gramineæ, palms, liliaceæ, amaryllidaceæ, irideæ, and also in the many dicotyledons, we meet with the so-called reserve-celluloses. We understand by the term *reserve-carbohydrates*, substances which appear as solid deposits on the cell-membrane of constructive tissue in seeds.

The dextrins are usually regarded as forming a particular class in the group of carbohydrates. They are, as has been stated, decomposition products, and are obviously mixtures of substances with different molecular weights. They form a transition stage between the "reserve-carbohydrates" and the "metabolic carbohydrates." By further hydrolysis they yield molecules of glucose.

Glycogen is to the animal organism what starch is to plants. We have to thank the French scientist Claude Bernard [1] for its discovery, who in 1848 noticed the high sugar content of liver, and found sugar only absent from it after prolonged starvation. A few years later, the same investigator succeeded in showing that the sugar observed in liver was not directly present as such, but was formed gradually from a preliminary state. He established the fact that by taking the liver from a dog right after it is killed, the blood being washed off and the washing continued in running water for forty minutes, then the last wash-water will no longer show the presence of sugar. Even if a piece of such liver is boiled in water, no sugar will be dissolved out of it. If, however, the liver is allowed

[1] See Bernard and Barreswil: Compt. rend. **27**, 514 (1848); also E. F. W. Pflüger: "Das Glykogen und seine Beziehungen zur Zuckerkrankheit," Bonn, Martin Hager (1905) and Max Cremer: "Physiologie des Glykogens," in Ergeb. Physiol. (Asher und Spiro) **1**, 803 (1902), Wiesbaden, published by T. F. Bergmann. A complete summary of Bernard's work is found in "L'œuvre de Claude Bernard," Paris, T. B. Bailliere et Fils.

to stand for twenty-four hours, a considerable quantity of sugar will then be found present. This suggested to Bernard that a substance must be present in liver which is difficultly soluble in water, but yielded sugar by the action of the liver substance, and this must be "living," as the following experiment showed. After thoroughly washing the liver, half of it was boiled, and from this it was not possible to obtain any more sugar, whereas sugar was slowly formed in the other piece. Bernard not only assumed the presence of a complicated substance in the liver from which the sugar was formed, but he actually succeeded in isolating such a substance.[1] The method employed by him for the preparation of glycogen is essentially the same as that of to-day. It depends upon the fact that alcohol precipitates glycogen from an alkaline solution of the organ. By dissolving it again in caustic potash and reprecipitating by alcohol, it is easy to purify the crude glycogen. In this way August Kekule[2] succeeded in preparing glycogen free from nitrogen and ash.

We shall later find that this skillful investigator, Bernard, not only discovered glycogen, but to him is also due the credit of clearly recognizing its biological significance.

Glycogen is closely related to starch not alone by acting as a reserve carbohydrate, but also as regards its formation. It is, however, not identical with starch, but sharply distinct from it. In common with the other members of this complicated group of carbohydrates, its empirical formula is represented by $(C_6H_{10}O_5)_x$. It is a fine, white, amorphous powder. We know absolutely nothing concerning its molecular weight.[3] It swells in cold water and apparently dissolves, although the solution shows a distinct opalescence. That an actual solution is not formed is shown by the fact that the glycogen will not diffuse through a parchment membrane. Furthermore, Gatin-Gružewska[4] has recently shown that glycogen in water behaves exactly like a colloid, migrating towards the anode. Glycogen is dextrorotary. Its solutions are colored by iodine yellowish-brown, reddish-brown to deep red according to the concentration. An alkaline solution of copper oxide dissolves it, but the solvent is not reduced.[5]

Quite like the other polysaccharides, glycogen is decomposed by boiling with dilute mineral acids into its simplest component, which in this case is exclusively grape-sugar. The breaking down of the complex

[1] Claude Bernard: Leçons sur la Physiologie et la Pathologie du Système nerveux, vol. i, p. 467 (1857). See also Gazette médicale, **28**, III (1857).

[2] Pharm. Zentrb. p. 300 (1858).

[3] Z. Gatin-Gružewska: Wanderung des Glykogens unter dem Einfluss des elektrischen Stromes. Pflüger's Arch. **103**, 287 (1904).

[4] Pflüger's Arch. **103**, 282 (1904).

[5] Concerning its quantitative estimation, see E. F. W. Pflüger, *loc. cit.* pp. 61 and 67, *et seq.*

molecule can take place, as with starch, step by step, and quite analogous products are obtained here. Diastatic ferments likewise attack glycogen. Among the decomposition products obtained by hydrolysis, dextrin and maltose have been identified with certainty. In its other relations it is exactly similar to starch, and here as in the case of the compounds of higher molecular weight formed from it (the dextrins, for example), we have no guarantee concerning their individuality, and similarly we do not really know whether glycogen itself is a simple compound or a mixture.

We do not yet know with sufficient certainty whether glycogen as such is deposited in the tissues, or whether at least a part of it may not be present in a combined state.

Glycogen is widely distributed in the animal kingdom, and is found in all sorts of different tissues.[1] One of its chief sources is the liver, in which it is deposited in the cell-substance. The nucleus is always free from it. The amount present depends greatly upon the condition of nourishment of the animal. The liver contains this polysaccharide even in its early stages of development,[2] although perhaps in small amounts. It is also found in organs corresponding to the liver in many invertebrates, thus, in crabs, mollusks, etc.

Detailed studies have been made concerning the distribution of glycogen in the liver of the Gasteropoda. It was found that the content of glycogen was dependent upon precisely the same conditions as with Vertebrata. In the case of Limax and Helix the entire glycogen content could be made disappear at the end of twenty to twenty-one days. After feeding, it reappeared in the course of nine or ten hours. It is first deposited in the connective tissue, and then in the epithelium of the liver. Starvation alone causes it to disappear. With the gasteropods the liver is the only place in which glycogen is deposited to any extent; in the other organs the amount is hardly worthy of consideration.

Also in the lower organisms, other than mollusks and gasteropods, glycogen is widely distributed. Bernard found it in the larvæ of flies, the grubs of many insects, in earth-worms and tape-worms, etc. Other authors have mentioned its occurrence in Echinoderms, Holothuria, Polyps, Sponges, etc.

Glycogen has likewise been identified in Protozoa (Vorticella, Opalina,

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[1] For the microchemical detection of glycogen, see Dietrich Barfurth: "Vergleichende histochemische Untersuchungen über das Glykogen," Arch. mikro. Anat. **25**, 259 (1895), and Edgar Gierke: "Das Glykogen in der Morphologie des Zellstoffwechsels," Habilib-Schrift, G. Fischer, Jena, 1905.

[2] E. Pflüger: Ueber den Glykogenhalt der fötalen Leber, Pflüger's Arch. **95**, 19 (1901), and Glykogengehalt der fötalen Leber und die Jodreaktion des Glykogens," *ibid.* **102**, 305 (1904).

Chilodon, Amœba, Rhizopoda) and also in fungi.[1] Clautrian,[2] as well as Harden and Young,[3] has carefully determined and studied the amount of glycogen in yeast.

The identification of the glycogen has in many cases not been perfect, and it is an open question as to whether some of the supposed glycogen has not been an entirely different substance. At all events, these substances belong, according to their biological relations, to the glycogen or starch group, or, as we may say, to the group of reserve-carbohydrates.[4]

In the vertebrates the muscles also serve to store glycogen. The various muscles contain different amounts, as shown by the following table:[5]

Animal.	Muscle.	Glycogen.
		Per cent.
Dog I	Biceps brachii	0.17
	Quadriceps femoris	0.53
Dog II	Biceps brachii	0.25
	Quadriceps femoris	0.32
Dog III	Dorsal musculature	0.135
	Adductors posterior	0.077
Rabbit I	Dorsal musculature	0.417
	Adductors posterior	0.444

It is found not only in striated but also in smooth muscle and in the muscle fibrils. The glycogen content in the muscles is dependent upon the condition of nourishment. We shall soon see that the glycogen of muscles has a particular function, and stands in direct relation to the performance of work by the musculature. Even in invertebrates, glycogen is not lacking in the muscular apparatus, and performs the same function.

Glycogen occurs furthermore, in the pancreas, in the small glands of the digestive apparatus, the lungs, kidneys, sexual glands, brain, epithelium, connective-tissue, and blood-[6] and lymph vessels.

B. Schöndorff[7] has determined the amount of glycogen in different

[1] Errera: "Das Epiplasma der Ascomyceten und das Glykogen der Pflanzen," Brüssel (1882), and Compt. rend. **101**, 253 (1885).

[2] Clautrian: "Chemische Untersuchungen über Glykogen," Mem. couronn. Acad. Roy. Belg. p. 53 (1895).

[3] Trans. Chem. Soc. **81** (1902).

[4] Concerning the occurrence of glycogen under pathological conditions, see O. Lubarsch in O. Lubarsch und R. Ostertag: Ergebnisse, **1**, Jahr. **2**, 166 (1895).

[5] August Cramer: Z. Biol. **24**, 78 (1888).

[6] It is a much disputed question whether blood-plasma itself contains glycogen, or whether the glycogen in blood is to be traced merely to that of the white blood-corpuscles. It looks as if glycogen might be present in the plasma, though ordinarily its presence is limited to the leucocytes.

[7] Pflüger's Arch. **99**, 191 (1903).

organs of dogs which were well fed with carbohydrates and meat shortly before their death. The following table gives a summary of the results obtained:

PER CENT OF GLYCOGEN IN THE ORGANS.

	Dog I.	II.	III.	IV.	V.	VI.	VII.
Blood	.005	0.002	...	...	0.0061	...	...
Liver	4.35	7.60	18.69	17.10	16.38	9.89	7.30
Muscle	0.72	0.88	2.54	3.23	3.72	2.53	0.76
Bone	0.18	0.39	1.00	1.31	1.76	0.97	0.27
Viscera	0.03	0.08	1.47	1.51	1.72	1.01	0.20
Skin	0.38	0.20	0.73	0.84	1.60	0.92	0.09
Heart	0.12	0.10	0.58	0.72	1.21	0.49	0.23
Brain	0.04	0.23	0.27	0.23	0.20	0.25	0.20

100 grams of glycogen in the body are distributed in the different parts of the dog as follows:

	Dog I.	II.	III.	IV.	V.	VI.	VII.	Average.
Blood	0.04	0.01	...	...	0.001	...	...	0.015
Liver	20.09	26.37	53.54	56.74	38.53	21.95	48.54	37.97
Muscle	62.55	58.31	31.22	29.00	38.93	53.76	35.83	44.23
Bone	5.36	10.32	6.81	7.29	12.88	11.30	10.77	9.25
Viscera	0.38	1.10	5.21	4.31	5.32	7.30	3.03	3.81
Skin	11.38	3.76	3.00	2.48	4.01	5.38	1.42	4.49
Heart	0.17	0.08	0.14	0.12	0.28	0.18	0.19	0.17
Brain	0.04	0.06	0.07	0.05	0.05	0.13	0.23	0.09

It is evident from the above table that the amount of glycogen present in the different organs varies greatly. At all events, it is never possible to draw conclusions concerning the amount of glycogen present in any given organ from a knowledge of the amount contained in another organ or even in the whole body.

Besides the carbohydrates which have been mentioned up to this point, there are quite a number of other compounds belonging to the group of polysaccharides which have been observed in blood, in milk, and especially in urine. They have been designated partly as animal gums,[1] and partly as dextrin-like substances,[2] etc. The last-mentioned are found in large quantities in the urine of diabetics, although it is quite possible that such products may be present to some extent even in normal urine, because boiling it with mineral acids causes the formation of humin substances,

[1] H. A. Landwehr: Zent. med. Wissensch. **21**, 369 (1885). See also K. Baisch: Z. physiol. Chem. **18**, 193 (1894); **19**, 339 (1895); and **20**, 249 (1895).

[2] Cf. K. v. Alfthan: Helingfors. Osakeyhtiö Weilin und Göös Aktiebolag. 1904.

which points to the presence of carbohydrates.[1] Our knowledge concerning these products is still indefinite, and the same may be said concerning their biological significance. It seems possible that in the case of these complicated carbohydrates in urine we have to do with products which have escaped a complete breaking down.

At this place we may mention the acid recently observed by P. A. Levene [2] in the preparation of nucleic acid from the spleen, which of itself has no reducing power, but acquires it after being boiled with acids. Levene called it *glucothionic acid*, and regards it as a sulphuric acid ester. It is not yet determined what the nature of the carbohydrate component is. John A. Mandel and P. A. Levene [3] succeeded in obtaining this acid also from the kidneys, liver, pancreas, and milk glands, although only in very small amounts. Apparently such sulphuric acid compounds of carbohydrate-like substances are quite widely distributed in the organism. Nothing is definitely known as to the relation that *chondroitin-sulphuric acid* [4] (prepared from cartilage and amyloid) bears to this group, and we are equally ignorant concerning the significance of these products.

Our knowledge concerning jecorin, first described by Drechsel [5] and found by him in the liver of a horse and later in that of a dolphin, and finally by Baldi [6] in the same organ and spleen of other animals, in the muscles and blood of the horse and in the human brain, is still very indefinite. Its constitution is unknown, but it contains sulphur, phosphorus, and a carbohydrate complex which Manasse [7] states to be glucose.[8] It is probably not a chemical individual, but rather a mixture of quite different products. At present there is not much known concerning its significance.[9]

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[1] Cf. Emil Abderhalden and Fritz Pregl: Z. physiol. Chem. **46,** 19 (1905).

[2] Z. physiol. Chem. **37,** 400 (1903).

[3] Z. physiol. Chem. **45,** 386 (1905).

[4] Carl Th. Mörner: Z. physiol. Chem. **20,** 357 (1895). See also Skand. Arch. Physiol. **1,** 210 (1899); Z. physiol. Chem. **23,** 311 (1897). N. P. Krawkow: Arch. exp. Path. Pharm. **40,** 195 (1898). R. Oddi: *ibid.* **33,** 376 (1894). O. Schmiedeberg: *ibid.* **28,** 355 (1891). A. Orgler and C. Neuberg: Z. physiol. Chem. **37,** 407 (1903).

[5] E. Drechsel: Ber. sächs Gesell. Wissensch. **1886,** p. 44, and "Beiträge zur Chemie erniger Seetiere," Z. Biol. **33,** 85 (1896).

[6] Baldi: Arch. Physiol. **1887,** Suppl. p. 100.

[7] Z. physiol. Chem. **20,** 478 (1895).

[8] B. Bing. Skand: Arch. Physiol. **9,** 166 (1900).

[9] See also J. Meinertz: Z. physiol. Chem. **46,** 376 (1905), and M. Siegfried u. H. Mark: *ibid.* **46,** 492 (1905).

LECTURE IV.

CARBOHYDRATES.

III.

Metabolism of Carbohydrates in Plant and Animal Organisms.

Formerly the biology of plants occupied a sharply distinct field from that of the animal organism. The two kingdoms were believed to be opposed to one another with regard to the transformations of energy and of force taking place within each. Plants were alone held to be capable of building up organic substances, i.e., to be capable of effecting syntheses. The animal system, on the other hand, served to break down such substances. In this way the animal and vegetable worlds acted in conjunction and formed a large unit. However, the more scientists penetrated into the intricacies of vegetable and animal metabolism, and in proportion to the comparative studies made, the more it became evident that there was no sharp line to be drawn between these two fields. When Wöhler in 1824 discovered that benzoic acid introduced into the animal body was not consumed nor eliminated as such, but was to be found in the urine combined with glycocoll in the form of hippuric acid, the path was broken, and for the first time a synthetical process was recognized as taking place in the animal organism.

In the following period, as we shall see, a large number of such syntheses were discovered as taking place in the organism of animals, and to-day there is no longer any doubt but that synthetical processes play an important part therein. To be sure, in importance, and, as far as we know, in variety also, they are far in the background as compared to the syntheses in plant organisms. On the other hand, plants utilize oxygen and produce carbon dioxide from more complicated compounds; in other words, they break down substances. In this way physiology has given a new and powerful support to the well-known common morphological outlines of the two kingdoms, so that to some extent the two fields have been placed upon a common basis although each retains a certain degree of independence.

Nothing supported the old conception of the sharp distinction between the synthetical processes of plants on the one hand, and the catabolic processes taking place in animal organisms on the other hand more than the

formation of carbohydrates in plants.[1] No synthesis is more wonderful than this. It determines the whole metabolism of the plant; it forms the support upon which rests the whole development or extinction not only of plants but of the animal world. By means of it the great mass of carbon which, in the form of carbon dioxide, is apparently withdrawn from metabolism as the final product in the combustion of organic substances, is carried back to it; thus the sugar synthesis, or what is commonly spoken of as the assimilation of carbonic acid by the organs of the plant containing chlorophyll, forms an important stage in the carbon cycle. The great deposits of coal, and the rocks which underlie the surface of the earth in vast layers, and are to a considerable extent composed of carbonates (principally of calcium and magnesium), belong to this cycle. The coal is formed from plant residue which formerly thrived on the carbon dioxide of the air; and by burning coal, carbon dioxide is again formed, so that the cycle can again take place. Carbon dioxide found combined with bases such as lime and magnesia likewise originated from the atmosphere. It is temporarily removed from the cycle only to return to it when, for example, it is replaced by the action of another acid, such as silicic acid. Oxygen, for the greater part, also makes the cycle with carbon.

The only important source of the carbon contained in plants is, in fact, as Ingenhousz[2] and then Theodor de Saussure[3] first showed, the carbon dioxide of the air. It is true that it has been observed that roots can take up carbonic acid from carbonates and bicarbonates in the soil, but the amount thus available is very slight. Carbon dioxide is taken up for the most part through the stomata (breathing-pores) of the leaves. The assimilation depends within certain limits upon the amount of carbon dioxide in the atmosphere, the temperature of the leaf, and the intensity of the illumination.[4] For every temperature there is a definite amount of carbon dioxide assimilation; in general, the optimum lies between 20° and 30° C.

In spite of numerous studies, it is still a problem as to what is first formed from the carbon dioxide. At present we understand merely the

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[1] Concerning the assimilation of carbonic acid, consult the text-books on botany, e.g., W. Pfeffer's "Pflanzenphysiologie, Ein Handbuch der Lehre vom Stoffwechsel und Kraftwechsel in der Pflanze," published by Engelmann in Leipsic. — Friedrich Czapek: "Biochemie der Pflanzen," published by Fischer in Jena.

[2] Ingenhousz: "Experiments upon Vegetables," London, 1779, and "Essays on the Foods of Plants and the Renovation of Soils" (1796).

[3] T. de Saussure: "Recherches chimiques sur la végétation," Paris, 1804. — Edmund O. v. Lippmann: "Die Chemie der Zuckerarten." — T. Sachs: "Geschichte des Botanik." — A. Hansen: "Geschichte der Assimilation."

[4] F. Frost Blackman and Gabrielle L. C. Matthaei: Proc. Roy. Soc. London, **76**, 402 (1905).

outward conditions under which the carbonic acid assimilation takes place. We know that cells containing chlorophyll are absolutely indispensable to the process. In order to disturb the condition of equilibrium in the carbon dioxide molecule and in those of the water required to form the assimilation products of carbon dioxide — in each of these compounds the affinity of carbon and hydrogen for oxygen is completely saturated — energy is necessary. The plant cells perform work in transforming kinetic energy into potential energy. It has been known for a long time, and proved experimentally, that cells containing chlorophyll are not of themselves capable of assimilating carbonic acid. The process takes place only by the aid of light. The light vibrations of the ether furnish the energy. All the rays of white light are not active in this respect. In fact, the so-called chemical, or actinic rays, of the ultra-violet and similarly the peculiar heat rays of the infra-red part of the spectrum have little or no power of furnishing the cells with the energy necessary for assimilation. The most active rays are the red, orange, and yellow.[1] These relations have been established by the work of Engelmann.[2] The essential moment, therefore, in the formation of organic substances in the plant cell is the transformation of radiant energy into chemical energy, a process which, as far as we know, takes place exclusively in cells containing chlorophyll. Chlorophyll takes part, thereby, in an important phase in the energy cycle. By its help, kinetic energy is transformed into potential energy, and when later the plant is eaten by the animal, this is eventually changed back into kinetic energy. Yet even in the vegetable world the last-mentioned process plays an important part, for we know of whole groups of plants which are not able to assimilate carbon dioxide themselves; these are the parasites which contain no chlorophyll, and, as regards their metabolism, they are closely related to the animal organism; while on the other hand, we have species belonging to the animal kingdom (vorticella, certain Flagellata, planarians, hydra, etc.), which assimilate carbonic acid, and set free oxygen, thus imitating plants in their metabolism. It has been found that the assimilation of carbonic acid in such cases is also due to the presence of the same agent, chlorophyll,

[1] The maximum assimilation for the bluish-green, fresh-water algæ and the red sea-algæ is effected by rays in other parts of the sun's spectrum. Engelmann has shown (*loc. cit.*) that the light rays act more strongly in proportion as they are absorbed by the color. In general, light complementary to the color of the plant is most active upon assimilation. Spectroscopic analysis shows that of light passing through different depths of water, the red rays are strongly absorbed, while the green and bluish-green are less so. This explains why at greater depths of water, the red and yellow forms prevail rather than those which are blue or bluish-green in color. Cf. W. Engelmann: Arch. Physiol. Suppl. 1902, p. 333. Gaidukow: *ibid.* p. 214.

[2] Th. W. Engelmann: Bot. Ztg. **1882,** 419; **1883,** 1, **1884,** 80; **1886,** 64; **1887,** 393, Pflüger's Arch. **25,** 285 (1881); *ibid.* **26,** 537 (1887); **27,** 485 (1882); **30,** 95 (1883).

which is present in plants; and in fact, the chlorophyll is not deposited free in the tissues of these animals, but the chlorophyll-bearers are those algæ which exist together with the animal cell and have a commensal existence. This sort of parasitism is met with very frequently, for instance in lichens,[1] which are composed of fungi and algæ. Again, we find the same sort of *symbiosis* in higher organisms. Undoubtedly, for example, the bacteria found in the alimentary canal illustrate this relation; they serve, as we shall see later on, to convert carbohydrates (cellulose, etc.) into a form capable of absorption.

By means of carbonic acid alone, the cell cannot build up organic substances; hydrogen, which forms an integral component of all the complicated compounds of the carbohydrate series which the plant organism produces, is lacking. Hydrogen is obtained by the plant chiefly from water in the soil. Herein the plant finds another source of oxygen. The way in which the plant cells utilize, by the help of chlorophyll and the sun's rays, this carbonic acid and water, or, in other words, the first products formed, is still a matter of conjecture. We know merely that all syntheses start with a reduction process; oxygen is set free. In fact, we can follow the assimilation of carbonic acid either by determining the loss of carbonic acid in a gas mixture, or by determining the oxygen evolved. The latter can be observed directly by immersing the parts of a plant in water. Engelmann[2] ingeniously made use of bacteria as a reagent for oxygen. If, for example, a thread of algæ and some aerobic bacteria are placed under an air-tight cover-glass, it will be noticed at first that the latter are very lively. If, now, the preparation is kept in the dark the motion of these bacteria will cease altogether after a time, showing that all the oxygen has been consumed. Then, on bringing the preparation to the light, the bacteria begin to become most active because the thread of algæ has begun to assimilate carbon dioxide and water with elimination of oxygen. By means of this very sensitive test as little as one billionth part of a milligram of oxygen may be detected. Engelmann, as already stated, has by means of this method studied the different parts of the solar spectrum, testing it with regard to its effect upon plant assimilation. Beÿerinck[3] later in a similar way made use of the luminescence of certain bacteria, which also depends upon the presence of free oxygen, for the detection of carbonic-acid assimilation.

With regard to the synthetical products first formed by the plant,

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[1] Schwendener: Nagelis Beiträge z. wissensch. Botanik H. 2, 3, and 4, Leipsic, 1860–1868. — de Bary: "Die Erscheinung der Symbiose," Strassburg, 1879. O. Hertwig: "Die Symbiose oder das Genossenschaftsleben in Tierreich," Jena, 1883.

[2] Bot. Ztg. *loc. cit.*

[3] *Ibid.* (**1890**) 744. See also Hans Molisch: "Die Lichtenwicklung in den Pflanzen, Naturwissenschaftliche Rundschau, **20**, 505 (1905).

different conjectures have been made, and the same is true concerning the action of chlorophyll. Some conceive it to have the action of a ferment,[1] others consider it as closely related to the first assimilation product assuming that it combines with the carbonic acid.

As long as we know practically nothing concerning the formation of chlorophyll, and still less about the plasma of the plant cell itself, and as long as we are unable to isolate satisfactorily any of the first products of the assimilation, the discussion of all such possibilities has only a relative value. For the present we can restrict ourselves to those hypotheses which seem plausible from a chemical standpoint. At the same time it does not follow necessarily that the plant organism carries out its syntheses in such a way, nor that the intermediate products which we assume are actually formed in the living plant cell. Above all, the impression should not be left that the assimilation of carbonic acid can take place only in one direction. It is indeed possible, and in fact probable, that other primary products of assimilation exist. It is undoubtedly certain that one of the first products of the assimilation is a carbohydrate, whether grape-sugar, starch, or perhaps a simpler sugar with fewer than six carbon atoms in the molecule. At all events, — and this is of greatest importance for understanding the whole metabolism of plants, and indirectly that of the animal organisms, — the carbohydrates stand at the center of all the synthetical processes taking place in the plant organism. We shall see later on that the fats, the albumins, and many other highly complicated compounds, evidently originate from carbohydrates, whether by constructive or destructive processes, or both acting together.

In discussing the first synthesis produced in the plant cell we meet with a highly important problem which we have already briefly touched upon.[2] All the carbon compounds which are either directly or indirectly produced by animal or plant organisms, are optically active; that is to say, they possess at least one asymmetric carbon atom. As we have already seen, most of these compounds are found in nature, only in the optically-active state. Now, the plant cell in carbon dioxide receives a carbon compound, which does not contain an asymmetric carbon atom. The

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[1] Jean Friedel (Compt. rend. **132**, 1138 (1901)) has tried to support this view by experiments. He brought together the glycerol extract from fresh leaves with finely powdered leaves which had been rapidly and carefully dried, and found oxygen was evolved by the action of light, and carbonic acid taken up. The experiments, however, are not conclusive, and have not been corroborated. Cf. M. Harroy: Compt. rend. **138**, 890 (1901).— R. O. Herzog: Z. physiol. Chem. **35**, 459 (1902). Again, Bach and Chodat have published a great deal of work summarized in Biochem. Zentrbl. **1**, 11, 417, and I, **12**, 457 (1903).

The relations are so complicated that it is hard to draw a conclusion at the present time concerning the work of these scientists.

[2] Cf. p. 15.

beginning of all the asymmetry in the organic world is centered in the assimilation of the carbon dioxide, for evidently it is here in the plant cell that the first asymmetric synthesis is carried out which continues constantly in the production of only asymmetric compounds.[1] It is indeed possible that chlorophyll, which is itself asymmetrically constituted, plays an important part in this asymmetric synthesis. The first appearance of asymmetry is, however, unexplained. Its existence evidently coincides with the formation of the first cell. It is possible, on the other hand, that the plant cell produces first of all an inactive compound, for example, an inactive sugar, and that asymmetry first develops by the cleavage of this compound.[2] The animal organism receives in turn with its food, this asymmetry of its body-substance from the plant world, partly directly, as in the case of the herbivora, and partly indirectly, as in the case of the carnivora. We shall find, moreover, that these relations are so suited to the animal organism that it sometimes directly disintegrates racemic compounds, and in many cases utilizes only one component and discards the other unchanged.

The first observed assimilation product of the carbon dioxide and water was starch, easily recognized by means of the iodine reaction. For a long time it was held to be the primary product of assimilation. Gradually it became a recognized fact that starch is a product formed secondarily from more simple components, and little by little the opinion gained ground that *d*-glucose was to be regarded as the primary product of assimilation. This assumption was supported by the discovery that leaves which have been preserved in the dark are capable of forming starch directly if solutions containing hexoses are placed upon them. In general, the following compounds are assimilated: — *d*-glucose, *d*-mannose, *d*-galactose, and *d*-fructose. Other similar compounds may be utilized; thus, mannitol

[1] Cf. Emil Fischer: "Die Chemie der Kohlehydrate und ihre Bedeutung für die Physiologie."

[2] Cf. A. Byk: Z. physiol. Chem. **49**, 641 (1904). Byk seeks to trace the formation of asymmetry back to circularly-polarized light which may be produced by the reflection of plane-polarized daylight by the surface of the sea. The revolution of the plane of polarization by the magnetism of the earth makes it impossible for equal amounts of the two forms of light to exist at any point on the earth, or upon the whole surface of the earth, or for any considerable period. It is indeed possible that such may be the explanation of the asymmetry of the first cells, but it is scarcely probable that it accounts for the continuous production of asymmetric molecules, for it is unreasonable to assume that one and the same kind of light is permanently in excess at one and the same point on the earth's surface; and it would seem likely, furthermore, that components of unlike optical products would be formed in different localities. Again, Byk's proof that by means of circularly-polarized light racemic compounds can be split off is not perfectly satisfactory. Furthermore, chlorophyll itself has optical properties. It undoubtedly changes the sun's rays of short wave lengths, which have no effect upon the assimilation, into active, longer wave lengths.

by leaves of the Oleaceæ, dulcite by those of the Evonymus; and interestingly enough it has been found in the case of all the plants examined that the assimilation takes place with those carbohydrates which they contain normally as reserve-substances.

The fact that the volume of carbon dioxide absorbed is equal to that of the oxygen evolved [1] has been cited to prove that a carbohydrate must be the first assimilation product.[2] Again, the relations of the heat effect observed coincides very satisfactorily with this assumption. On the other hand, it must be remembered that it has not been possible to carry out exact measurements in this direction. Side by side with the assimilation of carbon dioxide there is a constant absorption of oxygen and production of carbon dioxide. Both processes stand in a certain relation to one another, although the nature of this has never been determined. It has been repeatedly asserted that oils and fats may appear as the first products of assimilation. Inclusions of oil have been observed frequently in the chromatophores of certain plants; for example, in the Musaceæ, Cactaceæ, algæ, and especially the Vaucheria.[3] By the decomposition of the fat into glycerol and fatty acids, and the simultaneous partial reduction or oxidation of these cleavage products, sugars could be formed from the glycerol, and vegetable acids from the fatty acids. It was soon evident, however, that these fats and oils were not primary products of assimilation, but rather reserve-substances, the formation of which can be readily traced back to carbohydrates. Equally untenable proved Liebig's [4] hypothesis that the vegetable acids were formed as the first assimilation products from which the carbohydrates were obtained secondarily. At present, there are no known observations which are contrary to the assumption that the carbohydrates represent the entrance of the carbon from carbon dioxide into the general metabolism of the plant.

It is now a question as to how we shall explain this synthesis of carbohydrates — *d*-glucose, for example — from carbon dioxide and water. We have already mentioned [5] one hypothesis, namely, the assumption of Baeyer [6] that carbon dioxide by reduction is changed into formaldehyde, and by the condensation of the latter a sugar is formed:

$$CO_2 + H_2O \rightarrow HCHO + O_2.$$

Baeyer originally assumed that the carbon dioxide absorbed was first

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[1] Boussingault: Compt. rend. **53**, 862 (1861), and Holle: Flora, **118** (1877).

[2] This might take place as follows: $6\,CO_2 + 6\,H_2O = C_6H_{12}O_6 + 6\,O_2$.

[3] Paul Fleissig: "Ueber die physiologische Bedeutung der ölartigen Einschlüsse in der Vaucheria," Inaug. Diss. Basel, 1900.

[4] J. Liebig: Ann. **46**, 58 and 66 (1843).

[5] Cf. p. 15.

[6] Ber. **3**, 63 (1870).

changed into carbon monoxide and oxygen. The former was believed to combine with chlorophyll in much the same way as it does with hæmoglobin.

Bach [1] has recently modified somewhat this suggestion of Baeyer. According to him, the carbonic acid is first changed into percarbonic acid, water, and carbon. The percarbonic acid is then decomposed into carbon dioxide and hydrogen peroxide, while the latter forms with carbon and water the formaldehyde:

$$3\ H_2CO_3 = 2\ H_2CO_4 + H_2O + C,$$
$$2\ H_2CO_4 = 2\ CO_2 + 2\ H_2O_2 = 2\ CO_2 + 2\ H_2O + O_2,$$
$$H_2O + C = HCHO.$$

Naturally, a great many attempts have been made to isolate formaldehyde or related compounds from plants, especially from the green leaves. This has not been accomplished, however, up to the present time in a satisfactory way.[2] On the other hand, nothing has been shown that is contrary to the assumption that formaldehyde does actually represent the first intermediate product, for it is perfectly possible that the amount of aldehyde that is constantly being formed is so extremely small that it condenses so rapidly that it escapes detection. It has been brought forward in support of Baeyer's theory that certain plants show a considerable resistance towards formaldehyde. Thus Tréboux [3] found that *Elodea canadensis* will stand a 0.001 per cent solution. Algæ and young plants of *Sinapis alba* are said to be strikingly resistant towards formaldehyde.[4]

We saw in considering the artificial synthesis of carbohydrates that it was possible to form a sugar very easily from formaldehyde, and we can readily understand how, according to the number of molecules of formaldehyde entering into the reaction, sugars containing a different number of carbon atoms in the molecule may be obtained. It is, however, scarcely probable that such syntheses take place in this simple way. Thus, for example, according to all that we know at present, it is hardly to be expected that pentoses are formed directly as one of the first products of the condensation of formaldehyde. Apparently it is much more likely that such sugars are formed by the breaking down of higher sugars especially the hexoses. Such a process is likewise easy to represent, as, in

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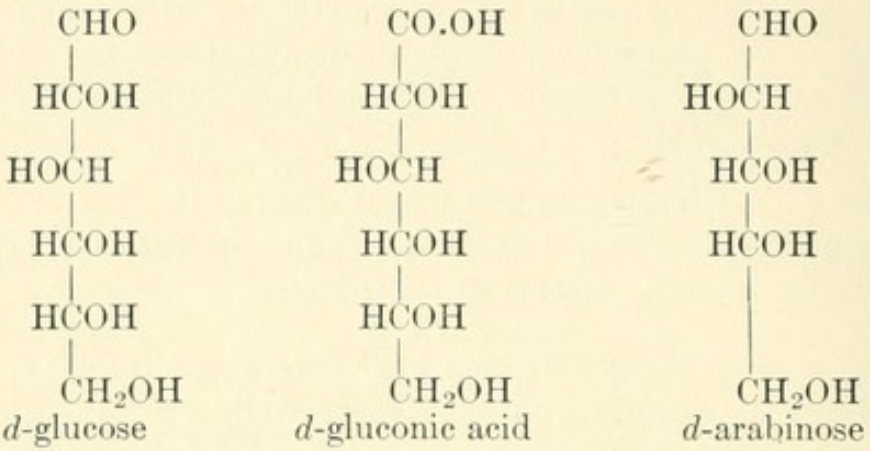
[1] Arch. sci. phys. nat. Gen. **5**, 401 (1898).

[2] H. Euler: Ber. **37**, 3411 (1904). Hans and Astrid Euler: Arkiv för Kemi. **1**, 347 (1904); Ber. **39**, 39 (1906), and *ibid.* **39**, 45 (1906). W. Loeb: Z. Elektrochem. **11**, 745 (1905).

[3] Tréboux: Flora, 73 (1903).

[4] R. Bouillac: Compt. rend. **135**, 1369 (1902). Bouillac and Giustiniani: *ibid.* **136**, 1155 (1903).

fact, O. Ruff has shown.[1] He prepared *d*-gluconic acid by the oxidation of *d*-glucose; its calcium salt he allowed to stand in the sunlight in the presence of ferric acetate, or he treated it with hydrogen peroxide and thus obtained *d*-arabinose.



d-glucose *d*-gluconic acid *d*-arabinose

At all events, — and this is of great importance, — we can explain chemically without difficulty the formation of the different members of the carbohydrate series from the hexoses, especially *d*-glucose.

Emil Fischer[2] has suggested that the glycerose discovered by him is perhaps the first assimilation product of carbonic acid by the plant cells containing chlorophyll. By the combination of two molecules of this, it is easy to understand how hexoses may be formed. Again, glycerose may be of great importance in other relations. We shall mention here merely the fact that it is closely related to glycerol, which is one of the components of the fats; and on the other hand, that from this point of the carbon dioxide assimilation the synthesis of albumin may start. Glycerose, as we shall show more fully later on, stands in close relation to certain of the decomposition products of albumin, namely alanine, serine, and cystine. Naturally it does not necessarily follow that either the formation of the fats or that of the proteins *must* begin with this hypothetical product of assimilation. It is indeed possible that glycerose appears merely as one of the decomposition products of *d*-glucose or starch, and then is used for syntheses by the plant cell.

All of these possibilities have been brought forward in order to bring the ground-plan of the whole carbon assimilation at least within the realms of our understanding, and to show the far-reaching value that purely chemical investigations, especially those of Emil Fischer, have upon the science of biology; for it is by this means only that the most important classes of substances — carbohydrates, fats and proteins — have been traced back to a common source. The consideration of these relations is

[1] Ber. **31,** 1573 (1898); **32,** 550 (1899). Otto Ruff and Ollendorf: *ibid.* **33,** 1798 (1900). Cf. A. Wohl: *ibid.* **26,** 730 (1893); **33,** 3666 (1899).

[2] Ber. **23,** 2138 (1890).

of great importance; and a detailed discussion is justifiable, furthermore, because it is very probable that also in the animal organism chemical changes take place by means of which a substance belonging to one class is changed into a compound of another, as, for example, a fat into a carbohydrate or conversely.

The animal organism is, in its entire existence, dependent upon this carbonic acid assimilation by the plants, for in this way it obtains all the organic compounds of complicated structure. Energy originating in the sun is thus obtained by the animal in the form of potential energy, from which the animal derives its kinetic energy and ability to perform work. The assimilation by the plant not only serves to furnish organic material for the animal organism, but to a certain extent it furnishes the oxygen which it requires for obtaining the kinetic energy again by combustion; the assimilation process being one of reduction in which oxygen is constantly being evolved. The oxygen in this way returns to the general cycle of the elements. This oxygen, after taking part in the metabolism of the animal, escapes chiefly as carbon dioxide and water, both of which are again utilized by the plant for the formation of other organic substances.

Let us turn now to those carbohydrates which are most important as food for the animal organism, namely, grape-sugar, cane-sugar, and starch. From the last two compounds the animal obtains all the carbohydrates that it needs. Let us follow these sugars on their way through the alimentary canal to their absorption and final assimilation. As an example, we shall choose starch, because it is here that the relations are the most complicated, and we shall be able to treat of the behavior of the simpler sugars in connection with the separate phases in the breaking down of the starch.

First of all starch — or, strictly speaking, the food containing it — is ground up with the saliva by the act of chewing. The saliva contains a diastatic ferment, ptyalin,[1] which converts the starch into dextrins and finally largely into maltose.[2] The latter is inverted by means of a

[1] Ptyalin is not found in the saliva of all animals, e.g., that of the carnivora. It would be well to drop the name ptyalin as it tends to give one the impression that there is only one ferment found in the saliva. At present we know only of its action, which coincides with that of various other ferments found in the animal and vegetable kingdoms. It is better, therefore, to speak of a *diastatic* or *amylolytic ferment*. We would be justified in using a special name for the ferment of unknown composition only when it has an unusual action; thus, for example, if the diastase in saliva led to different cleavage-products of starch than do the ferments of other origin.

[2] The earlier assumption, that glucose is formed directly, has been shown to be false. Cf. J. Seegen: Zent. med. Wissensch. **14**, 849 (1876), and Pflüger's Archiv. **19**, 106 (1879). Otto Nasse: *ibid.* **14**, 473 (1877). Musculus and v. Mering : Z. physiol. Chem. **1**, 395 (1877–78). *Ibid.* **2**, 403 (1878–79); *ibid.* **4**, 93 (1880). von Mering: *ibid.* **5**, 185 (1881). Brown and Heron: Ann. **199**, 165 (1879); **204**, 228 (1880). Külz and Vogel: Z. Biol. **31**, 108 (1895).

special ferment, called *glucase* (or *maltase*).[1] This last action plays a subordinate part in the total action of the saliva. The transformation of the starch past the dextrin stage into maltose does not take place so simply as might seem possible. A great many intermediate products have been described. At present there is no reason for going into the details here, partly because we are not sure whether some of these products are single substances, or mixtures of several constituents.

The human saliva, or rather the amylolytic ferment contained in it, does not attack raw starch to any extent. In almost every case the starch has already undergone a process of change which greatly facilitates the action of the diastase upon it. In most cases the starch has been cooked, which swells the grains. The fact that this is favorable to the action of the diastase can be easily shown by the following experiment: In one test-tube a few grains of ordinary starch are mixed with saliva, while in another the same saliva is allowed to act for an equal length of time upon starch paste. If at the end of a certain length of time iodine is added to test for unchanged starch, a much stronger coloration will be obtained in the first tube than in the second. If, on the other hand, we test for the sugar[2] formed, we shall this time obtain a better test in the other test-tube.

Leaving the mouth, the starch, together with some of its transformation products, all intimately mixed with the saliva, reaches the stomach. For a long time it was believed that the action of the diastase quickly stopped here on account of the acid reaction of the contents of the stomach. Free hydrochloric acid is especially unfavorable to the action of diastase; as little as 0.03 per cent prevents it from changing starch into sugar. Recent experiments[3] have shown, however, that the food reaching the stomach is not immediately mixed with the gastric juice as was formerly assumed, but on the contrary lies out of contact with this for some time. In the stomach itself there has not been found any agent capable of converting carbohydrates into sugar,[4] but, on the other hand, a not inconsiderable absorption of the simple sugar formed is known to take place here.

The main digestion of carbohydrates is effected, however, in the intestine by the action of a diastase from the pancreas. By means of it the starch — which for the greater part is still unchanged, or at least only very

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[1] M. C. Tebb: J. Physiol. **15**, 421 (1894). Hamburger: Pflüger's Archiv. **60**, 543 (1895).

[2] Naturally, the saliva, starch, and starch paste should be examined for sugar at the start.

[3] P. Grützner: Pflüger's Arch. **106**, 463 (1905).

[4] According to H. Friedenthal, the stomach juices of a dog contain a ferment which acts strongly in acid solutions. Archiv. (Anat. und) Physiol. **1899**, Suppl. 383.

incompletely broken down — is now acted upon completely, and in fact intermediate products are formed which are similar to, if not identical with, those produced by the saliva. The cleavage produced by the pancreatic diastase is believed to yield finally only maltose. This last, by the action of a particular ferment, *glucase* (also called maltase), is eventually decomposed into molecules of *d*-glucose. Here in the intestine the breaking down of cane-sugar also takes place for the most part. By means of the above-mentioned ferments, or at least by similar ones,[1] it is similarly inverted into its two components, dextrose and lævulose (*d*-glucose and *d*-fructose). In this way the carbohydrates in the food are prepared for absorption, which sets in as fast as the breaking down of the carbohydrates takes place.

We must now touch upon the question as to whether the more complicated carbohydrates, such as the dextrins, for example, are capable of direct absorption. Under normal conditions these substances are not taken up by the intestine, or at least such products are not met with beyond the intestines in the assimilatory tracts.[2] Cane-sugar and maltose can be absorbed directly. They are taken up more slowly than the simpler sugars, and in every case they must suffer cleavage before they are turned over to the blood; for if cane-sugar, avoiding the intestinal canal, is introduced directly into the blood, it suffers no further change, but is eliminated as such in the urine.[3]

The most important result of the successive changes which take place in the intestinal canal as regards the carbohydrates which we have studied up to this point, is the tendency to form by the action of a definite ferment the simplest building material, especially the hexoses, thus giving to the system a uniform material from which it can construct the substances of which the body is composed. It is evident from what has been said that the significance of the alimentary canal and of all the different organs connected with it does not consist solely in transforming the non-diffusible substances, which cannot be absorbed, such as starch, into products which are diffusible. Its task stretches far beyond this single action.[4] The molecules which are naturally foreign to the animal organism are destroyed and converted into a homogeneous indifferent material

[1] It is probable that the same ferment does not act upon both maltose and cane-sugar. Cane-sugar is not split up beyond the intestine, while maltose appears as a product from glycogen and is inverted.

[2] Von Mering (Arch. f. anat. und Physiol. **1877**, 379, 413) has found substances similar to dextrin in the blood of the portal vein after a diet very rich in carbohydrates. It has not been shown whether this is a normal occurrence.

[3] Cf. Fritz Voit: Deut. Arch. klin. Med. **58**, 523 (1897). Ernst Weinland: Z. Biol. **47**, 279 (1905).

[4] Cf. Emil Abderhalden: Zentrb. Stoffwechs. Verdauungskrankheit, **5**, No. 24, 647 (1904).

from which the organism is able to build up its own individual carbohydrates.

Before we attempt to follow the simple sugars on their way to the organs, or, in other words, to study the course taken in their absorption, we must consider what takes place in the case of a few compound carbohydrates which form an important part of our food. We refer to milk-sugar which occurs in milk, and the numerous carbohydrates other than starch that are obtained in vegetable nutriment, cellulose especially. Milk-sugar is unquestionably decomposed into its components while in the bowels in the case of animals accustomed to milk nourishment, especially during the age of suckling.[1] On the other hand, in many animals there seems to be no ferment present in the whole of the alimentary canal which is capable of splitting up milk-sugar. What happens to milk-sugar in such cases is not clear to us at present; probably it is further decomposed in the wall of the canal. The question as to the utilization of carbohydrate introduced into the organism in the form of cellulose is a very important one. Cellulose plays no part at all in the nourishment of the carnivora, and it is also unessential in the case of the omnivora, whereas in the case of the herbivora a not inconsiderable part of the carbohydrates contained in their nourishment is in the form of cellulose. Now, this compound is not acted upon by the saliva, nor by the juices of the stomach, pancreas, or intestine, provided we leave out of consideration the action of bacteria which are ever-present. It is here that the activity of certain micro-organisms present in the intestines comes into play, and this forms to some extent an example of symbiosis. The fact that cellulose is actually subject to a transformation in the bowels is proved by our being unable to find in the fæces the whole amount of cellulose which has been introduced into the system.[2] Outside of the organism, it has been found possible to dissolve as much as seventy per cent of cellulose by means of the intestinal juices from a horse; these are rich in bacteria.[3] Sugar is not formed by this process, but a considerable amount of gas is evolved. The mixture produced by the decomposition has an acid reaction. These products have been studied by Tappeiner.[4] He found that by the action of meat

[1] Cf. Röhmann and Nagano: Pflüger's Arch. **95** 60 (1903); Ernst Weinland: Z. Biol. **38**, 16, and 606 (1899); **40** (1900).

[2] The food-value of cellulose has not been determined definitely even as regards the herbivora. Cf. the following articles: — W. Henneberg and F. Stohmann: "Beiträge zu einer rationellen Fütterung der Wiederkauer," Braunschweig, 1860 and 1864. Z. Biol. **21**, 613 (1885). v. Knieriem: *ibid.* **21**, 67 (1885). Weiske, Schulze, and Flechsig: *ibid.* **22**, 373 (1886). E. Wolff: Landwirtsch. Jahrbücher, **49**, Suppl. III (1887). N. Zuntz: Pflüger's Arch. **49**, 477 (1891).

[3] Viktor Hofmeister: Arch. wissensch. und prakt. Heilkunde, **11**, 1 and 2 (1885).

[4] Z. Biol. **20**, 52 (1884); **24**, 105 (1888); and Hoppe-Seyler: Z. physiol. Chem. **10**, 401 (1886).

extract which he had inoculated with bacteria from the contents of the paunch upon absorbent cotton-wool, the following products were formed: carbonic acid, methane, and fatty acids (acetic, butyric, and valeric acids). It is perfectly possible that the breaking down of the cellulose takes place similarly in the intestinal canal. The behavior of cellulose here has, however, never been entirely explained. It is also possible that perhaps only a portion of the cellulose is decomposed in this way, while another portion may be acted upon differently perhaps by means of the epithelium of the canal itself, being transformed in such a way that it can be absorbed. Not only the herbivora are capable of utilizing cellulose, but the omnivora can make use of it at least to some extent. The investigations of v. Knieriem have shown that the human intestine is capable of dissolving a part of the tender cellulose from young vegetables. As much as forty per cent of the cellulose introduced into the system could not be detected in the fæces.[1]

Cellulose, especially in animals possessing a long intestine, — chiefly the herbivora, but also the omnivora, — plays still another characteristic part, as the following experiments show. If rabbits are fed with food containing no cellulose, they soon die. This is due to the fact that when cellulose is left out of the nourishment the intestine no longer experiences a certain mechanical irritation to which it has become accustomed. On this account the peristalsis becomes retarded, then the contents of the intestines accumulate, whereby putrefaction ensues, and eventually there is inflammation of the bowels. That this explanation is correct, is shown by the fact that the animals experimented with continue to live, if, instead of the cellulose, the animals are fed with horn shavings which are perfectly indigestible.[2]

The bacteria contained in the stomach and intestines attack not only cellulose but other carbohydrates as well. For this reason, the breaking down of the more complicated carbohydrates does not actually take place so simply as has been depicted. On the other hand, the decomposition brought about by means of bacteria is, in general, not very extensive, and depends very much upon the external conditions. The products formed by their action are chiefly lactic acid, formic acid, acetic acid, butyric acid, and alcohol with evolution of carbon dioxide, hydrogen, and methane.[3] There are, furthermore, other micro-organisms known, as, for example, *Bacterium thermo*,[4] which break down starch in very much the same way as this is accomplished by the diastase in saliva and pancreas, thus aiding the conversion of amylum into sugar.

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[1] *Loc. cit.*

[2] von Knieriem: *loc. cit.*

[3] Cf. H. Tappeiner: Z. Biol. **19**, 228 (1883).

[4] J. Wortmann: Z. physiol. Chem. **6**, 287 (1882).

The question has often been raised as to whether the micro-organisms of the alimentary canal are absolutely necessary for the perfect digestion of the food, or whether they should be regarded as true parasites. To decide this point, Nuttall and Thierfelder [1] have made the following experiment: By Cæsarean section, they removed guinea pigs from the uterus of the mother shortly before their normal birth, taking most careful antiseptic precautions, and placing them in a sterilized cage. Guinea pigs, unlike most of the related animals, come into the world in such a developed state that they are able to assimilate properly the food of the adult. It was found possible, as proved by later examination, to keep these little pigs perfectly sterile during the entire experiment (eight days) and to feed them with sterile food, crackers and milk, in a sterile environment. The animals experimented with gained in weight normally, thus proving that the animal organism could thrive when bacteria were absent. These experiments were especially valuable because they were performed with animals which are particularly likely to be infested with bacteria from their vegetable food. It may be said, on the other hand, however, that the experiment merely shows that guinea pigs can subsist upon crackers and milk in the absence of bacteria, but it does not necessarily follow that the result would have been the same if a food rich in cellulose had been fed them.

Schottelius [2] has arrived at quite different results from those of Nuttall and Thierfelder. He chose for his experiments chickens which were hatched under sterile conditions, kept in sterile places, and fed with sterile food. These animals, although they ate abundantly, had continuous hunger, and declined about as quickly as a starving animal. As soon as bacteria from hen fæces were mixed with the food the animals revived and increased in weight. Recently Moro [3] has carried out similar experiments with the larvæ of the mud-frog (*Pelobates fuscus*). He was able to keep them sterile for thirty-six days. It was found, however, that if the sterile larvæ were placed in water containing the fæces of the mother, the increase in weight and general development was much more rapid than was the case with larvæ kept sterile.

In discussing the carbohydrates we have mentioned the fact that the five-carbon sugars, the pentoses or their condensation products the pentosans which are so widely distributed in the vegetable kingdom, are not unimportant forms of nutriment, especially in the case of the herbivora. The researches of Stone [4] and of Weiske [5] have shown that the herbivora

[1] Z. physiol. Chem. **21**, 109 (1895–96); **22**, 62 (1896–97).
[2] Arch. Hyg. **34**, 210 (1899), and **42**, 48 (1902).
[3] Jahrb. für Kinderheilkunde, **62**, H. 4 (1905).
[4] Am. Chem. J. **14**, 9 (1902).
[5] Z. physiol. Chem. **20**, 489 (1895).

can utilize up to fifty or sixty per cent of the pentosans contained in vegetables. In these experiments a mixture of pentosans (araban, xylan, methylpentosan) was fed to the animals, but recently Slowtzow [1] has fed pure xylan to rabbits. In the excreta from 17.1 to 66.8 per cent of the pentosans were found in an unchanged condition. The remainder was undoubtedly utilized in the system after having been converted into the simpler sugars (pentoses).

From the intestine on, the simple sugars (e.g., *d*-glucose) are quickly absorbed, whether introduced into the system in this form, or obtained from the destruction of more complicated sugars. There are two ways in which the nutriment absorbed by the intestine can reach the general circulation. In the first place it can enter directly by means of the blood-vessels, in this case the branches of the portal vein. This is the path taken by salts, carbohydrates, and proteins. In this way they reach the liver, there to undergo certain important transformations, after which they are capable of being introduced into the general circulation of the blood. The second path is by way of the lymphatics, which conduct the absorbed substances, especially the fats, into the thoracic duct, from which they are led into the *Vena anonyma*, and thus into the general circulation.

The experiments showing that the absorption of carbohydrates as a matter of fact takes place in the first manner will be discussed later when we come to consider the absorption of fats.

In order to get some idea of the process by means of which sugar is transferred to the circulatory system, and in order to understand how large amounts of carbohydrates, e.g. 500 grams, can be transferred in a relatively short time to the blood-stream (especially into the portal vein) without materially increasing the sugar content of the blood, we must remember what an enormous surface for absorption is presented by the extremely fine network of blood-capillaries. Absorption takes place continuously hand in hand with the breaking down of the more complicated carbohydrates into simpler sugars. Although the tiny molecules of sugar are absorbed at thousands of places and pass into the blood, and although they are immediately carried away, it would seem that there must be an increase in the amount of sugar contained in the blood corresponding to the amount absorbed. That this is not the case — the normal amount of sugar is 0.5 to 1.5 grams per liter and remains constant — must be due to the fact that sugar is removed from the blood to the same extent that it is absorbed by it. This is, as a matter of fact, exactly what happens, and it is the liver which regulates the exchange of carbohydrates in the whole animal system. It intercepts the absorbed sugar, and keeps the sugar content in the blood constant. By means of the activity of the

[1] Z. physiol. Chem. **34**, 181 (1901). See also Rudzinski : *ibid.* **40**, 317 (1904).

liver cells, the molecules of *d*-glucose are made to unite together again with loss of water and the formation of a new polysaccharide, glycogen. In making this transformation the animal organism acts precisely like the plant in forming cellulose, the circulating sugar being removed from metabolism, stored up and protected from combustion in such a way that at any given moment it can be made "liquid" again. This stage in the metabolism of carbohydrates in the animal system can be demonstrated very simply by means of the following experiment: A number of rabbits may be kept from food for a length of time until it is known as a matter of experience that the glycogen content of the organs, especially the liver, has been brought as low as possible. This is the case at the end of about ten days. As we shall see later on, the consumption of glycogen can be accelerated greatly by muscular work (whether by actual body work — e.g. dogs running in a tread-mill — or by muscular convulsions produced by strychnine poisoning), and thus the time required for the experiment greatly shortened. A part of the animals experimented upon are then fed with a diet rich in carbohydrates. If now all the animals are killed, those that are starving as well as those which have been recently fed and are in the act of digesting the food, the former, when subjected to quantitative tests, will be found to contain only traces of glycogen in the liver, whereas the latter will contain a large amount.[1] This state of affairs is of regular occurrence, so that to-day there is scarcely any one who doubts that there is a direct connection between the carbohydrates taken up (the absorbed *d*-glucose) and the glycogen.[2] The fact that the liver actually forms glycogen directly from sugar has been demonstrated recently by Karl Grube.[3] Grube passed blood rich in sugar through the liver of a dog, and could detect a slight increase in the liver-glycogen. It is another question as to whether all carbohydrates, for example, the pentoses, are capable of forming glycogen in this way. We shall later on take up this point more in detail, but at this place it is of interest for us to know merely how the more important carbohydrates in food, namely, starch, cane-sugar, and grape-sugar, behave in this respect. Starch, as we have already seen, is decomposed eventually into molecules of *d*-glucose, and the same is true of saccharose. From the latter, however, not only *d*-glucose but an equal amount of *d*-fructose is formed, the latter being a ketohexose. The question that now arises is,

[1] Cf. F. W. Pavy: Phil. Transact. for 1860, p. 579, and Researches on the Nature and Treatment of Diabetes, London, 1862. Also The Physiology of Carbohydrates, 1894. See also Pflüger's Das Glycogen und seine Beziehung zur Zuckerkrankheit, and Cremer's Physiologie das Glykogens, Ergeb. Physiol. (Asher u. Spiro) **1**, p. 803 (1902.)

[2] Carl Voit has furthermore shown that subcutaneous introduction of *d*-glucose into rabbits caused an increase of up to eight per cent glycogen in the liver. Z. Biol. **28**, 245, 288 (1891). See also Erwin Voit: Z. Biol. **25**, 551 (1889).

[3] Pflüger's Arch. **107**, 490 (1905).

Does the latter become changed into glycogen, does it form a particular glycogen of its own, or is the fructose first changed into *d*-glucose,[1] and then, in common with the other glucoses, changed to glycogen? Until recently it was quite generally assumed that the last-mentioned process was carried out, but now certain facts have become known which will perhaps lead us to another conception. It has been established that, after the extirpation of the pancreas in dogs, not only does sugar appear in the urine, but at the same time the formation of glycogen in the liver is interfered with, and as a matter of fact this disturbance is much more pronounced when *d*-glucose is fed to the dog than in the case of fructose. The exact significance of this result is at present not clear.

When the food is rich in carbohydrates the liver cannot retain all of it as glycogen. The glycogen stored in the liver of man amounts at the most to 150 grams. The muscles can take up an equal amount provided they did not originally contain considerable. As this is often the case under normal conditions, however, we must answer the question as to what becomes of the sugar which is not disposed of as glycogen. A direct consumption of large amounts of sugar is not to be thought of; and on the other hand the sugar content of the organs, and of the blood especially, never exceeds certain well-established limits when the storage places for glycogen have been filled. Here for the first time, we meet with the question of the transformation of one food-stuff into another. Subsequently we shall have to study this closely, but at present we will merely mention that the excess of sugar is evidently disposed of as fat, a phenomenon which we meet with in the vegetable kingdom, and one which plays an important part in the depositing of nutriment in latent seeds, and conversely in its utilization at the time of germination.

Now what becomes of this stored-up glycogen? As we have seen, the glycogen gradually disappears if nourishment is withheld or work is performed. It was Claude Bernard [2] who first showed this relation between glycogen and muscular work. He found that the livers of hibernating animals during their winter's sleep contained large amounts of glycogen, and not only was the glycogen contained in the liver cells, but also in the muscular tissue and in the lungs. As soon as the animals awoke and

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[1] Such a transformation is explained to us by the work of C. A. Lobry de Bruyn and W. Alberda van Ehenstein, Ber. **28**, 3078 (1895), and Rec. trav. chim. **14**, 103, 156. These two authors have shown that glucose, fructose, and mannose can be easily transformed into one another in alkaline solution. The transformation of mannose into glucose is equally interesting as that of fructose into glucose, for the former is used as material for the formation of glycogen. Thus in Japan a natural manna is found which serves the inhabitant in the same way as starch does for us. Cf. Löw and Tsuji: Landwirtschaftliche Versuchstationen, **45**, 433. Also Haycraft: Z. physiol. Chem. **19**, 137 (1894).

[2] Compt. rend. **48**, 673 (1859).

began to move about, Bernard noticed that the glycogen disappeared. He furthermore observed that when the muscular tissues of well-nourished mammals or birds were at rest — whether voluntarily so or as a result of artificially severing the nerves in them — the glycogen content gradually increased, only to disappear again when the muscles were set at work.

Direct experiments were carried out by S. Weiss.[1] He compared the glycogen content of a frog's hind legs of which one had been tetanized almost to exhaustion while the other was under control and rested. The glycogen in the active muscles decreased from 24.27 to 50.43 per cent. Finally Th. Chandelon[2] has carried out the following experiment: In a rabbit he severed the sciatic and crural nerves, and at the end of from 2 to 5 days found in the paralyzed muscles an increase in glycogen amounting to from 5.51 to 172.4 per cent. Similarly Marcuse[3] made similar observations, and found the following glycogen values:

EXPERIMENT.	Per cent glycogen in the —	
	Unirritated Muscles.	Irritated Muscles.
I	0.748	0.539
III	0.749	0.461
IV	0.589	0.395
V	0.542	0.341
Average	0.657	0.434

Finally, the same result has been obtained by Edward Külz[4] in another way. He caused a well-nourished dog to draw a heavy cart. The animal weighed 45.500 kilograms, and was made to drag the cart for 9 hours and 40 minutes. The dog was then bled to death. The glycogen determination showed the presence of 52.053 grams, i.e., 1.16 grams per kilogram of the dog's weight. A well-nourished dog that is not tired shows a glycogen content of 38 grams per kilogram. For comparison, it may be mentioned that after 28 days of starvation a dog of about the same size as the above-mentioned showed but 1.5 grams of glycogen per kilogram. It is evident, therefore, that after about $9\frac{2}{3}$ hours of labor the glycogen stores were consumed to fully as great an extent as in the case of a dog starved for 28 days. Külz then repeated the experiment with three other dogs and with the same result.

A further confirmation of the fact that the carbohydrates serve as an important source of muscular energy is shown by the interesting experi-

[1] Sitzber. Akad. Wiss. Wien. **64**, Abt. **1**.

[2] Pflüger's Arch. **13**, 626 (1876).

[3] Pflüger's Arch. **39**, 425 (1886). See also Edward Manché, Z. Biol. **25**, 163 (1889).

[4] Beiträge zur Kenntniss des Glykogens, p. 41 (1891).

ment of Fick and Wislicenus.[1] These scientists attempted to find out what substances were chiefly decomposed as a result of strenuous muscular work. At that time Liebig's[2] theory prevailed that the muscles performed work at the cost of albuminous substances, and they decided to test this experimentally. If Liebig's theory were correct, it was to be expected that the elimination of nitrogen would be increased as a result of muscular activity. Fick and Wislicenus climbed Mount Faulhorn, 1956 meters above the Lake of Brienz, which was the starting-point. For seventeen hours before the start, during the ascent (which required six hours), and for six hours following, care was taken to eat only food which was free from nitrogen. All the urine passed during the ascent and the six hours following was carefully collected and the nitrogen accurately determined. From the results obtained by chemical analysis it was found that Fick had decomposed 38.3 grams of albumin, and Wislicenus 37 grams. These amounts of albumin correspond to about 250 heat units in each case, or 106,000 kilograms of work. If we estimate the actual work performed in climbing the mountain we arrive at the following values. Wislicenus weighed 76 kilograms. By simply raising this weight to the height of the mountain peak, $76 \times 1956 = 148{,}656$ kilograms of work was performed. These values suffice to show that the work could not have been at the expense of albumin alone. This fact is still more striking when we remember that the above value of albumin in heat units is too high, for it is based upon the assumption that the carbon is completely changed to CO_2 and the hydrogen to H_2O. Now, as a matter of fact, nowhere near this amount of energy is obtained by the consumption of albumin in the animal organism, for a part of the carbon, some of the hydrogen, and the greater part of the nitrogen, are eliminated in the form of urea. In man, the amount of urea formed is as a rule equal to one-third the weight of albumin decomposed. Therefore from the above heat value of albumin we must deduct one-third the heat of combustion of the same weight of urea. On the other hand, as a matter of fact, the scientists performed much more work than we have estimated. Fick and Wislicenus estimate the amount of work performed by their circulatory and respiratory apparatus as 30,000 kilogrammeters. Furthermore, it must be taken into consideration that in every motion, in every step, work is performed which is transformed into heat and lost as far as the work performed is concerned. According to Helmholtz, only one-fifth of the actual heat of combustion is transformed into external work. It is perfectly certain, therefore, that we can safely conclude from the above experiment that albumin alone is not the source of muscular work, but, on the other hand, we are not justified in concluding

[1] Vierteljahresschrift des Züricher naturforschenden Gesellsch. **10**, 317 (1865).

[2] Chemische Briefe (1857). Cf. also Ann. **153** 1, and **157** (1870); C. Voit: Z. Biol. **6**, 305 (1870); Schenck: Arch, exper. Path. Pharm. **2**, 21 (1874).

that the source is to be sought entirely in substances free from nitrogen. At all events, however, Liebig's theory is untenable.

It remained for C. Voit [1] to establish a more exact proof of the theory that muscular work is performed chiefly at the expense of substances free from nitrogen. He caused a dog to run in a tread-mill, and compared the amount of nitrogen in the urine which was passed during the working period with that passed during rest, both before and after working. It was found that the amount of nitrogen eliminated during twenty-four hours of a working period was but slightly, if at all, in excess of that eliminated during the resting periods. O. Kellner [2] tried a similar experiment with horses, and obtained the same results when the animals experimented upon were fed with an abundance of carbohydrates. If this was not the case, the amount of nitrogen eliminated was considerably more. Finally, Voit [3] performed corresponding experiments with human beings, and determined not only the amount of nitrogen eliminated, but at the same time estimated the carbon dioxide, and indirectly the absorption of oxygen.[4]

The increased absorption of oxygen and elimination of carbon dioxide have also been observed in direct experiments upon the muscles themselves, by comparing the amount of these gases contained in the venous blood of a resting muscle and of one that has been tetanized.[5] The inactive muscle takes up more oxygen from the blood than it gives back to the latter in the form of carbonic acid gas. Obviously the muscular cells retain oxygen in some form or other. We can, in fact, speak of a *storing up of oxygen*. This stored-up oxygen again appears after violent exercise, for then the muscle gives up to the blood more oxygen as carbon dioxide than it takes up from the blood as free oxygen. Yet it is also true that the muscle takes up more oxygen from the blood during exercise than in periods of rest, for venous blood contains during work less oxygen and more carbonic acid than when the muscle is at rest. That evidently not only an oxidation process but a hydrolytic decomposition as well should be regarded as the source of muscular work will be shown later.

We must now answer the question as to how the glycogen is decomposed and in what way it is utilized in muscular work. Furthermore, we are interested to know what connection there is between the principal

[1] Z. Biol. **2**, 307 and 339 (1866).

[2] Landw. Jahrb. **8**, 701 (1879), and **9**, 651 (1880).

[3] Z. Biol. **2**, 307, 488 (1866).

[4] This was known to Lavoisier: Seguin and Lavoisier: Mém. acad. Sciences, 688 and 696 (1789). See also Sondén and Tigerstedt: Skand. Arch. Physiol. **6**, 181 (1895). O. Krummacher: Z. Biol. **33**, 117 (1896) Zuntz, Frentzel, and Loeb: Arch. (Anat. und) Physiol. **541** (1894). Speck: *ibid.* 465 (1895).

[5] Ludwig and Sczelkow: Sitzber. Akad. Wiss. Wien. **45**, 171 (1862). Max v. Frey: Arch. Anat. Physiol. **533** (1885).

store of glycogen, namely liver-glycogen, and the consumption of carbohydrates by the muscles. According to all our experience up to the present time, glycogen is not directly oxidized, but is previously decomposed into *d*-glucose. This is shown directly by the experiments of J. Ranke [1] and of Otto Nasse.[2] The former examined the sugar content of the hind legs of a number of frogs, one leg being tetanized and the other at rest. Several determinations showed 0.058 per cent sugar in the dry substance of the resting muscle, and 0.082 per cent in the tetanized muscle. The sugar content, therefore, was increased 41 per cent in the latter case. The hydrolysis of glycogen is evidently caused, as Magendie [3] has shown, by a ferment which in its action coincides with that of diastase, which is already known to us. In the liver also the stores of glycogen are decomposed in this way.[4] Here, besides *d*-glucose, the intermediate products dextrin and maltose have been detected. They are probably formed in the breaking down of glycogen in the muscles, but their presence has never been established with certainty. The fact that the hydrolysis of glycogen in the liver is not to be considered as a result of the activity of the cells, but rather that it is due to a ferment which can be separated from the liver cells, was clearly shown as long ago as 1873 by von Wittich.[5] He proved that it was possible to extract by means of glycerol from liver which was completely freed from blood and hardened by alcohol a ferment which was capable of hydrolyzing glycogen. von Wittich showed that this ferment belonged to the liver cells rather than to the blood by again and again obtaining the diastatic ferment after repeated, thorough washings of the liver. It is also possible, as Pavy has shown, to treat liver with alcohol, dry it, and preserve it indefinitely. If such a preparation is digested with water, the diastatic reaction will be obtained invariably. On the other hand, the objection may be raised to this line of reasoning, that the formation of sugar is perhaps due to the action of micro-organisms, an assumption which in the light of recent experience obtained by working with organs and their extracts does not seem improbable.[6] E. Salkowski,[7]

[1] Tetanus, p. 168, Leipsic, 1865.

[2] Pflüger's Arch. **2,** 97 (1869).

[3] Compt. rend. **23,** 189 (1846).

[4] Musculus and v. Mering: Z. physiol. Chem. **2,** 416 (1878–79). E. W. Pavy: The Physiology of Carbohydrates, London, 1894. Külz and Vogel: Z. Biol. **31,** 108 (1895).

[5] Pflüger's Arch. **7,** 28 (1873).

[6] For an explanation of the other view, that the sugar formation from glycogen is caused by the life process of the liver cells, see M. Foster: Text-book of Physiology, appendix by Sheridan Lea, pp. 58, 98. Noël Paton: Hepatic Glycogenesis, Trans. Roy. Soc. 1894, and Phil. Trans. **185** B, 233 (1894).

[7] Deut. Med. Wochschr. No. **16,** 1888; Arch. Physiol. **554** (1890); Zent. med. Wissensch. Jg. **27,** No. **13,** 227 (1889). See also Otto Nasse: Rostocker Ztg. No. **105** (1889). Salkowski: Z. klin. Med. p. 90 (1891), and Pflüger's Arch. **56,** 339 and 351 (1894). Arthus and Huber: Arch. de Physiol. **651** (1892).

however, has shown that this sugar formation will also take place in chloroform water. Since aqueous solutions of chloroform prevent protoplasmic action including the action of micro-organisms, this experiment proves that the formation of sugar from glycogen is not due to the action of bacteria, and further that the liver cells themselves are not the cause, but that the hydrolysis of glycogen is to be traced to the action of a soluble ferment. Salkowski clearly established this important fact by means of the following experiment: He removed the liver from a rabbit which had taken into its stomach seventeen hours before death ten grams of cane-sugar dissolved in water. After taking out the gall bladder and the large bile ducts, the liver was cut up into fine pieces and triturated. Two portions of the same weight of liver-pulp were taken, one of which was placed directly in a bottle filled with chloroform water; the other portion was boiled in water first, and then treated with the same amount of chloroform water. After sixty-eight hours' digestion, the glycogen and sugar were determined in each of the extracts. The first extract showed a large amount of sugar and no glycogen, whereas the extract of the boiled liver contained considerable glycogen and only very little sugar. In the first experiment there were found 48.28 grams of sugar; in the second, 3.65 grams.

As we have seen, the glycogen content of the liver stands in a definite relation to that of the muscles. After the muscles have performed hard work, we find that not only does the glycogen in them disappear, but that in the liver as well. This leads us to believe that the liver serves as a central storage place which is capable of feeding all the other stores in the organism. The transfer of the liver-glycogen takes place by means of the blood, and in the form of *d*-glucose as we have seen. Now the organism strives to a remarkable degree, even during starvation, to maintain a constant amount of sugar in the blood. If the glycogen in the muscles is used up, the muscle cells then strive to form glycogen from the sugar in the blood. This would cause a diminution in the sugar content of the blood, except for the fact that as soon as this sugar is removed, then hepatic glycogen is decomposed into *d*-glucose and removed by the blood.[1] It has been stated that the higher products of the hydrolysis of glycogen, namely dextrin and maltose, are likewise transported by the blood; but how far such observations are correct, or what the extent to which this takes place, cannot be decided at present. At all events, such statements have

[1] The assumption made by J. Seegen (Die Zuckerbildung im Tierkörper, ihr Umfang und ihre Bedeutung, Berlin, 1890, and Studien über Stoffwechsel im Tierkörper, Berlin, 1887), that blood-sugar is formed from proteins in the nourishment, and that on the other hand the liver-glycogen originates from the fats, is not supported by the facts. Cf. R. Böhm and F. A. Hoffmann: Pflüger's Arch. **23**, 205 (1880). H. Girard: Pflüger's Archiv. **41**, 294 (1887). E. Cavazzani: Arch. Anat. Physiol. **539** (1898).

lost much of their significance since it has become known that the blood-serum contains a ferment which is capable of converting glycogen and starch into dextrose.[1] The fact that the muscles are capable of forming glycogen from glucose has been proved by Külz.[2] By subcutaneous injection of sugar into frogs with extirpated livers he was able to establish the fact that there was an increase of muscle-glycogen.

That the sugar content of the blood is directly dependent upon the liver is shown by the fact that ablation of the liver causes the amount of sugar in the blood to diminish and finally disappear.[3]

Although we have outlined the way carbohydrates break down in the alimentary canal, the absorption of their hydrolytic products and their destiny in the animal organism from the time of their being stored up as glycogen on to their change back into *d*-glucose, still we have failed to give an exact picture of the manner in which the glucose formed is eventually consumed. We are, indeed, acquainted with the end-products, carbon dioxide and water, and know that an oxidation takes place, but we are still in doubt concerning the intermediate products. The destruction of the sugar has been traced by Lépine [4] and others to the action of a glucolytic ferment in the blood and in the tissues. Claude Bernard [5] had previously shown that the sugar content of blood gradually diminishes on standing. More recently it has been found that ferments of similar action are present in almost all of the organs. It is hard to decide whether in all cases there is no coöperation of micro-organisms, and as to what part this decomposition of *d*-glucose plays in the living tissue. At all events, there is at present no justification for the assumption that *all* of the sugar decomposition is caused by the action of the ferment mentioned. In this connection we will refer to the work of Stoklasa.[6] He obtained from the expressed juices of all sorts of different organs (muscles, liver, lungs, pancreas) by precipitation with alcohol-ether, ferments which produced alcoholic fermentation in a sterilized sugar solution without the aid of bacteria. The proportion of carbon dioxide and alcohol formed was the same as in the fermentation brought about by the zymase in yeast. The decomposition

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[1] M. Bial: Pflüger's Arch. **52**, 137 (1892), and **54**, 73 (1893). Cf. also Röhmann, Ber. **25**, 3654 (1892).

[2] Pflüger's Arch. **24**, 64 (1881).

[3] Cf. Tangl and Harley: Pflüger's Arch. **61**, 551 (1895). Pavy and Siau: J. Physiol. **29**, 375 (1903). Minkowski: Arch. exp. Path. Pharm. **21**, 41 (1886). Schenk: Pflüger's Arch. **57**, 553 (1894).

[4] Compt. rend. **110**, 742 (1890); **110**, 1314; **112**, 146 (1891); **112**, 411; **112**, 604; **112**, 1185 and 1414; **113**, 118 (1891); **120**, 139 (1895). Lépine: Le ferment glycolytique et la pathogénie du diabète, Paris, 1891. Cf. Nasse and Framm: Pflüger's Arch. **63**, 203 (1896).

[5] Leçons sur le diabète (1878).

[6] Hofmeister's Beitr. **3**, 460 (1903); Ber. **36**, 4058 (1903); Pflüger's Archiv. **101**, 311 (1904); Zentr. Physiol. **17**, 465 (1903); and Ber. **38**, 664 (1905).

of the carbohydrate did not stop with the formation of the products named, but acetic and formic acids were formed with consumption of oxygen. It is at present hard to tell what part the alcoholic fermentation plays in the living organism, or whether, in fact, it has any significance. At all events, Stoklasa's observations include the possibility of energy being produced without oxygen being supplied. The fact that the animal organism evidently makes use of such simple decompositions as a source of energy is shown by the interesting experiments of Hermann, Pflüger, and Bunge. Hermann [1] proved that a piece of extirpated muscle, from which no more oxygen could be pumped out, could work in an atmosphere free from oxygen and produce carbonic acid. Hermann at the same time detected the formation of an acid (lactic acid). Pflüger [2] succeeded in keeping a frog alive for twenty-five hours at a temperature a few degrees above the freezing-point of water in an atmosphere free from oxygen, during which time the animal evolved a considerable amount of carbonic acid gas. Finally G. V. Bunge [3] showed that a parasitic-worm of the cat, *Ascaris mystax*, could survive for four or five days in a medium absolutely devoid of oxygen, and move around in a very lively manner during that time. It should by no means be concluded from these very interesting experiments, that the animal organism performs its muscular work solely at the expense of the energy set free by hydrolytic decompositions. The amount of living force produced in this way would be altogether too small. On the other hand, it is conceivable that the cell, by means of a partial breaking down, that is to say, by a hydrolysis and subsequent oxidation of the decomposition products, can increase when necessary the amount of energy available. Thus 100 grams of glucose when completely oxidized to carbon dioxide and water yield 3939 calories (= 1,674,000 kilogram-meters of work). By alcoholic fermentation, i.e., the hydrolysis of 100 grams glucose into carbon dioxide and ethyl alcohol, only 372 calories (= 158,100 kilogram-meters of work) are liberated.

As a result of work the muscular tissue, which is amphoteric, becomes of acid reaction. This change is, at least to some extent, due to the formation of lactic acid. Formerly it was believed that there was a direct connection between the formation of the latter and the decomposition of carbohydrates. More recently, however, this view has been strongly combated. At present it has not been definitely decided as to what relation the lactic acid bears to the performance of work by the muscles. It is possible that its presence is due to a hydrolysis caused by the absence of a sufficient amount of oxygen. On the other hand, it is also conceivable that the formation of lactic acid has nothing whatever to do

[1] Untersuchungen über den Stoffwechsel der Muskeln, Berlin, 1867.

[2] Pflüger's Arch. **10**, 251 (1875).

[3] Z. physiol. Chem. **8**, 48 (1883–84); **12**, 565 (1888); **14**, 318 (1889).

with the combustion of carbohydrates in the muscle, but results from the breaking down of protein. We shall see later on that alanine, which is formed by the hydrolysis of albumin, stands in close relation to lactic acid.[1]

The *rôle* of the carbohydrates in the animal organism is by no means limited to the production of muscular energy. Above all they are to be considered as a source of heat. Thus it is possible to cause the glycogen stores to disappear by merely chilling the animal.[2] The carbohydrates, without doubt, take an active part in the life process of the individual cells. They take part also in their building up. At present we know nothing concerning the way they occur in the organism and concerning their union in the cell complex. We have already mentioned the occurrence of pentoses, especially xylose, which is in the nucleoproteids. We shall later on see that there are indications of the hexoses also being used as building material for the nuclei of the cells.

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[1] Cf. Astaschewsky: Z. physiol. Chem. **4**, 397 (1880). Warren: Pflüger's Arch. **24**, 391 (1881). Heffter: Arch. exper. Path. Pharm. **31**, 225 (1893). Werther: Pflüger's Arch. **46**, 63 (1890). Spiro: Z. physiol. Chem. **1**, 111 (1877–78). Zillesen: Z. physiol. Chem. **15**, 387 (1891). Araki: Z. physiol. Chem. **15**, 335 and 546 (1891), and Z. physiol. Chem. **16**, 453 (1892). Hoppe-Seyler: Z. physiol. Chem. **19**, 476 (1894).

[2] E. Külz: Pflüger's Arch. **24**, 14 (1881).

LECTURE V.

CARBOHYDRATES.

IV.

Building Up and Breaking Down of Carbohydrates in the Animal Organism.

We found in the last lecture that the blood under varying physiological conditions always maintained a constant sugar-content. This does not increase when the food is rich in carbohydrates, nor decrease materially during starvation. This phenomenon is explicable only by the assumption that there is an extraordinarily delicate regulating mechanism which works on the one hand in conjunction with the organs containing the stored-up sugar, and on the other hand with the places where sugar is consumed. If for any reason the amount of sugar in the blood increases above the normal, then sugar appears in the urine. This may take place, for example, when an excessive amount of sugar has been introduced into the alimentary canal. In such a case it is not always possible to remove the sugar fast enough from the general metabolism by converting it into glycogen or fat. This ability of storing up sugar is a restricted one.[1] The maximum amount of sugar which it is possible for the system to assimilate is known as the *limit of assimilation*, and the elimination of sugar which takes place when this is exceeded is spoken of as *alimentary glucosuria*. The limit varies with different forms of sugar and for different individuals. In general, the danger of giving the blood an over-supply of sugar under normal conditions of nourishment is but slight, because under normal conditions the bulk of the carbohydrates are taken into the system either as starch or as cane-sugar. There is no danger of these forms of sugar being suddenly broken down in the alimentary canal; on the contrary, their decomposition always takes place gradually from one stage to another so that this in itself serves in a measure to regulate the absorption of sugar.

Under certain conditions the assimilation limit for carbohydrates can be reduced greatly for a time. This is particularly true of the central organ of carbohydrate metabolism, namely the liver. Claude Bernard[2] recognized the fact that the storing up of sugar in the form of glycogen

[1] Cf. Franz Hofmeister: Arch. exp. Path. Pharm. **25**, 240 (1889).

[2] Leçons (Cours du semestre d'hiver), 1854–55, p. 289.

and its transformation into glucose, both changes being functions of the liver cells,— partly directly and partly indirectly,— were dependent upon the nervous system. He showed this by means of the following classical experiment, which was first performed upon rabbits. If a rabbit is injured at a certain place in the medulla, sugar appears in the urine after a short time. This region is bounded above by the origin of the two auditory nerves, and below by a line connecting the places of origin of the vagus nerves. The experiment is carried out in this way: After tying the rabbit, the point of a trocar is placed in the median line upon the *os occipitale* exactly at the *Protuberantia occipitalis superior*, and then pushed through carefully until the *Pars basillaris* is reached. The instrument thus bores through the skull, the cerebellum, and the posterior and median columns of the medulla. Two hours after the operation sugar appears in the urine. The elimination of sugar, however, in such cases is not lasting, usually disappearing at the end of five or six hours. With dogs, however, this glucosuria lasts longer. Claude Bernard in one case found it to last for a week. In such cases the amount of sugar contained in the urine is not large, usually amounting to merely two or three per cent.[1] Bernard shows the cause of the sugar elimination to be an excessive amount of blood-sugar. He found, instead of the customary 0.1 to 0.15 per cent, more than 0.3 per cent. This operation also has the same result when performed with birds [2] or with frogs.[3]

An observation made by F. W. Dock [4] is of considerable importance for complete understanding of this kind of glucosuria. He found that the above operation succeeded only with well-nourished animals, i.e., with those which possessed stored-up glycogen. Naunyn [5] arrived at the same conclusion. He showed that the success of the so-called "diabetic puncture" depended wholly upon the state of nourishment of the animal experimented upon. Dissection of the animal after the operation always showed that the liver was free from glycogen. The fact that the liver in this case loses the power of storing up sugar in the form of glycogen is shown by the following experiment: If a solution of *d*-glucose is injected into the mesenteric vein of a dog whose liver has been deprived as far as possible of glycogen by starvation, only very small amounts of sugar will subsequently be found in the urine. If the same experiment is repeated with another animal which has undergone the "diabetic puncture," a marked glucosuria ensues soon after the injection of the sugar.[6]

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[1] Hédon: Diabète, Dictionnaire de Physiol. **4**, 812.
[2] M. Bernhardt: Virchow's Arch. **59**, 407 (1874).
[3] M. Schiff: Untersuchungen über die Zuckerbildung, Würzburg, 1859.
[4] Pflüger's Archiv. **5**, 571 (1872).
[5] Arch. exper. Path. Pharm. **3**, 85 (1875).
[6] Cf. P. Levine: Zentr. Physiol. **8**, 397 (1894).

The question now arises as to what the connection is between the "diabetic puncture" and the flooding of the organism with sugar. Claude Bernard, by means of the following experiment, showed that the vagus nerves participate in this.[1] If the "diabetic puncture" is performed after cutting these nerves at the neck, it is as effective as when the nerves remained intact. On stimulating the peripheric stumps of a vagus nerve, no glucosuria ensues. It appears immediately, however, if the central end, i.e., the end connected with the medulla oblongata, is stimulated. In such experiments Bernard showed that the whole body of the animal experimented upon was flooded with sugar. The greatest quantity was found in the hepatic veins. Bernard showed, furthermore, that cutting the vagus nerves at the neck resulted in making the liver free from sugar. He concludes from all these experiments that in the medulla oblongata there is a center which regulates the transformation of sugar by the liver. Communication, i.e., the excitement, is provided by means of the vagus nerve. The sugar formation caused by stimulation of the central stump of the vagi nerve, Bernard conceives to be a reflex action;[2] and it is the pulmonary branches of the vagus which contain the fibers acting upon the sugar center, for if the vagi are cut above the liver and below the lungs, no influence upon the sugar formation of the liver was observed.

Now how does this sugar center exert its influence upon the liver? Bernard cut through the spinal cord at different places below the medulla oblongata, and found that the path of communication must lie in the upper parts of the spinal cord; for if it was severed below the lower dorsal vertebræ, the sugar formation in the liver was not affected. C. Eckhard,[3] who confirmed this conclusion, found that after severing both the vagi and sympathetic nerves in the neck, a successful diabetic puncture could be made. After severing the splanchnic nerves, however, it ceased to be operative. This indicates that the diabetic puncture acts upon the transformation of the carbohydrates in the liver by nerve impluses passing along the path of the splanchnic nerves.

We must assume, therefore, that the sugar formation in the liver is regulated directly by a center in the medulla oblongata. The vagi nerves conduct the *centripetal* impulses, and the splanchnic nerves the *centrifugal*. Later on, when we come to discuss digestion, and especially the dependence of the secretion of the digestive glands upon certain nervous influences, the above hypothesis will no longer appeal to us as remarkable.

Thus far we have left the matter unsettled as to whether the liver alone gives up sugar after the "diabetic puncture," or whether the sugar in the

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[1] Cf. C. Eckhard: Beit. Anat. Physiol. **8**, 77 (1879).

[2] Cf. E. F. Pflüger: Das Glykogen, *loc. cit.* 386.

[3] Beitr. Anat. Physiol. **4**, 138.

urine also comes from other organs. The experiments of Moos[1] and of Moritz Schiff[2] have proved that only the sugar formation in the liver is affected. If the vessels of the liver are all tied, the diabetic puncture becomes inoperative. This is particularly well shown by the experiments of Schiff, who took eight frogs of the same size, and produced glucosuria in all of them. At the end of from two to four and three-quarters hours sugar could be detected in the urine. The livers of all the animals experimented upon were then exposed, drawn out through the abdominal wound, and all the vessels and bile ducts encircled with a slip-knot of thread. In four cases the knots were drawn tight, while in the other four they were not. In the latter case the glucosuria continued, while in the former it gradually diminished, so that at the end of three hours the urine was free from sugar.

It is not yet clear how this increased sugar formation is brought about. There are several conceivable possibilities. The fact that the glycogen stored up in the liver is suddenly converted into glucose, would make it seem probable that the diastatic action has been considerably increased above the normal. It is also conceivable that there is an increased production of diastase, or, on the other hand, it is possible that under normal conditions the glycogen is not, as ordinaly assumed, deposited in the cells as a foreign body, but rather in the form of a loose chemical combination, and that the diastase begins to act upon glycogen as soon as it is set free. By exerting certain influences upon the liver cells, — whether by the diabetic puncture, or whether by some other excitement of the sugar center, — all of the glycogen is perhaps set free from its loose chemical combination, and subjected to the action of diastase, which found no point of attack as long as the glycogen was in a combined state. Claude Bernard believed that the increased formation of glucose was due to an increased blood flow, which was probably caused by the fact that the diabetic puncture had an effect upon the vaso-motor center. He had in mind the increased blood flow which accompanies the increased secretion of saliva by the submaxillary[3] gland on stimulating the fibers in the chorda tympani. R. Heidenhain, however, has shown that an increased secretion of saliva may take place without an increase in the blood flow, and that the chorda tympani evidently possesses certain specific secretory fibers. Analogously, it is possible that the splanchnic nerves contain fibers which exert an influence upon the formation of glucose in the liver.

Closely connected with the so-called "diabetic puncture" we have another observation to consider. If a one per cent. solution of common

[1] Arch. wissenschaftl. Heilkunde, **4**, 37.

[2] Untersuchungen über die Zuckerbildung in der Leber, p. 76, Würzburg, 1859.

[3] Pflüger's Arch. **5**, 309 (1872); **9**, 335 (1874).

salt [1] is introduced into the vascular system, glucosuria ensues. If, on the other hand, the splanchnic nerves are severed, the salt infusion becomes ineffective. Morphia [2] behaves similarly. Quite recently the exact connection between these experiments and the glucosuria produced by the "diabetic puncture" has been developed by Martin H. Fischer.[3] He showed, first of all, that instead of the one-sixth-molar solution of common salt which he injected into the circulatory system of rabbits at the rate of 75 to 100 c.c. per minute, one-sixth-molar solutions of other sodium salts — e.g., NaBr, NaI, $NaNO_3$ — could be used with the same effect.[4] Fischer showed, furthermore, that the cause of the glucosuria was not a permanent injury produced in any organ, for he could cure the disease by the use of a solution of calcium chloride. A renewed injection of the common salt would, however, again lead to glucosuria. The greater the concentration of the solution of sodium salt employed, the sooner the sugar appeared in the urine.

Fischer, now, sought to determine upon which tissues the solution of salt acted in producing this glucosuria. He remembered at the same time the experiments of Külz, who observed that glucosuria was only produced when the splanchnic nerves remained intact. Fischer found that after cutting these nerves, he could not produce glucosuria, and, furthermore, if the glucosuria was already established before the nerves were severed, it would disappear shortly after the operation. This suggested the thought that probably the salt solution produced some such action as that produced by the vagi nerves upon the sugar center. Fischer sought to localize the action of the common salt as much as possible. For this purpose he tied the axillary artery in some rabbits, and injected the solution of salt into the central end of this artery so that the salt passed on through the vertebral artery directly to the medulla oblongata. When the salt solution was injected in this way, the sugar appeared in the urine somewhat more quickly than when the same amount of the same solution was introduced into a peripheral vessel. Furthermore, the glucosuria was more severe and was more lasting. As much as 7.3 per cent of urine sugar was found in the urine. These experiments make it seem probable that the salts introduced into the circulation act upon the same center as that of the "diabetic puncture."

There are other poisons known which will produce glucosuria, strychnine, for example. The poisonous effect of this substance is not obtained, however, if the spinal cord, with the exception of the upper portions, is extirpated. Since strychnine does not cause elimination of sugar in the

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[1] C. Eckhard: Beit. Anat. Physiol. **8**, 77 (1879).

[2] Külz: Eckhard's Beiträge, **6**, 177 (1872).

[3] University of California Publications, Physiology, **1**, 77 (1903), and **1**, 87 (1904).

[4] LiCl, KCl, and $SrCl_2$, produced glucosuria; NH_4Cl did not.

urine of frogs with extirpated livers, it is probable that the glucosuria is to be attributed to a disturbance of the liver function. It might be supposed that the sugar elimination in the urine is a result of the tetanus produced by the strychnine poisoning. That this is not the case is shown by the fact that animals poisoned with large amounts of strychnine — large doses, instead of producing tetanus, cause paralysis of the motor nerves — are also subject to glucosuria, and, as a matter of fact, in a more severe form than in the case of animals with tetanus, caused by smaller doses of strychnine. The simplest explanation of this last fact is that during tetanus sugar is consumed. Other poisons, such as phosphorus, arsenic, uranium salts, corrosive sublimate, carbon monoxide (illuminating-gas), amyl nitrite, curari, chloral, nitrobenzene, chloroform, acetone vapors, ether, etc., will also cause glucosuria.[1] At present we know nothing more definite concerning the mode of action of these various substances. It should not, however, be assumed that they all have the same point of attack.

The glucosuria produced by the glucoside phloridzin has been studied with especial thoroughness.[2] If from one to three grams of this substance is administered to a dog for each kilogram of its body weight, glucosuria results. In the case of starving animals, after the injection of from 0.3 to 2.5 grams of phloridzin, the sugar can be found in the urine. Von Mering, the discoverer of this form of glucosuria, also studied the effect of the decomposition products of phloridzin, and found that phloretin alone was active in this respect, whereas phloroglucinol and phloretic acid were inactive.

All the other glucosurias which we have discussed up to this point have been caused by a glucohemia, i.e., a flooding of the organism and especially of the blood with sugar. The elimination of sugar through the kidneys is a result of a self-regulating mechanism. By eliminating the excess of sugar the organism seeks to bring the sugar content of the blood back to the normal. Even the glucosuria produced by phloridzin has been explained in this way,[3] although Mering himself and many other later investigators have failed to detect any increase in the amount of sugar contained in the blood even when the ureter was tied. Phloridzin glucosuria is also

[1] Cf. Langendorff: Arch. Anat. Physiol. 138 (1887). F. Gürtler: Inaug. Dissert. Königsberg, 1886. Araki: Z. physiol. Chem. **17**, 311 (1893). Luchsinger: Inaug. Dissert. Zürich, 1875. Senff: Inaug. Dissert. Dorpat, 1869. Straub: Arch. exper. Path. Pharm. **38**, 139 (1897). Rosenstein: *ibid.* **40**, 363 (1898). Araki: Z. physiol. Chem. **15**, 351 (1891); **19**, 422, 476 (1894); also Bernard: Leçons sur la diabète et la glycosurie animale. Paris, 1877.

[2] v. Mering: Ueber Diabetes mellitus. Verhandl. Kongr. innere Med., 1886 u. 1887; Z. klin. Med. **14**, 405 (1888), and **16**, 431 (1889). v. Mering and Minkowski: Zentr. klin. Med. **10**, 393 (1889). Max Cremer: Z. Biol. **29**, 175 (1893). Cremer and Ritter: *ibid.* **29**, 256 (1893).

[3] Cf. Pavy: J. Physiol. **20**, xix-xxii (1896). S. Leone: Gazz. internaz. di med. prat. Vol. **3**, 21.

shown to be different from the other forms by the investigations under the leadership of Minkowski, carried out by Andreas Thiel [1] and by von Mering.[2] These investigators have shown that geese with extirpated livers eliminate sugar after the injection of phloridzin, whereas we have seen that with the other forms of glucosuria it is essentially the liver alone which takes part in the sugar-formation.

Much more important than the results of these last experiments [3] is the above-mentioned fact that most investigators have failed to find any evidence of glucohemia in the case of phloridzin-glucosuria. The conclusion has, therefore, been drawn that the elimination of sugar after phloridzin poisoning is due to the abnormal permeability of the kidney epithelium for sugar. Thus the sugar in the blood would become less and less, and indirectly the liver, and perhaps the other places where glycogen is stored, would be compelled to give up sugar. To support the assumption, that the glucosuria produced by phloridzin is of a renal nature, N. Zuntz [4] has published a proof, which is not, however, conclusive. He injected, by means of a trocar, a solution of phloridzin through the walls of the renal artery into the blood-stream. The kidney to which the glucoside was carried directly was the first to eliminate sugar. Pflüger [5] believes that it is not impossible that the first separation of sugar may be due to a decomposition of the phloridzin itself. At present we cannot be sure as to what is the correct explanation of phloridzin glucosuria. Only this is known, — the matter is by no means so simple as has been assumed.[6] Above all, it must be mentioned that starving animals will also eliminate sugar after the introduction of phloridzin, and repeated additions of the glucoside result in renewed glucosuria,[7] and to such a degree that the question as to whether food other than carbohydrates can give rise to the sugar formation now presents itself.

Our knowledge concerning the formation of sugar in the animal organism was considerably increased by the discovery of von Mering and Minkowski [8] in the year 1889 that dogs always suffered from severe glucosuria after *complete extirpation of the pancreas*. If a small part of the pancreas is left

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[1] Inaug. Dissert. Königsberg, 1887.

[2] *Loc. cit.* Z. klin. Med. **14,** 415 (1888). Cf. Minkowski and Thiel: Arch. exp. Path. Pharm. **23,** 142 (1887).

[3] For a critical discussion see E. Pflüger: Das Glykogen und seine Beziehungen zur Zuckerkrankheit (1905).

[4] Arch. Anat. Physiol. **1895,** 570.

[5] *Loc. cit.* p. 539.

[6] Cf. Carl Jakobj: Arch. exper. Path. Pharm. **35,** 213 (1895).

[7] Cf. Lusk: Z. Biol. **42,** 31 (1901). O. Loewi: Arch. exper. Path. Pharm. **47,** 48 (1902).

[8] *Ibid.* **26,** 371 (1890). O. Minkowski: Untersuchungen über den Diabetes mellitus, Leipzig, 1893.

in the body, the glucosuria is either prevented or lessened. As Pflüger [1] has shown, an animal which has been wholly deprived of its pancreas behaves outwardly quite differently from one which has a slight residue of the gland left in the body. Pflüger observed that after a total extirpation the sugar elimination invariably appeared within the first twenty-four hours, and lasted continuously until the death of the animal, even when it received no further nourishment. The symptoms peculiar to a partial extirpation, such as polydipsia (excessive thirst), polyphagia (voracity), and polyuria (excessive urination), were either entirely lacking or barely indicated. Usually the elimination of sugar takes place very quickly after the extirpation of the pancreas. Thus Bierry and Gatin-Gružewska obtained the following results from four experiments:

	Weight of Dog.	End of Operation.	Appearance of Dextrose.
Experiment No. 1	10 kg.	1 o'clock	3 o'clock
Experiment No. 2	14 kg.	4 o'clock	5 .35 o'clock
Experiment No. 3	20 kg.	1 o'clock	3 .30 o'clock
Experiment No. 4	14 .3 kg.	10 .30 o'clock	3 .30 o'clock

The elimination of sugar in the urine has likewise been observed after total pancreas extirpation in Selachier,[2] frogs [3] and in birds.[4] The results obtained after partial extirpation are not as uniform. Sometimes glucosuria is observed, sometimes not. This might indicate that all the parts of the pancreas gland are not of the same nature, so that in one operation more of the tissue which partakes in the sugar formation is carried away than in another. Numerous experiments by different observers in this direction have, however, established the fact that all parts of the pancreas tend to increase the sugar content of the system, and thus prevent glucosuria. The true cause of the divergence in the results obtained by different investigators is probably to be traced to the different methods of operation employed.[5] Indeed, the glucosurias produced by partial and

[1] Pflüger's Arch. **106,** 181. Cf. Sandmeyer: Z. Biol. **31,** 12 (1895). Cf. also E. W. Pflüger: Das Glycogen, etc., *loc. cit.*

[2] V. Diamare: Zentr. Physiol. **20,** 617 (1906).

[3] Cf. Aldehoff: Z. Biol. **28,** 293 (1891), and Marcuse: Arch. Anat. Physiol. 539 (1894).

[4] W. Kausch: Arch. exper. Path. Pharm. **37,** 274 (1896); **39,** 219 (1897). O. Minkowski: Arch. exper. Path. Pharm. **31,** 85 (1893). Cf. v. Diamare: Zentr. Physiol. **19,** 545 (1905). Cremer and Ritter: Z. Biol. **28,** 459 (1891).

[5] Cf. De Renzi and Reale: Berliner klin. Wochenschr. No. **23** (1892). J. Thiroloix: Diabête-pancréatique, p. 95 (1892). von Mering and Minkowski: Diabetes mellitus nach Pancreasexstirpation, p. 12 (Leipsic, 1899). W. Sandmeyer: Z. Biol. **31,** 74 and 85 (1894). E. Hédon: Travaux de physiologie, 1–150, Paris, 1898.

total extirpation of the gland are very similar, and only show gradual differentiations. Also, it must not be forgotten that the pancreatic gland, like other organs, is in its entire function without question dependent upon nervous influences, and that under certain conditions disturbances and injuries in the region of the nerves which are connected with the pancreas may produce glucosuria. The fact that the operation of itself does not cause elimination of sugar, has often been shown, neither does the extirpation of the solar plexus, or at least not permanently. Before we take up the explanation of this disturbance in the metabolism of carbohydrates produced by extirpation of the pancreas, it may be well to describe briefly the phenomena exhibited by the organism after it has been deprived of the gland. In general, dogs do not survive the operation very long; at best they can be kept alive only two or three weeks. Pflüger found the cause of death to be extensive suppuration. According to him, it was not the lack of the gland, nor the glucosuria produced, which caused the death, but rather that the wound did not heal on account of the sugar in the tissues. That this view is correct is shown by the fact that if a piece of the pancreatic gland is left in the abdominal cavity, glucosuria ensues only after this piece is dead, and such dogs live much longer. By dissection of such a dog, Pflüger [1] found an extensive atrophy. The individual organs did not show much signs of disease. The fatty tissue had disappeared. The liver showed a remarkable condition. All the other organs, except the brain, heart, and kidneys, which retain their weight even during inanition, had lost considerably in weight; the liver, on the contrary, had increased. Its weight represented 4.77 per cent of the body weight. Normally, the liver, according to Pavy,[2] amounts to from 3 to 4.7 per cent of the body weight, and after 28 days of starvation the value falls to 1.5 per cent. The composition of the liver was found to be as follows:

	Per cent.
Dry substance in the fresh liver	24.2
Fat in the fresh liver	2.7
Fat in the dry substance	11.2
Water in the fresh liver after extraction of the fat	78.3
Dry substance in the fresh liver after extraction of the fat	21.7
Nitrogen in the fresh liver	3.2
Nitrogen in the dry liver	13.2
Nitrogen in the dry liver after extraction of the fat	14.9

[1] Pflüger's Archiv. **108**, 115 (1905).

[2] Phil. Trans. for 1860, p. 579. Researches on the Nature and Treatment of Diabetes, London, 1862.

The liver contained 0.0259 gram of glycogen. This shows that it had retained the power of forming glycogen.

Pflüger also examined the muscles, and, as in the case of the liver, found values which were not different from the normal. The only striking fact was the high percentage of water. In spite of this agreement of composition in the case of the liver and muscles (which obviously play the most important part in the metabolism of carbohydrates) with the values obtained under normal conditions, it is on no account permissible to draw the conclusion that as a matter of fact there has been no change in the materials which go to make up these tissues. As we shall see subsequently, our methods of analysis are not sensitive enough, and our knowledge of the cell-constituents is far too inadequate for us to attempt to answer such questions with exactness. It is of importance, first of all, to know that so far as we are now able to judge, the liver, like the organs indispensable to life [e.g., the brain, heart, and kidneys], is protected during inanition at the cost of all other tissues. From this the conclusion may be drawn that evidently the liver is not simply cut off from the metabolism of carbohydrates during the whole duration of glucosuria, but, on the contrary, is continually acting vigorously. It gives up the large amounts of sugar which are found in the urine, and in it evidently takes place, as we shall see, the transformation into sugar of substances not belonging to the carbohydrate group.

The fact that the sugar content of the blood rises after total extirpation of the pancreas, while at the same time the glycogen content of the organs, the liver especially, remains low, is a matter of considerable importance. A true glucohemia naturally ensues which causes glucosuria. Thus the cause is the same as in all the other cases of sugar elimination, which have been observed up to the present time, phloridzin glucosuria possibly forming an exception. We are now ready to take up the question as to what causes the glucohemia. It is a matter of fact that it appears as soon as the pancreatic gland is removed, which suggests the thought that the loss of the function of this gland is the cause of the observed disturbance. First of all we must remember that the pancreas plays an important part in the digestion taking place in the alimentary canal. We have seen that the breaking down of starch in the bowels is brought about principally by the action of the diastase from the pancreatic gland. On the other hand, it is certain that on taking away the ferments of the pancreas the absorption and assimilation of all the remaining foods, and consequently the whole metabolism, must suffer. We have then to decide, first of all, whether the glucosuria produced by the removal of the pancreas can be traced to the absence of the digestive ferments. This must be answered in the negative, because, for one thing, if the ducts of the pancreas are ligated, glucohemia does not develop. Then again the greater part of the

gland may be removed, and, in fact, that portion directly connected with the intestine, and glucosuria does not take place as long as a small part of the tissue of the gland still remains in the body. A further proof that pancreatic-glucosuria is not intimately connected with digestion is shown by the fact that in the case of total extirpation of the gland, sugar still appears in the urine after prolonged starvation, i.e., after the stomach and intestines have become empty. Furthermore, it is not possible to influence the existing glucosuria by feeding the gland to the animal. It is interesting that after a partial elimination of the pancreas the assimilation limit for sugar is considerably decreased. This is shown by the fact that an animal experimented upon which did not show glucosuria would eliminate sugar in the urine when the amount of carbohydrates fed to it was increased, especially if the food contained glucose.

It is particularly significant that a small piece of the gland tissue usually suffices to keep the entire metabolism of carbohydrates in normal paths. This was shown very strikingly by the experiments of Minkowski, which proved conclusively that it was not the severe operation itself, but the total removal of the pancreas function, that caused the great disturbance in the metabolism of carbohydrates. In dogs the lowest part of the descending branch of the pancreas does not grow together with the duodenum, but lies free in the mesentery. Minkowski separated this piece from the remaining tissue of the pancreas so that it was still in connection with the mesentery and without disturbing its supply of blood and lymph vessels. This piece of pancreas was then drawn out through the abdominal cavity, grafted under the skin, and allowed to heal together with the wound. After the animal had survived this operation, the abdomen was again opened, and all of the rest of the pancreas removed from the body. Thus only the small portion of the gland which had been transplanted under the skin remained in the animal, and yet glucosuria did not ensue. If, however, this part of the pancreas was finally removed, sugar appeared in the urine at once.

The fact that, in many cases, a small portion of the pancreas left in the body serves to prevent the appearance of glucohemia, suggests to us other possibilities. It is perfectly conceivable that the pancreatic gland serves, like the liver, for example, to neutralize injurious substances, and especially those which interfere with the metabolism of carbohydrates. When the pancreas is removed, these products perhaps pass unhindered into the circulation, and prevent the normal breaking down of sugar. If this view were correct, it would follow that the injection of the blood from an animal suffering from glucosuria into the circulation of a healthy animal would inevitably produce the same disease. That this is not the case was shown by the direct experiments of Minkowski and von Mering.

The only remaining explanation of pancreatic-glucosuria would seem to

lie in the assumption that the pancreatic gland produces a substance which either directly or indirectly influences the metabolism of carbohydrates. It is altogether out of the question to imagine that the sugar in the blood undergoes any change by passing through the gland. The regulation of the carbohydrate metabolism must take place indirectly, i.e., the tissue of the pancreas influences in some way or other the organs whose task it is to build up or to consume the sugar. This assumption has gradually gained ground, especially after the experimental explanation of Lèpine concerning pancreatic-glucosuria had been found untenable. Lèpine,[1] as has been mentioned, discovered the presence of a ferment in the blood which was able to decompose sugar. This glucolytic ferment was assumed to be produced by the pancreatic gland. The ferment was supposed to pass continually through the thoracic duct into the blood and circulate in this attached to the white corpuscles. Lèpine found that in animals deprived of the pancreas there was a considerable diminution of the amount of this ferment, or, in other words, the power of the blood to consume sugar was considerably diminished. Thus the assumption was made that pancreatic-glucosuria was a result of the fact that the ferment was not formed after the pancreas was removed. Lèpine's views, however, were soon contradicted, and to-day the hypothesis may be said to be completely shattered. De Dominicis,[2] for one, showed that in animals suffering from pancreatic-glucosuria there was no diminution in the elimination of sugar when blood from the portal vein of normal animals, which according to Lèpine would be rich in the ferment, was injected into them. On the contrary, the result was that the glucosuria became more severe. Arthus [3] found that the blood contained in ligated vessels was not capable of decomposing sugar, and therefore contained no glucolytic ferment. Moreover, from many sides it has been shown that glucolysis appears as a post-mortem phenomenon, and that it has any connection with the metabolism of carbohydrates in the living organism has been flatly denied. In fact, the whole theory of glucolysis in the blood rests upon an extremely slight foundation, and a great deal may be said against it. Lèpine in his experiment failed entirely to take into consideration many phenomena which appear after extirpation of the pancreas, such as, for example, the fact that after the operation the liver loses its glycogen. In this connection, Marcuse's [4] observation is interesting that glucosuria in frogs, resulting from extirpation of the liver, disappeared if at the same time the pancreas was removed.

In the last lecture, we found that the muscles are the chief consumers

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[1] Compt. rend. **113,** 729 (1891); *ibid.* **113,** 1014 (1891).

[2] Wiener med. Wochschr. 42–45 (1898).

[3] Arch. Physiol. **1891,** 425; **1892,** 337.

[4] Z. klin. Med. **26,** 225 (1894). Cf. A. Montuori: Arch. ital. biol. **25** (1896).

of sugar. Their necessary supply of carbohydrates is regulated by the liver. It would seem possible, therefore, that the function of the liver might be disturbed in some way by the removal of the pancreas. The liver stores up the resorbed sugar in the form of glycogen. It might seem possible that the liver in an animal deprived of the pancreas is no longer able to retain the resorbed sugar, i.e., withdraw it from the general metabolism, so that in this way the blood is flooded with sugar. Now Pflüger, as we have seen, showed that the liver, even after long-continued glucosuria, is still capable of forming glycogen. At least the ability is not entirely wanting. Furthermore, this assumption does not explain the fact that starving animals also exhibit glucohemia. This latter fact suggests to us another way in which the liver-function may be disturbed, namely, with regard to the *decomposition of glycogen*. Hand in hand with the consumption of sugar in the muscles, there takes place the transformation of the stored-up glycogen into sugar. An extremely fine regulating mechanism prevents large amounts of glycogen being suddenly decomposed in such a way that the blood would be flooded with sugar. We have already met with this regulation in discussing the glucosuria produced by the " diabetic puncture," and the elimination of sugar in the urine caused by the injection of salt solution into the circulation. We saw at that time how evident it was that nervous influences had a great deal to do with keeping the glycogen condition of the liver along definite paths. What is unexplained is merely how the breaking down of the glycogen takes place. It is a result of diastatic action. It is inexplicable why the diastase, which is evidently present in the liver, at one time attacks the glycogen and at another leaves it alone, unless we assume that either the glycogen is present in a condition such that it cannot be acted upon by the ferment, i.e., is in some form of loose chemical combination, or that the diastase becomes active only at the time it is required.[1] We are acquainted with many ferments, as we shall eventually see, which are secreted by the cells in an inactive condition. In such cases the presence of another substance usually formed by an entirely different kind of cells is necessary in order to make the ferment " active." Such processes have not been sufficiently studied in the case of diastase. We may assume, however, that by union with some sort of substance, perhaps the protoplasm of the cells, the diastase is inactive and becomes free only at such a time when it is needed. At

[1] The assumption that the breaking down and building up of glycogen takes place in the same way that, for example, a hydrolysis or a synthesis may be brought about artificially by the action of one and the same ferment according to the concentration ratio (cf. p. 38) is not well established at present, for the products formed artificially are not those expected, but their isomers; and furthermore, it is not known that the living cell contains the condition established in chemical experiments. Cf. Hofmeister's Die Chemische Organisation des Zelle. Viewig and Sohn, Braunschweig, 1901.

present our knowledge in this direction is still too slight for us to attempt to discuss in the light of experimental data any disturbance in the glycogen decomposition. We merely wish to point out the possibility, and to suggest that there is a certain analogy between pancreatic-glucosuria and the disturbances in the formation of sugar which have been taken up previously (diabetic puncture, etc.).

Glucohemia might also become established by the failure of the muscles to consume sugar. Unfortunately we know very little concerning the manner in which the muscles consume sugar, and consequently practically nothing concerning the possibilities of disturbing this function. In analogy to other processes, it has been suggested that this may also be due to the action of a ferment, a conception which is perfectly plausible, for we have here to deal with either a direct or indirect protoplasmic action. Recently O. Cohnheim [1] has studied this problem. He showed that the juice obtained from the pancreatic gland by high pressure was not capable of decomposing sugar. On the other hand, sugar was not attacked by the expressed juice from the muscles. Cohnheim found, however, that on bringing together the juices from both of these organs, glucolysis took place at once. He explained this fact by considering the analogy with observations made with other ferments, and assuming that the muscles produce a ferment which is inactive, i.e., incapable by itself of attacking sugar. This muscular ferment is activated by a substance obtained from the pancreatic gland and brought to it by the blood circulation. This would readily explain pancreatic-glucosuria. It must be remembered, however, that this conception does not explain all of the phenomena observed in pancreatic-glucosuria; thus, for example, it does not account at all for the fact that the glycogen stores of the liver disappear. On the other hand, there is absolutely no reason for assuming that the pancreas has only one function with regard to the metabolism of carbohydrates. It is, indeed, possible that it has different effects upon different organs, and that, furthermore, when the functions and metabolic effect of the different organs become disturbed, they again bring into play secondary influences of the organs upon one another, so that one disturbance causes a number of complications.

If we summarize all that we know positively concerning the cause of the glucohemia resulting from extirpation of the pancreas, we may say that we have to deal with a disturbance in the regulation of the transformation of sugar, and that evidently the pancreatic gland gives up to the blood some substance which regulates the metabolism of carbohydrates. This function of the pancreas, in contrast to its other function of forming and

[1] Z. physiol. Chem. **39**, 336 (1903); **42**, 401 (1904); **43**, 547 (1905). For objections to Cohnheim's conclusions, see Claus and Embden: Pankreas und Glycolyse, Hofmeister's Beiträge, **6**, 214, 343 (1905).

secreting the digestive ferment, is spoken of as that of an *internal secretion*. An internal secretion is something formed within a glandular organ and given off to the blood or lymph. The ordinary pancreatic juice is called an external secretion.

The discovery by Langerhans [1] of a peculiar segregation of cells in the pancreatic gland led to discussion as to whether the gland possesses particular cells for its various functions. These cell-forms — called *islands of Langerhans* — which stand out very sharply from the other cells in the gland differ from the latter not only in outward appearance, but, by the fact that, unlike the ordinary secretory cells, they have no connection with the exit ducts from the gland. More recently Diamare and Kuliabko [2] have taken up anew the question as to the significance of these cells. They studied the pancreatic glands of the *Teleostei* because in these animals the islands of Langerhans are relatively large, and preparations of them may be easily made free from the other cells of the pancreas tissue. They found that only the ordinary cells of the gland produced an amylolytic ferment, while the cells of the islands of Langerhans possessed the power of destroying dextrose. This sums up all that we know concerning these islands of Langerhans, and it remains undecided as to whether they form an internal secretion or not. We shall come back to this point in the discussion of diabetes.

Changes in the pancreatic gland had been observed before the discovery of pancreatic glucosuria, namely in the so-called *diabetes mellitus* of man. Although, in discussing the phenomena of this pathological degeneration, we are leaving the proper field of physiological chemistry, we will, nevertheless, take it up more or less in detail, because in this disease we have in a certain sense an experiment brought about by Nature, which serves to give us some insight into the normal metabolism of carbohydrates. We shall, however, discuss the disease only in so far as it is directly or indirectly connected with the metabolism of carbohydrates, and leave the discussion of the remaining clinical symptoms of this very interesting disease to the text-books on clinical medicine.

Diabetes has been known for a long time.[3] The Indian and Arabian physician of the Middle Ages recognized the fact that associated with the disease was the elimination of a sweet substance in the urine. It remained for Thénard, in 1806, to isolate this sweet substance; Chevreul crystallized

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[1] Beiträge zur mikroskopischen Anatomie der Bauchspeicheldrüsen, Berlin. Dissert. 1869.

[2] Zentr. Physiol. **18**, 432 (1904).

[3] Cf. Max Salomon: Geschichte der Glukosurie von Hippokrates bis zum Anfang des 19 Jahrhunderts, Deutsches Arch. klin. Med. **8**, 489 (1871), and E. O. v. Lippmann: Zur Geschichte des diabetischen Zucker. Chem.-Ztg. **29**, 1197 (1905).

it in 1815; while Bouchardat [1] and Peligot [2] succeeded in identifying it as dextrose, or grape-sugar, in 1838.

The cause of this disease has for a long time been attributed to a marked glucohemia. This naturally does not account for the whole nature of the disease, but is only one of many symptoms. It may be caused in a number of different ways; and, according to all we know at present regarding diabetes, there is no longer any doubt that diabetes does not represent a single disease, but rather that the glucohemia, or rather the resulting glucosuria, is merely a symptom most readily recognized, and is produced by the most varied pathological conditions. For this reason it would be out of the question to attempt to find a common cause of glucohemia. The disturbance in the metabolism of the carbohydrates varies in different cases.

We distinguish between a light and severe form of diabetes. In some cases sugar is eliminated in the urine only after the patient has partaken of starch or of glucose. In such cases there is no noticeable glucosuria if the diet is restricted to meat and fats. These mild forms show all stages of alimentary glucosuria, and make it seem probable that the limit of an assimilation for carbohydrates has been considerably diminished. In many cases sugar is found in the urine only when carbohydrates are eaten on an empty stomach. Often muscular work suffices to arrest the sugar elimination. In other cases, the glucosuria lasts only while the absorption of sugar continues in the intestine. The cause of this kind of diabetes is generally attributed to a weakening of the liver function. The latter is obviously not able to work over the sugar quickly enough into glycogen. It permits too much sugar to get into the circulation. Thus a glucohemia results, which after a time is compensated by the elimination of sugar by the kidneys, only to appear again from the same cause as before. Against this assumption the objection has been raised that the liver may suffer most severe changes, without the appearance of sugar in the urine. This is, however, not a serious objection, for we know that the liver has quite a number of different functions, of which each is to a certain extent independent of the others, so that one function by itself may be disturbed. It does not follow that every disease of the liver will attack that part of the cells which participates in the regulation of carbohydrate metabolism. A general weakening of the activity of the liver cells can cause diabetes; thus it is met with in persons of undermined constitution. Possibly the fact established by Hofmeister [3] that dogs, after all forms of nourishment had been withheld for a considerable time, eliminated sugar in the urine,

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[1] Compt. rend. **6**, 337 (1838).

[2] *Ibid.* **7**, 106 (1838).

[3] Arch. exp. Path Pharmak. **26**, 355 (1890).

shows that many forms of glucosuria belong in this category. It is not impossible, but on the contrary extremely probable that many of these light forms of diabetes result from conditions similar to those resulting from the diabetic puncture, etc. The only difference here is probably that in the case of the operation we have but a single shock to the system, whereas here there is evidently a permanent irritation of the sugar center. This coincides with the fact that many persons afflicted with the disease are very nervous.

Between these light forms of diabetes and the more severe types, there are, as we have said, all stages of intermediate types, and not infrequently the former change into the latter. In the first case the disease is not of a very bad character, and appears chiefly in elderly people, producing symptoms which are easily understood, but in the more serious types we are astonished at the severity of the disease. How great the disturbance in the carbohydrate metabolism is, may be illustrated by the fact that the elimination of sugar continues even after carbohydrates are entirely withheld from the diet, and the patient eats, for example, only meat and fat.

Let us now turn our attention to the main symptom of diabetes, the glucohemia. Why does this exist? There are *a priori* two possibilities. On the one hand, the amount of sugar formed may be abnormally large; or, on the other hand, the sugar produced normally may not be consumed as it should be, and thus lies unutilized in the tissues only to be removed finally from the organism as a waste-product. The last explanation is really the more plausible; for although we can easily conceive that there might be temporarily an increased formation of sugar, possibly from fat or perhaps from albumin, it is not easy for us to understand how this could take place continuously. Furthermore, the whole behavior of the patient does not correspond to any such assumption. Fats and albumin agree with him. With their help and the removal of carbohydrates from the diet, it is possible greatly to diminish the elimination of sugar. On the other hand, the glucosuria immediately becomes more severe if carbohydrates are fed to the sick. In these severe cases we cannot account for the facts by assuming that the liver has lost its power of storing up sugar in the form of glycogen. The disease continues during a period of fasting, long after all carbohydrates have left the alimentary canal.

The fact that the liver actually retains its ability of storing up sugar in the form of glycogen is proved by the fact that glycogen has been repeatedly found in the livers of those who have suffered from severe diabetes.[1] This, however, is not always the case. Sometimes the liver

[1] Cf. Kühne: Virchow's Arch. **32**, 536 (1865). Jaffé: *ibid.* **36**, 20 (1866). Külz: Pflüger's Arch. **13**, 267 (1876). J. v. Mering: *ibid.* **14**, 274 (1877). Abeles: Zentr. med. Wissensch. **23**, 449 (1885). F. Th. Frerichs: Ueber den Diabetes, Berlin, 1884.

contains glycogen, and sometimes it does not contain a trace of this polysaccharide. This is not astonishing when we remember that the name *diabetes* includes quite a number of different diseases, all of which show the common symptom of glucohemia.

We will now attempt to answer the question as to whether diabetes can be explained entirely on the assumption that it is due to a diseased pancreas. It is certainly interesting to compare diabetes with the glucosuria produced by extirpation of the pancreas. It has been shown in many cases that the pancreas of diabetics was diseased, and there is absolutely no doubt but that there are forms of diabetes which originate in a disturbance of the functions of the pancreatic gland. Recently changes have been noticed, particularly at the islands of Langerhans, and also many degenerations have been observed, e.g., hyaline degeneration. At the present time it is impossible to decide whether the conclusion may be drawn that there is positively a connection between the cells of the Langerhans group and the metabolism of carbohydrates. At all events, the fact that in many cases of diabetes the post-mortem examination shows these cells to be absolutely normal, does not necessarily show that any such assumption is false, for it is not possible to trace all forms of diabetes back to a common cause; and furthermore, the fact that there is no noticeable histological sign of a change having taken place in the tissues, does not prove that the cells have suffered nothing as regards their functional activity. Perhaps the researches of Diamare and Kuliabko [1] will eventually settle this question.

Let us return to the question as to the sugar formed not being consumed. It is possible that diabetics in general have a limited capacity for oxidation. On the other hand, the fact that the other forms of nourishment are taken care of normally speaks *a priori* against any such assumption. The following experiments are a direct proof that the organism of diabetics possesses its full oxidation capacity. O. Schultzen [2] found that diabetics readily consume the alkali salts of lactic and the vegetable acids, also inosit and mannitol. M. Nencki and N. Sieber [3] observed that patients suffering from severe diabetes could take care of the difficultly-oxidizable benzene just as well as the healthy organism could. Direct respiration experiments likewise showed that the respiratory exchange in such cases — severe cases of diabetes — did not vary from the proper physiological relations. It is true that von Pettenkofer and C. Voit [4] found that there was a considerable diminution in the inspired oxygen and expired carbon dioxide, and drew the conclusion that this was due to a decreased capacity for oxidation; but this conclusion was later abandoned by Voit [5] himself,

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[1] *Loc. cit.*

[2] Ber. klin. Wochschr. No. 35 (1875).

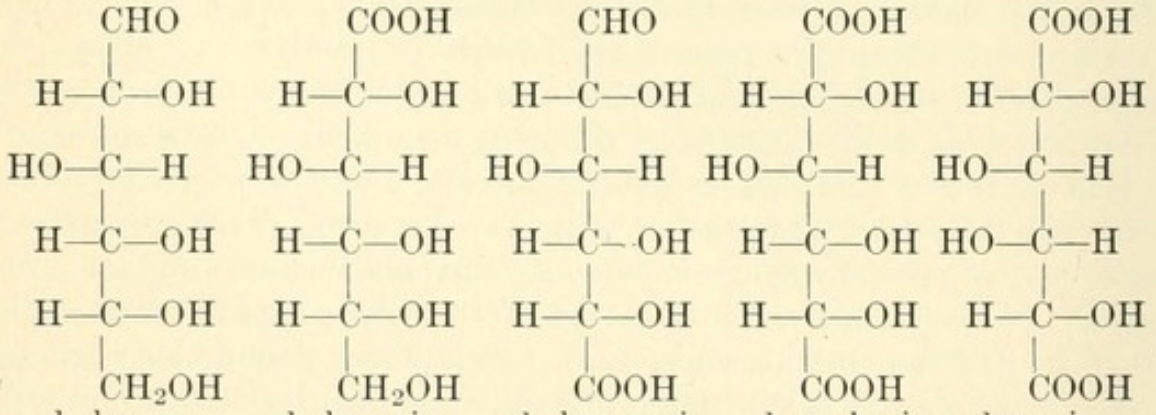
[3] J. pr. Chem. N. F. **26**, 35 (1882).

[4] Z. Biol. **3**, 380 (1867).

[5] Physiol. allg. Stoffwechsels und der Ernährung (1881), p. 227, *et seq.*

who found that the limited absorption of oxygen was a result of the disturbed carbohydrate metabolism, rather than the cause of it. The absorption of oxygen depends upon the combustion taking place in the body. Leo,[1] as well as Weintraud and Laves,[2] has finally shown that the amount of absorbed oxygen is the same with healthy people that it is with those suffering from diabetes of equal weight and condition of nourishment, and finally that the apparent decrease in the elimination of carbonic acid is caused by the faulty decomposition of carbohydrates.

Of especial importance as regards the cause of the insufficient consumption of the sugar formed in the organism are the experiments performed by O. Baumgarten[3] under the direction of von Mering. Baumgarten, at von Mering's suggestion, tried some feeding experiments upon diabetics, and upon dogs with removed pancreas, employing substances which are regarded as decomposition or oxidation products of the sugars. It was found that *d*-gluconic acid, *d*-saccharic acid, mucic acid, glucuronic acid, glucosamine-hydrochloride, succinic acid, *d*-tartaric acid, salicylic aldehyde and vanillin, were consumed by diabetics just as readily as by healthy individuals. The following summary illustrates the relation between some of these products and *d*-glucose (also called dextrose, or grape-sugar):



These experiments show that the organism of diabetics can decompose in the same way as the healthy organism, substances which in their aldehydic nature are closely related to dextrose, and are to be regarded as direct oxidation products of dextrose; in other words, a very slight oxidation of the sugar molecule suffices to enable the organism of the diabetic to attack it. In this way the views expressed by Scheremetjewski,[4] Schultzen,[5] A. Cantani,[6] and of Nencki and Sieber gain ground;

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[1] Z. klin. Med. **19** (1890). [2] Z. physiol. Chem. **19**, 603 (1894); **19**, 629 (1894).

[3] Z. exper. Path. u. Therapie, **2**, 53 (1905).

[4] Arbeiten aus dem physiol. Institut zu Leipzig, 1868.

[5] *Loc. cit.* [6] Du Diabetes mellitus, Berlin, 1877.

and, as a matter of fact, it seems probable that the principal cause of glucohemia and the resulting glucosuria is that the organism has lost the power of splitting the sugar molecule. The complete combustion of the glucose molecule is, according to this view, always dependent upon a previous hydrolysis, or some attack which loosens up the sugar molecule and disturbs the condition of equilibrium; without some such action the combustion cannot take place in the organism. This assumption is substantiated by the fact that glucose is very readily consumed by the healthy organism, even more readily than is the case with other foods. Thus in phosphorus poisoning, the oxidizing power is lost to a considerable extent without the appearance of glucosuria.

We have again come back to the undecided question concerning the normal breaking down of sugar. The answer depends upon the explanation of the inability of diabetics to break up glucose. It is easy to conceive that the principal places of sugar consumption, the muscles, produce a ferment which serves first of all to loosen up the glucose molecule and thus prepare it for oxidation. That the glucose is not itself directly oxidized is very plausible. In this way, the sugar stores in the organism are in a measure protected. Only at the moment of the expenditure of energy do the muscular cells prepare the dextrose molecule for consumption. As to why the diabetic has lost this power, whether the ferment which starts the process is missing, or whether he has lost the power of activating this ferment, are questions which cannot be answered positively at the present time. An important observation has been made that when cane-sugar is taken into the system often only one half of the molecule appears subsequently in the urine. This is due to the fact that many diabetics are able to oxidize and assimilate fructose without difficulty.[1] In fact, because the liver can in such cases take care of fructose and not of glucose, it has been proposed to feed diabetics a carbohydrate, such as inulin, which on being hydrolysed yields fructose rather than glucose. Unfortunately, however, the above carbohydrate is so difficultly digestible, that the experiment has not met with much success.[2] At all events, it is most remarkable that the liver should transfer fructose into glycogen, a process which according to the generally accepted opinion involves the intermediate formation of glucose; and yet should not be able to use the glucose which it receives as such.

[1] E. Külz: Beiträge zur Pathologie und Therapie des Diabetes mellitus, p. 130, Marburg, 1874. Worm-Müller: Pflüger's Arch. **34,** 576 (1884); **36,** 172 (1885). F. Hofmeister: Arch. exper. Path. Pharm. **25,** 240 (1889). J. Haycraft: Z. physiol. Chem. **19,** 137 (1894). Minkowski: Arch. exper. Path. Pharm. **31,** 158 (1898). Sandmeyer: Z. Biol. **31,** 12 (31) (1894). Fr. Voit: Z. Biol. **29,** 147 (1892). Socin: Wie verhalten sich Diabetiker Lävulose- und Milchzuckerfütterung gegenüber? Dissert. Strassburg, 1894.

[2] Sandmeyer: *loc. cit.* and Miura: Z. Biol. **32,** 279 (1895).

The assumption that in some way or other the muscles have lost the power of preparing sugar for consumption does not explain the cause of diabetes, for the real beginning of the chain of disturbances which constantly brings into play new effects is not, it is certain, to be attributed originally to a faulty combustion of sugar; in fact, the combustion of sugar is not entirely lost in the organism of diabetics. We come back here, as in the case of most disturbances in metabolism, to the individual cells themselves and their dependence upon the nervous system. Even if we attribute the breaking down and formation of glucose entirely to the action of ferments, we fail even then to get around the responsibility of the cells, for it is they which form the ferment, and the production of the latter seems in many cases to be dependent upon nervous influences. Now if, furthermore, we add to this the fact that many ferments are known which are secreted by the cells in an inactive condition, and are only activated by means of a second ferment produced by other cells — often those of another organ — then it becomes easy for us to understand how disturbances may originate at countless places in the whole mechanism. These all together may accomplish the final effect, namely, a glucohemia in consequence of the non-consumption of a part at least of the glucose, whether on account of a disturbance in the nervous system, or the fact that the activating agent (perhaps furnished by the pancreas) is lacking, or whether it may be because the muscular cells themselves are diseased. In discussing these possibilities, it must be brought forward once again that in glucohemia we have merely a symptom, a consequence and not a cause, which without question, by means of the resulting changes in the composition of the tissue and of the blood, may effect all sorts of secondary disturbances, and which again, by a continuous *circulus vitiosus*, tends to diminish the life energy of the body cells and aggravate more and more the nature of the disease. In investigating the causes of pathological phenomena, the endeavor must be more and more to study the purely functional disturbances on the basis of biological experiments, for doubtless such may be present without there being any indication of morphological changes discernible by present methods of investigation.

We have learned to consider glucosuria as an essential symptom of all forms of diabetes. It can attain quite remarkable proportions: — in a single day as much as a kilogram (2.2 pounds) of sugar may be eliminated. Besides glucose we occasionally meet with an elimination of other kinds of sugar; for example, fructose and certain pentoses. Furthermore, in the urine of diabetics, often higher molecular sugars, such as maltose and dextrin-like compounds, have been detected without our being able to draw any conclusions from the occurrence of these products of an evidently incomplete combustion, as to the nature of the disturbance in the metabolism of carbohydrates. In severe cases of diabetes the urine has a

peculiar, fruity odor which was noticed by the older physicians. This is due, as C. Gerhardt [1] supposed and v. Jaksch[2] has shown, to the presence of acetoacetic acid $(CH_3CO)CH_2 . COOH$. Besides this compound acetone $CH_3 . CO . CH_3$ and, as Minkowski [3] has shown, β-hydroxy-butyric acid $CH_3CH(OH)CH_2 . COOH$, are also present in many cases. All three of these compounds, as a glance at their structural formulæ will show, stand in direct relation to one another. Acetoacetic acid is evidently formed by the oxidation of β-hydroxy-butyric acid:

$$\underset{\beta\text{-hydroxy-butyric acid}}{* CH_3.CH(OH) . CH_2 . COOH} + O = \underset{\text{acetoacetic acid}}{(CH_3 . CO) CH_2 . COOH} + H_2O$$

From acetoacetic acid, acetone is readily formed by loss of carbon dioxide:

$$\underset{\text{acetoacetic acid}}{(CH_3 . CO)CH_2 . COOH} = \underset{\text{acetone}}{CH_3 . CO . CH_3} + CO_2$$

The fact that in many cases β-hydroxy-butyric acid is not found in the urine when the other two compounds are present, is not remarkable, for we can easily understand that in one case the former is oxidized and in another case is not. β-hydroxy-butyric acid contains, as the above formula indicates (*), an asymmetric carbon atom, — i.e., it is optically active; as a matter of fact, the left-rotating form is always eliminated.

It has been attempted repeatedly to establish the origin of these compounds in the urine, and especially their significance as regards disease. It is above all worth mentioning that the occurrence of acetone in the urine (*acetonuria*) is not peculiar to diabetes. To some extent it has been observed in the urine of many fever patients. Acetone and acetoacetic acid have also been found after long-continued inanition.[4] Acetone is also eliminated in small amounts when healthy individuals are fed exclusively with albumin and fat. All of the acetone does not leave the system through the kidneys, but a part is given off by the lungs. The question as to the origin of these so-called *acetone bodies* [5] has been the subject of considerable controversy. The simplest explanation is that they are in some way directly connected with the disturbance in the metabolism of carbohydrates,[6] for the observation that acetonuria occurs after inanition

[1] Wiener med. Presse, **28,** 673 (1865). Cf. Petters Prager Vierteljahresschrift, **55,** 81 (1857). Jaksch: Z. physiol. Chem. **7,** 485 (1883).

[2] Ber. 15, 1496 (1882).

[3] Zentr. med. Wiss. 242 (1884). Külz: Arch. exper. Path. Pharm. **18,** 291 (1884); Z. Biol. **20,** 165 (1884); **23,** 329 (1887). O. Minkowski: Arch. exper. Path. Pharm. **31,** 183 (1893). Araki: Z. physiol. Chem. **18,** 1 (1894).

[4] von Jaksch: Ueber Azetonurie und Diazeturie, Berlin, 1895. Fr. Müller: Berlin. klin. Wochschr. 428 (1887).

[5] H. C. Geelmuyden: Z. physiol. Chem. **23,** 431 (1897).

[6] Cf. F. Maignon: Compt. rend. **140,** 1124 (1905).

does not disagree with Hofmeister's [1] experiment showing that glucosuria is produced in starving dogs. It has been proved, however, that an existing acetonuria is diminished and even stopped by limiting the supply of carbohydrates, while, on the other hand, it is a matter of experience that when carbohydrates are entirely removed from the diet of diabetics the direct consequence is often an acetonuria. For this reason it has been attempted to ascribe the appearance of these acetone bodies to other sources, to albumin especially. The appearance of the acetone bodies is, according to one view, due to the progressive decomposition of albumin taking place in severe types of diabetes. Now there is no parallel between the elimination of nitrogen and acetone bodies. In starvation experiments, for example, on account of the lessened consumption of albumin during the first days, the amount of acetone eliminated increases.[2] According to Weintraud,[3] diabetics can be in equilibrium as regards nitrogen, or may even add to their nitrogen without the elimination of acetone being affected in the slightest. The observation made by Magnus-Levy [4] is likewise contrary to the assumption that the acetone bodies result from albumin; in a case of diabetes there were 262 grams of albumin decomposed, and 342 grams of β-hydroxy-butyric acid eliminated. This does not show that acetone cannot be formed from albumin, for there may be more than a single source; and then again the discussion of this question brings us once more to the possibility of one food-stuff being converted into another. Furthermore, it must be admitted that our present knowledge concerning the intermediate decomposition of albumin is still very limited. In no case does it follow necessarily that the amount of nitrogen and of sulphur eliminated is to be taken as a measure for the total decomposition of the albumin introduced into the body. Again the point may well be raised that the carbon eliminated in the urine represents only a part of the carbon contained in the albumin, and the remaining carbon can be retained in the system for a considerable time after all of the nitrogen and sulphur have been eliminated.[5] Again there is much to be said in favor of fat being the mother-substance of the eliminated acetone. This agrees with the fact that during starvation for a time the organism performs its tasks at the expense of its own fat, while, at the same time, the elimination of acetone increases. The fact that feeding carbohydrates lessens the ace-

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[1] *Loc. cit.*

[2] Cf. Giuseppe Satta: Hofmeister's Beiträge, **6,** 1 (21), and **6,** 376 (1904).

[3] Arch. exper. Path. Pharm. **34,** 169 (1894).

[4] *Ibid.* **42,** 149 (1899), and **45,** 389 (1902).

[5] Acetone has been obtained in the laboratory to a slight extent by the oxidation of albumin. These experiments, however, have no relation to the formation of acetone in the body. The amount formed is far too small, and the possible source in fats or carbohydrate is not excluded.

tonuria is explained by a decreased decomposition of fat. If large quantities of albumin are fed to the organism, the elimination of the acetone bodies is likewise abated. Conversely, if the diet is made to consist of fat with exclusion of carbohydrates, more acetone is formed. Schwarz [1] observed, moreover, that the blood of diabetics always contains an excessive amount of fat.

For the present, we must leave the question of the source, or, perhaps more correctly speaking, the sources, of the acetone bodies as undecided. We know just as little concerning their place of formation.

The occurrence of acetone bodies in the blood has for a long time been considered to have considerable significance concerning the course of diabetes. It has been assumed that the presence of acids decreases the alkalinity of the blood, and thus brings about severe disturbances. This is, in general, not the case, because the organism can form ammonia which by combining with acids keeps the reaction of the blood, and thereby that of the tissues, within normal limits. The fact that the interchange of oxygen is not materially altered during the elimination of considerable amounts of these aldehyde bodies, speaks in favor of this assumption. Sometimes, however, there takes place a rapid formation of large amounts of these acetone bodies, resulting in considerable injury to the whole organism, namely in *coma diabeticum*. By this name is meant a complex of symptoms which very often occurs, in case intercurrent disease has not carried off the patient, at the period near the death of the diabetic. The condition is characterized by progressive dyspnœa, somnolence, and sinking of the body-temperature. In many cases the patient recovers, the acetone bodies are again eliminated in the urine, and the organism has time to combat the acid by the formation of ammonia. After some time the symptoms reoccur with increased severity, until finally the patient dies as a result of such an attack. The first explanation of this was in attributing a specific poisonous action to the acetone bodies. This is, however, as direct experiments with the separate substances has shown, not the case.[2] Obviously we have here a true instance of acid poisoning. The blood and tissue react acid after a diabetic has died in coma. There takes place, together with this change in the reaction of the blood, a diminution in the processes of oxidation. The β-hydroxy-butyric acid is no longer oxidized to acetoacetic acid, or at least only to a slight extent. Both of these acids unite with the free alkali in the blood. This explains the fact that the amount of carbonic acid in the blood sinks during the coma. The fact that there is less oxidation in the organism during this period is proved by the fact that various products of the hydrolysis of albumin are now

[1] Deut. Arch. klin. Med. **76** (1903).

[2] F. T. Frerichs: Z. klin. Med. **6,** 3 (1883). P. Albertoni: Arch. exper Path. Pharm. **18,** 218 (1884). Stadelmann: *ibid.* **17,** 419 and 443 (1883).

found in the urine.[1] The general symptoms during *coma diabeticum*, as Walter [2] showed, have great similarity with those observed during experimental acid poisoning. He injected dilute hydrochloric acid into the stomach of rabbits — animals which, in contrast to all other animals which have been investigated, are not able to combat acid by the formation of ammonia — and dyspnœa soon appeared. The carbonic acid content of the blood was greatly lessened, and the ammonia in the urine considerably increased. By subcutaneous introduction of bicarbonate of soda the symptoms were relieved, and the animal revived.

The formation of these acetone bodies is not restricted to diabetes. It takes place also under certain conditions in all the different kinds of glucosuria. There is always some disturbance in the metabolism of carbohydrates, but there is not necessarily a direct relation between the two symptoms. It must not be forgotten that actually such a deep-seated disturbance of metabolism as exists both in the mild and chronic forms of glucohemia can never exist by itself, i.e., be limited in its effect to one class of substances. Since the metabolism of the organism may be traced back eventually to the metabolism of its cells, it is easy to see how much the whole metabolism must suffer if one group of its most important nourishing and building materials becomes afflicted. It is readily comprehensible that in the course of time the metabolic disturbance becomes general, and the metabolism of the fats and albumins suffers as well. It is from this point of view that we must regard the patient suffering from a severe type of diabetes, in order to get some idea of the whole scope of the disturbance. It is not alone the loss in energy which goes on during the constant carrying away of large amounts of sugar, and which indeed may be compensated to some extent by other food-stuffs, that governs the whole disease and makes it so serious, but the general derangement of the entire metabolism. The abnormal composition of the blood and of the lymph gives rise to many secondary phenomena, the resistance of the tissues and cells to infection is diminished (the numerous tissues furnish a favorite nutriment for certain forms of life — furunculi, colonies of aspergilli, etc.) and thus one trouble follows another, eventually giving to the disease of diabetes its peculiar characteristics.

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[1] Abderhalden: Z. physiol. Chem. **44**, 17 (1905).

[2] Arch. exper. Path. Pharm. **7**, 148 (1877).

LECTURE VI.

FATS — LECITHIN — CHOLESTEROL.

Among the foods of the animal organism, the fats occupy a peculiarly important place, due to their high calorific value. They also play an important *rôle* in the plant, and especially in the animal kingdom, as reserve material. The animal organism stores tremendous reserves of vital energy in the form of fats.

The most important locations are the intermuscular connective tissue, the fatty tissues of the abdominal cavity, and the subcutaneous connective tissue. Small deposits occur in every organ and cell. These fat reserves vary much in size, depending on nutritive conditions, so that no definite statement can be made regarding the fat content of the individual organs.

In the vegetable kingdom the fats are also widely distributed, and act as reserve stores, yet never to the same extent as in the animal organism. Fats are deposited in dormant parts, as in seeds, in which we first find the accumulations of carbohydrates, then fats; often they occur together. Here the fat does not occur in the form in which we meet it in the animal tissues. It is very finely disseminated throughout the protoplasm, only in isolated cases occurring deposited as crystalline aggregates in the nutritive cells. Supplies of fat have occasionally been found in stalks embedded in the soil; onions, tubers, and roots, and similiarly the shoots and branches of bushes in winter as well as the leaves of evergreen have shown a reserve supply of fat.

The fats are composed entirely of the elements carbon, hydrogen, and oxygen. The natural fats belong to the group of neutral fats. Free fatty acids are found only in small quantities. The neutral fats are, in general, to be considered [1] as esters of glycerol and monobasic fatty acids; i.e., in the triatomic alcohol, glycerol, the hydrogen atoms in all three hydroxyl groups are substituted by fatty acid-radicals (R):

$$\begin{array}{l} CH_2O \,.\, R \\ | \\ CHO \,.\, R \\ | \\ \underbrace{CH_2O}_{\text{Glycerol residue}} \,.\, \underbrace{R}_{\text{Fatty acid radical}} \end{array}$$

[1] Cf. Chevreul: Chem. Untersuchungen über Tierische Fette. Paris, 1823.

The three fatty acids, oleic, $C_{18}H_{34}O_2$, stearic, $C_{18}H_{36}O_2$, and palmitic, $C_{16}H_{32}O_2$, play the principal rôle in the formation of fats. The last two belong to the series of normal saturated fatty acids, $C_nH_{2n}O_2$, whose lower members are represented by formic, acetic, and propionic acids. Oleic acid, however, is an unsaturated fatty acid: $C_8H_{17} . HC : CH (CH_2)_7 . COOH$.

These fatty acids combine with the triatomic alcohol, glycerol, (commonly called glycerin) splitting off water. We speak of *tripalmitin*, *tristearin*, and *triolein* according to the nature of the fatty acid concerned:

$$\begin{array}{lll} CH_2OH & C_{15}H_{31}COOH & CH_2 . O . CO . C_{15}H_{31} \\ | & & | \\ CH . OH + C_{15}H_{31}COOH = & CH . O . CO . C_{15}H_{31} & + 3\,H_2O \\ | & & | \\ CH_2OH & C_{15}H_{31}COOH & CH_2 . O . CO . C_{15}H_{31} \\ \text{Glycerol} & \text{Palmitic acid} & \text{Tripalmitin} \end{array}$$

Tripalmitin and tristearin are solid at the ordinary temperature, while triolein, on the other hand, is liquid. As fats are mainly mixtures of the triglycerides, the solid or liquid condition of these depends entirely on the quantity of triolein present. Besides these separate triglycerides there are also mixtures, for instance, *dipalmito-olein*, a fat whose glycerol base is united to two molecules of palmitic and one molecule of oleic acid; again, we have *distearopalmitin*. Aside from the fatty acids mentioned, there are the volatile members of the normal series; i.e., butyric, caproic (*hexoic*), caprylic (*octoic*), and capric (*decoic*) acids. Thus in vegetable fats almost all the different members of the normal fatty acid series have been found. Even the higher fatty acids, such as lauric ($C_{12}H_{24}O_2$), myristic ($C_{14}H_{28}O_2$), and arachidic ($C_{20}H_{40}O_2$), as well as individual oxy-fatty acids, have been observed. The latter have been isolated with certainty only in the vegetable kingdom, while in the animal body the fatty acids may combine with the higher alcohols instead of with glycerin. Rohmann[1] has shown that in the secretions from the oil-bags of birds a portion of the fatty acids is combined with octadecyl alcohol, $CH_3 . (CH_2)_{16} . CH_2OH$. The palmitic acid ester of cetyl alcohol, $C_{16}H_{33}OH$, from spermaceti, or cetin, has long been known to occur in the cranium of the sperm whale. The palmitic acid ester of myricyl alcohol ($C_{30}H_{61}OH$) is found in beeswax. Analogous combinations are widely distributed in nature. In only a few cases, however, has their identification been positive.

While triolein and the glycerides of the lower fatty acids constitute the major part of the vegetable fats, tripalmitin and tristearin predominate in animal fat. An exception occurs only in the fat supply of cold-blooded animals, which is very rich in triolein, as a consequence of which it remains in a fluid state at temperatures which would cause solidification among

[1] Hofmeister's Beiträge, **5**, 110 (1904).

warm-blooded animals. Among the latter there are many differences, depending on the species. For instance, human fat melts at about 25° C., mutton-tallow at about 50° C., and fat of the horse at about 65° C.

The fats decompose according to their composition, by taking on water, into fatty acids and glycerol. This hydrolysis can be accomplished by various agents, for instance by boiling with dilute mineral acids, or with alkalies especially in the presence of alcohol, and, above all, by the action of specific and widely distributed plant and animal ferments known in this case as lipases. The process of decomposing fats is called *saponification.*[1] If this be accomplished by the action of free bases, we do not obtain free fatty acids, but their salts. These are called *soaps.* Although the sodium and potassium soaps are easily soluble in water, the fatty acid salts of the alkaline earths (calcium, barium, and magnesium soaps) are insoluble. The neutral fats are perfectly insoluble in water. If we shake them long and vigorously with water, we obtain an emulsion, which, however, soon disappears, while the fat separates again on the surface of the water. Absolutely neutral fats, i.e., fats which do not contain a trace of free fatty acid, cannot be emulsified even by shaking with dilute, alkaline-carbonate solutions. When any free fatty acids are present, however, an extremely fine, permanent emulsion is obtained.

With few exceptions fats are of little influence in plant economy,[2] being of far less importance than the carbohydrates. As a whole, they form reserve material. In seeds, like *Helianthus* and *Curcubita*, we notice the stored-up fat quickly disappears on germination. Concurrently we observe an appearance of free fatty acid. A splitting of fat occurs, as was first shown by Sigmund,[3] by the action of a specific ferment, *lipase*, also called *steapsin.* More recently, extensive investigations have been made on the very active lipase occurring in the seed of the castor-oil plant.[4] This ferment is active in a weakly acid solution. The saponification of the fat evidently changes it into such a condition that it can penetrate into the protoplasm, and through the cell-walls. Small amounts of fatty acid in the presence of alkali suffice to produce an emulsion. It has been demonstrated experimentally that these extremely minute globules are capable of penetrating the cell-membranes, although it has not been decided definitely how large a quantity of the neutral fat is saponified. Again, nothing definite is known at present concerning the further behavior of the fatty-acids, though Sachs has shown that their disappearance coin-

[1] J. Gad: Arch. Anat. Physiol. **1878**, 179–187.

[2] Cf. F. Czapek: Biochemie d. Pflanzen, vol. i, pp. 115–126. Jena, 1905.

[3] W. Sigmund: Sitzungsber. d. Wiener Akad. **99**, 407 (1890); **100**, 328 (1891); **101**, 549 (1892).

[4] W. Connstein, E. Hoyer, and H. Wartenberg: Ber. **35**, 3988 (1902). Connstein: Arch. Anat. Physiol. **1905**.

cides with an increase in the amount of carbohydrates. We shall come back to this point later on.

Let us now follow the course of the fat, which is received by the animal organism in its food, as it passes through the alimentary canal and through the paths of absorption. Fat passes the entrance to the alimentary tract (i.e., the mouth) in a perfectly unaltered condition. Saliva has no action upon it, and, although saponification begins in the stomach, the extent to which this is accomplished is still much in dispute.[1] The digestion of fat while in the stomach is of small moment, for the action of the ferment is soon lessened, and eventually stops entirely, on account of the acid reaction of the stomach juices. The lipase requires, moreover, for its maximum efficiency that the fats shall be in an emulsified state, a condition which is rarely fulfilled in the stomach. The action of the gastric juices is, however, of indirect importance, because the fat of meat is set free by the digestion of the connective tissue. For these reasons there is no absorption worth mentioning of the fats while they remain in the stomach. The real digestion of fat sets in when it reaches the intestine. Here, first of all, it undergoes a purely physical change. By the action of the free fatty acids, and on account of the presence of the alkali salts in the intestinal and pancreatic juices and the bile, the fat is first of all subdivided. The fatty acids, which are deposited everywhere between the tiny particles of fat, react with the alkaline carbonates present. Soaps result which now tear the tiny particles apart, making them still finer, a process which is assisted by the carbon dioxide set free by the neutralization. An emulsion is formed. The purpose of this process may be twofold. We can imagine that the epithelial cells of the intestine absorb these fine globules directly in the same manner as is done by the plant cells. Again, it is perfectly possible that the chief advantage of the emulsion is to present an enormously large surface to the lipase, thus facilitating its action.

We are absolutely certain that ingested fat is decomposed into fatty acids and glycerol. The fatty acids unite as much as possible with the alkali present, thus forming soaps. A question yet to be settled is the extent of saponification; i.e., how much of the total fat in the food is entirely decomposed. Although Pflüger[2] assumes that the hydrolysis must be complete, i.e., that only fatty acids and glycerol are available for assimilation, other investigators believe that only a portion of the fat is

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[1] Cash: Arch. Anat. Physiol. **1880**, 323. Ogata: *ibid.* **1881**, 515. Volhard: München. med. Wochschr. **5** and **6** (1900); Z. klin. Med. **42**, 414; Verhandl. deut. Naturf. Aerzte, **1901**, II, 2d half p. 43. A. Zinsser: Hofmeister's Beiträge, **7**, 31 (1905). A. Fromme: *ibid.* **7**, 51 (1905).

[2] Pflüger's Arch. **80**, 111 (1900); *ibid.* **81**, 375 (1900); *ibid.* **82**, 303 (1900); *ibid.* **85**, 1 (1901); **86**, 1 (1901); **89**, 211 (1902).

saponified, while another portion, consisting of finely divided globules, is absorbed in this state. In spite of numerous investigations a clear conception of fat digestion is not yet at hand. Radijewski,[1] in investigations with dogs, showed that soap can be assimilated, and converted into fats. To show that a complete hydrolysis of fat occurs, the work of Connstein[2] has been cited. He fed a dog with lanolin. This is not saponified by the usual saponifying agents, although it forms an extremely fine emulsion when rubbed up with water. 97.5 per cent of the lanolin administered in this form was evacuated unchanged, in the fæces.

Many fats require complete decomposition to make them available for assimilation. Such fats are the ones whose melting-points lie above the body-temperature. I. Munk[3] has shown that 90 per cent of mutton-tallow, melting at 49°, was utilized by dogs. Spermaceti, melting at 53°, was also assimilated. The rapidity and extent of digestion are certainly influenced by the melting-points of the fats. The fats of lowest melting-points are utilized soonest and in the largest quantities.

Microscopic investigations have been made in the attempt to estimate the extent of the fat decomposition in the alimentary tract, by determining at what place in the epithelial cells and walls of the intestine the fat is present as such. In the first place we must state that the walls of the intestine are a large factor in the assimilation of fats. In it the fatty acids and glycerol molecules are reunited so that large quantities of fatty acids and glycerol are prevented from entering the body. I. Munk[4] has shown this very clearly. He fed dogs with pure fatty acids, and found an increase in the neutral fat, but no free fatty acid in the lymph taken from the thoracic duct.

That the synthesis takes place in the walls of the intestine is certain from the investigations of Perewoznikoff.[5] He fed fatty acid and glycerin to a fasting dog, and obtained in the epithelial cells of the intestine the same microscopic appearances as when he administered fats. Will[6] and C. A. Ewald[7] even observed a fat formation from glycerol and fatty acids in a dissected intestine. Microscopical studies of fat assimilation have not given uniform results. Some observers have found the basal edges of the intestinal epithelial entirely homogeneous and free from fat globules during the absorption of the fats, and could indicate their presence only

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[1] Virchow's Arch. **43**, 268 (1868).

[2] Connstein: Arch. Anat. Physiol. **1899**, 30; Die med. Woche, No. 15, 1900.

[3] Virchow's Arch. **95**, 407 (1884). Arnschink: Z. Biol. **26**, 434 (1890). O. Frank, Arch. Anat. Physiol. **1894**, 308. F. Müller: Z. klin. Med. **12**, 45 (1887).

[4] Virchow's Arch. **95**, 431 (1884); *ibid.* **123**, 230 and 484 (1891).

[5] Zentr. med. Wissensch. **1874**, 851.

[6] Pflüger's Arch. **20**, 255 (1879).

[7] Arch. Anat. Physiol. **95**, 407 (1884).

in the second third of the cells; other investigators[1] have noted globules in the basal edge. The results do not enable us to arrive at any decision. It has also been stated that an active ingestion of unchanged fat globules takes place.[2] The epithelial cells are supposed to send out protoplasmic processes which surround and absorb the fat globules. The leucocytes have also been credited with a direct activity in the assimilation of the fats. They are believed to migrate in the intestinal lumens and saturate themselves with fat. Finally, there exists the possibility that other methods may exist for fat assimilation. Aside from the fat absorption by the cells, there remains the possibility of intercellular absorption.

For the time being, we can only state with certainty that fat is emulsified in the intestines, and that there is always a decomposition of fat into glycerol and fatty acids. The only question is as regards the amount of fat saponified. Undoubtedly the decomposed fat is recombined in the walls of the intestine, so that only neutral fats are introduced into the organism.

Many factors participate in the process of fat absorption. One of the most important is the pancreas. It furnishes, on the one hand, the alkaline fluid which is so necessary for the emulsification of the fats, and, again, it supplies the fat-splitting ferment, which produces the saponification. If the amounts of the pancreatic juice be diminished, either by extirpation of the gland, or through ligating the ducts of the pancreas, an appreciable reduction in fat absorption takes place. It is not entirely abolished, for Abelmann[3] has shown an absorption of 28–53 per cent of milk-fat under these conditions. Sandmeyer has shown that, if dogs whose pancreatic glands have been removed are fed with finely chopped pancreas, the amount of fat absorbed can be increased.

It is still a question whether the absence of lipase, which results on the removal of the pancreatic juice, is the cause of the diminished fat assimilation. If it be true that a copious emulsification is sufficient to cause a fat absorption, we must conclude that this can take place without the pancreatic lipase being necessarily present, because fatty acids are certainly set free in the stomach, and fats can also be decomposed by bacterial action. The formation of an emulsion might, on the other hand, tend to prevent the diminution in amount of the alkaline pancreatic juice. If we saturate an emulsion of fat with an acid, we observe that the emulsion

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[1] Cf. I. Munk: Zentr. Physiol. **14**, 6/7, 121, 152, 409 (1900). Heidenhain: Pflüger's Arch. **43**, Sup. 85 (1888). Kischensky: Zentr. allg. Path. u. path. Anat. Heft **1**, 1902; Beiträge z. path. Anat. u. z. allg. Path. **32** (1902).

[2] Cf. Zawarykin: Pflüger's Arch. **31**, 231 (1883). Heidenhain: *loc. cit.* L. V. Thanhoffer: *ibid.* **8**, 391 (1874). R. Wiedersheim: Festsch. 56 Versam. deut. Naturfors. Aerzte, 1883.

[3] Abelmann: Inaug. Diss. Dorpat, 1890. Cf. W. Sandmeyer: Z. Biol. **31**, 12 (1894).

gradually disappears. Large oil-drops are formed, which collect on the surface of the liquid. It is very probable that the diminished fat absorption is largely dependent on the reduced alkalinity of the pancreatic juice, on account of the acid contents of the stomach preventing the formation of an emulsion in the duodenum.

Although Teichmann [1] has shown that if the pancreatic duct of a dog be ligated the absorption of fat is not appreciably affected, this does not invalidate our assumption. The secretions in the small intestines of the herbivora occur in greater alkalinity than is the case with the carnivora. That milk-fat is even assimilated in the absence of pancreatic juice is possibly explained by the following circumstances. If milk is coagulated by means of rennet, and the coagulum then dissolved in pepsin-hydrochloric acid, we obtain a very stable, acid fat-emulsion. It is rather difficult to obtain a clear conception of the actual relations of fat assimilation, so long as the conditions of fat digestion are so little understood. It is, of course, possible that the stomach lipase continues to act in the duodenum even when the alkaline pancreatic juice diminishes, and in this way a part of the fat is decomposed. We are certainly not justified in concluding, from the fact that fat assimilation proceeds in the absence of lipase, that neutral fats are directly absorbed.

The bile especially is of great importance in the absorption of fats. Formerly, a direct influence on the intestinal epithelial cells was assigned to it. It was supposed to stimulate them to assimilation. The function of the bile, however, has been shown by Pflüger [2] to consist of the ability to produce solutions of the fatty acids and soaps. Large quantities of stearic and palmitic acids were dissolved by a mixture of bile and sodium carbonate. The cholates of the bile dissolve even the magnesium and lime soaps which are insoluble in water. That the bile exerts a considerable influence on the absorption of fat is shown by the following observations: Dastre [3] ligated the bile duct of a dog, and made a fistula between the gall bladder and the middle of the small intestine. With a diet rich in fat the lacteals showed a milky turbidity below this fistula. The bile acting alone does not seem capable of causing a fat assimilation, although it acts more in conjunction with the pancreatic juice. This can be shown very beautifully by an experiment upon a rabbit. In this animal the bile duct joins the small intestine about ten centimeters above the pancreatic duct. Between the junctions of these two passages the chyle vessels remain clear and transparent, even on a diet rich in fat. It is only below where the pancreatic juice enters that we notice the turbid milky streams of fat-bearing chyle.

[1] Inaug. Diss. Breslau, **1891.**

[2] E. Pflüger: Pflüger's Arch. **88,** 299, 431 (1902); **90,** 1 (1902).

[3] A. Dastre: Arch. phys. norm. et path. V. **22,** 315 (1890).

Let us follow the progress of the fat, that has been absorbed by the walls of the intestine, and which has undoubtedly been re-formed to some extent, in its further passage through the organism. If we examine the viscera of a fasting or starving animal, we observe the chyle proceeding in a transparent vessel from the intestine to the mesenteric lymphatics. We obtain an entirely different appearance if we feed the animal a diet rich in fat just prior to death. The lacteals are then plainly visible. They have become milky and opaque. If we investigate their nature, we find that they are permeated with fat, even if no fat, as such, but only fatty acids have been administered. In the latter case the glycerin was necessarily missing for a fat synthesis, which must therefore be provided by the organism in some other manner. If we make a fistula at the entrance of the thoracic duct into the vena anonyma of a dog, we can estimate the amount of chyle which escapes in a given period of time. In a mixed diet we do not observe any increase in the quantity of chyle. Its appearance only changes when the food contains fat. Although ordinarily transparent, it then becomes white and opaque. In this process of digestion the fats behave differently from other food materials, all of which are poured directly into the blood-stream, and thence conveyed to the liver. The chyle itself retains the fat in the form of a finely divided emulsion.

I. Munk and Rosenstein [1] observed in a girl, who was afflicted with a fistula of the thoracic duct, that over 60 per cent of the fat consumed flowed out of the fistula in less than twelve hours. Only about one twenty-fifth of the fat administered had been saponified. Certainly, all fats do not follow such an indirect course, for on feeding a diet rich in fat a direct transmission into the blood-stream occurs. If the thoracic duct is ligated, larger quantities of fat are carried into the blood. I. Munk and Friedenthal [2] found that after a liberal consumption of cream, the fat content of the blood increased to six times the normal. As much as this had passed into the blood, although only 32–48 per cent of the fat had been assimilated. Fat also appeared in the blood after administering fatty acids, about four-fifths of these having been converted into normal fat.

The amount of fat absorbed depends, as previously indicated, on its composition. For instance, 97.7 per cent of olive oil is utilized, and 97.5 per cent of fats, which melt at temperatures between 25–34 degrees (goose-grease and lard). On the other hand, 90–91.5 per cent of mutton-tallow, melting at 49–51 degrees, and only about 15 per cent of spermaceti melting at 53 degrees, are absorbed by human beings.

Pettenkofer and Voit [3] as well as Rubner [4] have studied the absorption

[1] Virchow's Arch. **123**, 230 and 484 (1891); Arch. Anat. Physiol. **1890**, 376 and 581.
[2] Zent. Physiol. **15**, 297 (1901).
[3] Z. Biol. **9**, 1 (1873). [4] *Ibid.* **15**, 115 (1879).

of fats. The former found that a dog, weighing 35 kilograms, assimilated 98 per cent of 350 grams of fat administered in a day. Rubner maintains that the intestine of a human being can absorb a like quantity. As a rule, however, 100–120 grams is about as much as the system can stand.

The blood also shows changes after the absorption of fat. Chyle, charged with fat, is continually poured into the blood-stream through the thoracic duct. The blood, and especially its plasma, quickly shows an increased fat-content. This is especially true if the fat absorbed is large in amount, and is indicated by a milky turbidity, of an otherwise clear plasma. Often a distinct separation of drops of fat on the surface can be obtained by placing such plasma in the centrifugal. Ultimately the excess of fat again disappears from the blood. The process by which this is accomplished has not yet been demonstrated. The globules of fat do not migrate through the capillary walls. It is possible that the leucocytes have some function here. At one time it was considered certain that the blood contained a lipase. Why it should be present, however, was not clear, because, through the syntheses in the intestine, the organism protects itself against free fatty acids. Why, then, should the fat be decomposed again in the blood? This hypothesis has been abandoned, but on the other hand the investigations of Connstein and Michaelis [1] have shown that blood possesses the ability to transform fats into unknown substances, which are soluble in water and capable of dialysis. This process is dependent upon the presence of oxygen, and seems to require the interaction of the red corpuscles. A part of the absorbed fat is taken directly to the tissue-cells and consumed.

The unused fat is stored away as such. This is evident from the following experiments. Franz Hofmann [2] allowed a dog to fast until it was devoid of fat. The beginning of this period can be ascertained by estimating the amount of excreted nitrogen. The starving animal utilizes its stores of glycogen and then of fat, keeping its albumin intact as long as possible. If the fat supply is used up, a rapid decomposition of albumin takes place. The nitrogen elimination increases immediately. This occurs in from four to six weeks. Hofmann then fed the animal under inspection with considerable bacon and little meat. The amounts of fat and albumin given were accurately determined. It was found that in five days this dog assimilated 1854 grams fat, and 254 grams albumin; and stored up 1353 grams fat. This proved that fat in food is utilized to increase the fat supply in the body. Pettenkofer and Voit [3] reached the same conclu-

[1] Sitzungsber. Akad. Wissensch. zu Berlin, 771, 1896; Pflüger's Arch. **65**, 473 (1897); **69**, 76 (1897). Cf. Ergebnisse d. Physiologie, **3**, 1, 194.

[2] F. Hofmann: Z. Biol. **8**, 153 (1872).

[3] *Loc. cit.* Z. Biol. **9**, 1 (1873).

sion by another method. They determined the amounts of excreted products from a dog, which had been given a liberal fat diet, with but little meat. They found that all the nitrogen appeared again in the excretions, but that all the carbon did not do so. I. Munk [1] could also obtain storage of fat in a starved dog, by fats, or even fatty acids.

The proof that nutrient fat and stored-up fat possess direct interrelations, has also been obtained in still another manner. For fourteen days I. Munk [1] fed a dog, weighing 16 kilograms after nineteen days' fasting, on fatty acids obtained from mutton-tallow. The weight of the animal, which had been reduced 32 per cent during the previous fasting period, then showed an increase of 17 per cent. On dissecting the animal, a very large fat addition was noted. On " trying out " this, about 1100 grams of fat were obtained, which was solid at room temperature, and melted at 40° C. It is well known that mutton-tallow melts at this temperature, while fat from a normal dog would possess a far lower melting-point (about 20° C.). I. Munk used rape-seed oil in a second experiment. A fat was obtained which melted at 23° C. while at 14° C. a granular crystalline deposit separated. The fat obtained showed 82.4 per cent oleic acid, and 12.3 per cent fatty acids. Normal dog-fat contains only 63.8 per cent oleic acid, and 28.8 per cent solid acids. Rape-seed oil contains erucic acid ($C_{22}H_{42}O_2$). This was isolated from the above dog-fat.

It is very remarkable, that a food-stuff, and especially a vegetable one, should determine the composition of an animal tissue. We shall see later, that the decomposition of the organic food-stuffs not only makes it possible for them to be absorbed, but also enables the organism to select the material necessary for its own development.[2] In fact, the fatty tissues, together with glycogen, maintain a distinct individuality when compared with the other substances of the tissues. Both are reserve-materials which the organism stores up, in order to utilize them when needed. We do not, however, desire to place glycogen and fat in the same category. Glycogen is of far more importance in metabolism, than is fat in the true fatty tissues. It is continually being used up in metabolism, and also being constantly redeposited. The fat supplies in the liver, and possibly in other organs also, act similarly to glycogen. They likewise undergo quick changes. The fatty-tissue proper, however, is a true tissue under ordinary circumstances. Aside from its function as a reserve material it serves for other purposes, e.g., a purely mechanical one (like the fat in the eye-socket, etc.), and again as a non-conductor of heat. It must not be inferred that the fat is deposited as a dead mass entirely protected from metabolism.

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[1] Arch. f. (Anat. u.) Physiol. **1883,** 273; Virchow's Arch. **95,** 407 (1884). Cf. G. Rosenfeld: Verh. d. 17, Kong. f. in. Med. **503** (1899).

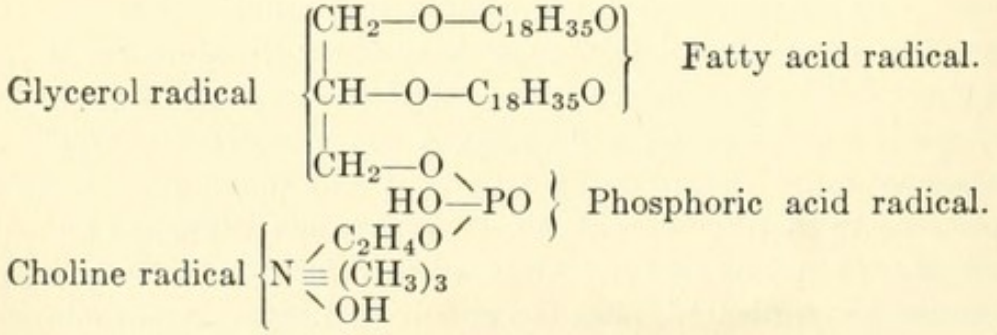
[2] Cf. E. Abderhalden: Zentr. Stoffwechsel- Verdauungskrankheiten, **5,** No. 24, 647 (1904).

On the contrary, on leaving the blood-stream in a diffusible condition, at present not understood, it is taken up by certain cells, and there converted into fat. The fat-cells themselves are supplied with an efficient resisting membrane. It withstands the action of alcohol and ether, and is not dissolved by acetic and dilute mineral acids. The fat-cells also contain a yellow color principle. Like all cells they contain protoplasm, for which, however, there is only a limited space if the fat supply be large. The fatty tissue is closely interwoven with that of the network of blood-vessels, so that this valuable material may be quickly utilized when necessary. We do not know how this reserve material is liquefied. It has not yet been determined in what form the fat leaves the cell and enters the blood-stream. Nervous influences undoubtedly control these large fat supplies, so that the fat cells are in this way kept in unbroken relations with the general metabolism. It is not improbable that the fat-containing cells of the connective tissue (i.e., the protoplasm which they contain) play in the metabolism of the fats, a rôle similar to that taken by the cells of the liver in carbohydrate metabolism. As the latter build up glycogen from grape-sugar and so protect the excess of carbohydrate from oxidation, and in case of need either directly or indirectly bring about cleavage, so the fat-cells withdraw from the blood the excess of valuable fat material, retaining it for a time, in order to set it free again for oxidation at the required moment. The animal organism has an efficient supply of reserve-material in the fat. It possesses double the calorific value of carbohydrates and of proteins. One gram of albumin gives 4.1 calories, one gram carbohydrate gives likewise 4.1 calories, while one gram fat, on the other hand, gives 9.3 calories. In fasting, the fat supply is very quickly drawn upon. Ordinarily the normal organism keeps this supply very constant. Gradually, equilibrium is established to a certain extent between the amount of the food which is used as fuel and that used for the replacement of tissue. This, of course, applies only to the mature organism. In man, frequently, this equilibrium is disturbed. More fat is in many cases being added constantly, so that the fat deposits grow far beyond the physiological requirements, and finally a condition results which is variously known under the names of adiposity, obesity, and polysarchia. It is impossible to distinguish sharply between such *fleshiness* and a physiological reserve supply of fat. Only when difficult breathing and faulty heart-action are indicated, does the condition become a pathological one. The causes of obesity are unknown. Various conditions can lead to this same result. There is unquestionably a *physiological* obesity, which arises from a rich diet, and a *pathological* obesity, which occurs in spite of all precautions. The latter form belongs to the class of metabolic derangements, and can be traced to an abnormal metabolism of the cells. It is certain, that, until we are better acquainted with the subject of physiological

fat assimilation, we cannot get an exact explanation of the causes of obesity. We are confronted by a condition which leads to many secondary symptoms; above all we have to remember that a very large amount of tissue has to be supplied with blood so that unusual strains are placed upon the heart. The condition of those afflicted with obesity undoubtedly depends upon their ability to satisfy these demands.

The functions of fats in the animal organism are not restricted to the part that they play as direct or indirect nourishment. In the growing individual the fat which is in the cells, and also otherwise distributed in the true fatty tissues, and the substances which are closely related to it, play a part the importance of which we cannot yet fully estimate. We are acquainted with many materials which are absorbed in a water-soluble condition, and in this form they penetrate the cells. On the other hand, we also know of many substances which are entirely insoluble in water, but which are, nevertheless, easily taken up. The fats may in these instances act as solvents. Although a substance is soluble in water, it may be more so in oils; and this property may exert an influence on cellular assimilation, and permit the cell to exercise a selection according to its constitution and condition. This suggestion has been made by H. Meyer [1] and by Overton [2] to explain the action of certain narcotics. It is possible, and in fact, even probable, that such relations are important for cell-metabolism, under ordinary circumstances.

Closely related to the fats, and the functions which we have just considered, is the *lecithin group*. These, also, are combinations of glycerol with fatty acids. Here only two hydroxyls are substituted by fatty acids in the tri-atomic glycerol, while the third is replaced by a phosphoric acid molecule which is also combined with the base, choline. The following formula gives an idea of the constitution of lecithin, also called distearyl-lecithin:[3]

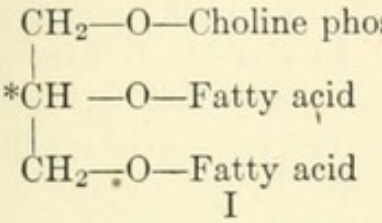


[1] Arch. exp. Path. Pharm. **42**, 109 (1899).

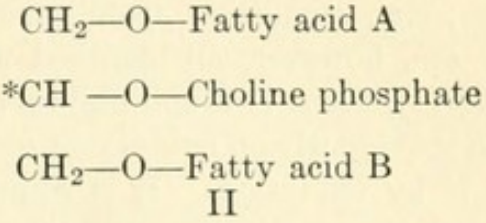
[2] Studien u. d. Narkose, etc., Jena, **1901**. Cf. also H. J. Hamburger: Osmotischer Druck u. Ionenlehre in d. Medicin. Wiesbaden, J. F. Bergmann, 1904, vol. iii, 242.

[3] Cf. Diakonow: Zent. med. Wissensch. **1868**, 438. F. Hundeshagen: J. pr. Med. **28**, 219 (1883). E. Gilson: Z. physiol. Chem. **12**, 585 (1888). A. Strecker: Ann. **148**, 77 (1868).

On saponification with alkalies, we obtain fatty acids, glycerol, phosphoric acid, and choline. Dilute acids have little action on lecithin. The fatty-acid component varies. We are acquainted with lecithins containing stearic, palmitic, and oleic acids. Even two different acids may participate in the constitution. We have not yet succeeded in preparing lecithin synthetically. As it is optically active, it must contain an asymmetric carbon atom. We are justified in making certain deductions regarding the method of grouping of the glycerol and combined radicals, as indicated by R. Willstädter and Karl Lüdecke.[1] The following formulæ are possible ones:



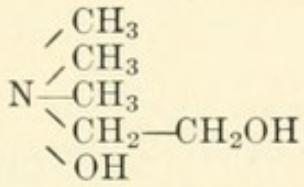
I



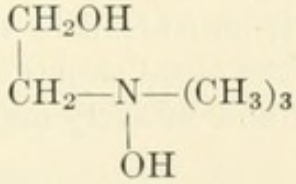
II

Formula II only contains an asymmetric carbon atom when the two fatty acids are different. The investigators mentioned decided in favor of formula I, because they succeeded in obtaining an optically active glycerophosphoric acid by hydrolysis. This is only possible when the molecule has the following grouping: $HO \cdot CH_2—\overset{*}{C}H \cdot OH—CH_2 \cdot O \cdot PO_3H_2$.

The base choline is of much interest. It is a quaternary-ammonium base, and has the following constitution:



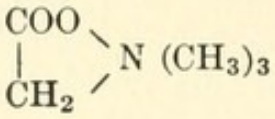
It is, therefore, to be considered as trimethylhydroxyethylammonium hydroxide. Wurtz[2] proved this by synthesis. He combined ethylene oxide, C_2H_4O, trimethylamine $N(CH_3)_3$ and water. Choline can also be derived from glycol, as shown by the following formula:



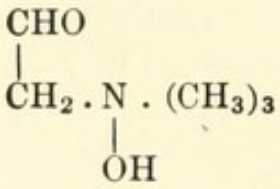
[1] R. Willstätter and K. Lüdecke: Ber. **37**, 3753 (1904).

[2] Ann. Sup. **6**, 116 and 197 (1868). Cf. M. Krüger and P. Bergell: Ber. **36**, 2901 (1903).

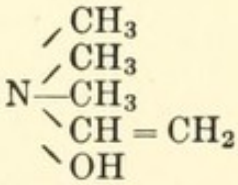
In aqueous solution choline breaks down into glycol and trimethylamine. It has also been found in a free state in plants. It is closely related to another base, also found in plants, and especially in sugar-beets, known as betaïne, or oxyneurine. Its formula is :



It has been obtained from choline by oxidation. Other bases have been isolated from various plants, which in part have been given characteristic names; e.g., amanitine, from toad-stools; fagine, from buchu seeds, etc. They are, however, all identical with choline. In toad-stools (*Amanita Muscaria*), there is found besides choline, another base called muscarine,[1] which is evidently an oxidation product of choline, and can also be obtained from it by oxidation. It is commonly considered to be an aldehyde, although its constitution has not yet been established positively:



Closely related to these is neurine, which has been isolated from the brain by Liebreich.[2] Its composition is that of trimethylvinylammonium-hydroxide :



The second component of lecithin, the glycero-phosphoric acid, is easily produced by uniting glycerol and phosphoric acid.

The lecithins are widely distributed in the plant and animal kingdoms. We could truly say that every cell contains lecithin. It occurs particularly in animal tissues, in the brain, nerves, fish-eggs, yolk of eggs, and in spermatozoa. It is also found in the muscles and the blood (in the plasma as well as in the blood corpuscles)[3] in the lymph and leucocytes; in fact, in every cell and in every organ. We find lecithin very widely

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[1] O. Schmiedeberg and E. Harnack: Arch. exp. Path. Pharmak. **6**, 101 (1877).

[2] Ann. **134**, 29 (1865).

[3] Cf. E. Abderhalden: Z. physiol. Chem. **25**, 65 (1898).

distributed in the vegetable world, more especially in seeds. During germination the lecithin content increases.[1]

In digestion, lecithin acts in an analogous manner to the fats; in fact, it resembles these very closely in every respect. It forms an emulsion with water. It partly resembles a colloid. Lecithin is decomposed by lipase into glycero-phosphoric acid, free fatty acids and choline; it is not certain that the decomposition of lecithin in the alimentary tract is complete, nor that unchanged lecithin can be directly absorbed. It is rather to be assumed that its components are separately turned over to the organism for further use.

The wide distribution of lecithin leads us to conclude justly that it is of great importance to the animal organism. We, however, know little about its function at present. From its constitution we can indeed assume that it acts as an intermediary body between various groups of compounds. We easily recognize its relation to the fats, from which it perhaps derives two components, the fatty acids and glycerol. On the other hand, lecithin evidently acts as a bridge to the very important nucleïns. It is possible that lecithin plays a leading part in the internal metabolism of the cells. To a certain extent it represents the fat of the cells. Furthermore, it unites the inorganic foods with the organic ones. The nucleïns possibly obtain their phosphoric acid from lecithin.

We do not know anything at present concerning the occurrence of lecithin in the organs. It may be there in the free state, or it may enter into numerous combinations. Many lecithides have been described, but as lecithin has the property of readily enclosing other substances, e.g., albumin, all such claims should, for the moment, be regarded with considerable skepticism.

The following experiments [2] may possibly give us some conception of the functions of lecithin, even if only indirectly. If we remove every trace of serum from the blood corpuscles by means of a centrifugal machine, and careful washing with physiological sodium chloride solution, the corpuscles are not dissolved by the cobra poison of the Naja snake, when suspended in an isotonic sodium chloride solution. The process of dissolving the blood corpuscles in such a way is called *hemolysis*, and the poisons causing this are *hemolytic*. If the serum is not separated from the blood corpuscles they immediately go into solution on adding cobra poison; i.e., the hemoglobin diffuses from the blood corpuscles into the surrounding medium. We can show the influence of serum in a better way by taking thoroughly-washed blood corpuscles, suspending them in a

[1] Cf. E. Schulze and A. Lickiernik: Z. physiol. Chem. **15**, 405 (1891).

[2] Cf. S. Flexner and H. Noguchi: J. Exp. Med. **6**, No. 3 (1902). P. Kyes: Berl. klin. Wochensch. 38/39 (1902), Nos. 2–4 (1903); Z. physiol. Chem. **41**, 273 (1904). E. Abderhalden and Le Count: Z. exp. Path. Therap. **2**, 199 (1905).

sodium chloride solution, and adding only one drop of serum to this, after having previously shown that cobra poison alone had not caused hemolysis. S. Flexner and H. Noguchi, who first observed this fact, and noticed it also with other poisons (*tetanustoxin*, *solanin*, *saponin*, etc.), rightly concluded that some substance was undoubtedly present in serum, which made it possible for the cobra poison to act on the hemoglobin of the corpuscles. P. Kyes then succeeded in showing that lecithin could be substituted in place of serum. Minute traces are sufficient to cause hemolysis. Lecithin alone, when used in small quantities, does not act hemolytically, but lecithin and the cobra poison together do so. This is not the place to dwell upon this interesting biological phenomenon and its explanation. We must content ourselves with the knowledge that lecithin possesses the capacity of accelerating the activity of poisons. Many interesting questions are suggested by this fact. It is entirely possible that lecithin also acts as an accelerator in the animal cells, and even on the intracellular ferments. As a result of recent investigations we are forced to conclude that the ferments as a whole are not released from the cells in their active form, but that they require the influence of a second substance to develop their activity. With such an hypothesis we can easily explain the action of ferments in the cells.

To lecithin is ascribed a large influence in the construction of the cell-walls, and also in the resorption of the cells. What was said concerning the fat contents of cells is also applicable to this case. Lecithins act as solvents.

There is another substance, which, although not at all related to lecithin chemically, is like lecithin indispensable to all cells. This is **cholesterol.** Its various modifications are widely distributed in the vegetable kingdom. Vegetable cholesterins are designated **phytosterols.** In the animal organism it is found in all cells, in the blood, lymph, etc. It occurs in exceptionally large amounts in the brain and nerve tissues. In the gall bladder it often gives rise to the formation of calculi, although this is almost always a secondary effect, and a result of disease of the bladder (catarrh, etc.) It forms white, fatty-feeling crystals with a pearly luster. It is absolutely insoluble in water. Sometimes cholesterol occurs in the free condition, as in the blood corpuscles;[1] then again it forms *ester* combinations. For instance, it is united with fatty acids[2] in the blood. Schulze isolated an isomer of cholesterin from wool fat, called **isocholesterol.**[3]

The way cholesterol is formed is still unknown to us. We do not at present know its constitution. All that we knew up to within a short

[1] E. Abderhalden: *loc. cit.*

[2] K. Hürthle: Z. physiol. Chem. **21,** 331 (1895-96). E. Hepner: Pflüger's Arch. **1898,** 73.

[3] Ber. **5,** 1075 (1872), and **6,** 251 (1873).

time, is that the formula $C_{27}H_{44}O$, or $C_{27}H_{46}O$, may be assigned to it and that the molecule contains a double bond and also an alcohol hydroxyl. Recent investigations [1] have shown that cholesterol belongs to a group of chemical compounds widely distributed in the vegetable kingdom, but not hitherto found in the animal economy. Cholesterol is evidently a terpene. The animal organism, therefore, contains hydro-aromatic compounds.

At present, cholesterol is considered to occupy an isolated position in the animal kingdom. In the vegetable world we could easily understand its formation from the terpenes in a number of ways. From its constitution, it hardly seems possible that cholesterol originates in the animal organism. Animal cholesterol is undoubtedly vegetable cholesterol which has been utilized by the animal organism for its requirements. We know absolutely nothing about its decomposition in the animal body. Bondzyński and Humnickni [2] have isolated a substance similiar to cholesterol from human fæces, which does not possess a double bond, but has two atoms of hydrogen more than cholesterol. About one gram of this compound is excreted daily. It has been called " dihydro-cholesterol," or coprosterol. The reduction is undoubtedly brought about by the activity of putrefactive bacteria.

We know practically nothing of the significance of cholesterol in the animal organism. Its general occurrence leads us to conclude that it is of great importance in cell metabolism. We cannot possibly consider it as a decomposition product. We only know of one definite property of cholesterol. This relates to the hemolytic action of lecithin and cobra poison. We have seen that lecithin accelerates the activity of cobra poison. Conversely, cholesterol retards the action of lecithin. We have seen that if we add snake venom to blood corpuscles, suspended in water and freed from serum, no hemolysis results; when, however, a trace of lecithin is added, hemolysis quickly follows. If we then add a minute quantity of cholesterol suspended in methyl alcohol, the lecithin, which previously had caused the cobra poison to become active, is now without effect. The blood corpuscles are not dissolved. Lecithin and cholesterol occur in all cells, and especially in the blood corpuscles. It is probable that they also show their antagonism towards one another in these. We are acquainted with various kinds of blood, whose corpuscles are dissolved by cobra poison alone; others require the presence of lecithin. It is perfectly possible, and even probable, that lecithin is present in these different kinds of blood corpuscles in different states of combination,

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[1] A. Windaus: Ber. **36,** 3752 (1903); **37,** 2027 (1904); **37,** 3699 (1904); **37,** 4753 (1904). O. Diels and E. Abderhalden: *ibid.* **36,** 3177 (1903); **37,** 3092 (1904). Cf. also G. Stein: Inaug. Diss. Freiburg, 1905.

[2] St. Bondzyński and V. Humnicki: Z. physiol. Chem. **22,** 396 (1896–97); Ber. **29,** 476 (1896). Müller: Z. physiol. Chem. **29,** 129 (1900).

or that cholesterol is present in a different form, or perhaps to a different extent in one case than in the other. Perhaps when the cholesterol is in a combined state lecithin may act normally; and conversely, lecithin may be in some such state of combination that it is less active, so that in the different processes of the cell at one time lecithin acts freely, while at another it does not.

The terpenes, and especially the cyclic terpenes, are very widely distributed in the vegetable kingdom. Plant secretions are largely composed of these. We are acquainted with a large number of the members of this class. Limonene and pinene are most widely distributed. At present we cannot say anything [1] regarding their functions or their origin.

[1] Cf. F. Czapek: Biochemie der Pflanzen, G. Fischer, Jena, 1905, vol. ii, p. 658.

LECTURE VII.

ALBUMINS OR PROTEINS.

Elementary Composition. Simple Substances or Mixtures. Classification.

The albumins, or proteins, occupy a distinct position among our organic foods. They are indispensable, and cannot be replaced by either the carbohydrates or the fats. They are large factors in cell-formation, and possess just as important relations to the animal organism as do the carbohydrates to the plants. We shall see later that the animal organism obtains all its albumin requirements from the vegetables. With the herbivora this requirement is supplied directly; with the carnivora, indirectly.

The albumins present a well characterized group of compounds. They differ essentially from the carbohydrates and the fats in their elementary composition. Besides the elements C, H, and O, they invariably contain nitrogen, and, as far as our present knowledge is concerned, also sulphur. These five elements are found in proteins[1] in closely agreeing amounts. The carbon varies from 50–55 per cent, hydrogen from 6.5–7.3 per cent, nitrogen from 15–17.6 per cent, oxygen from 19–24 per cent, and the sulphur from 0.3–2.4 per cent. These figures, of course, mean but little, and give us no conception of the composition of the individual constituents of proteins. This fact must be thoroughly appreciated, because unfortunately many far-reaching conclusions regarding the constitution and identity of albuminous bodies have been made on the basis of the elementary analysis.

Before we proceed to discuss the composition of the proteins, we are confronted with the question: Are we justified in considering albumin itself as a well-defined, chemical individual? We shall see later that we are acquainted with a large number of different proteins, which vary according to their mode of formation, and, in part, their place of occurrence. They all possess the common characteristic of not being able to diffuse

[1] The term **proteid** has been used in English as the equivalent for **albuminous substances** (German, Eiweisskörper), although Hammarsten, Neumeister, and other European authors have designated as proteids what may be called "compound proteids." It has seemed best, however, to follow the German text and to designate the whole group as that of the proteins.— Translators.

through animal or vegetable membranes. They belong to that class of bodies designated by Graham [1] as "colloids." If the colloid be liquid, we call it a *sol;* while if it be solid, we designate it as a *gel.* Liquid and solid gelatin represent these two phases. If the colloid is distributed throughout water, in appearance practically dissolved, we call it in the hydrosol condition. We are acquainted with many such colloidal substances among the inorganic compounds. Silicic acid is a good example of this. If a solution of sodium silicate is treated with an excess of hydrochloric acid, the silicic acid set free, remains apparently in solution. If this is then transferred to a dialyzer, the excess of hydrochloric acid and the sodium chloride produced in the reaction diffuses into the liquid — in this case distilled water — which is placed on the other side of the membrane. The silicic acid, on the other hand, remains behind, in the form of a tough, viscous mass, which can be coagulated by introducing a few bubbles of carbon dioxide gas. "Albumin solutions" act in an analogous manner. If we transfer blood serum, which occurs as a pale yellow, clear liquid, to a dialyzer, a floculent precipitate quickly separates out. This is the globin of the serum, which separates, the salt which had held it in "solution," having been withdrawn.

A question widely discussed, is this : Is the colloid occurring in the sol form to be considered as an actual solution, or as a suspension? It is variously answered. As the albumin solutions conduct the electric current, the products in "solution" appear as both anions and cations, it has been assumed that an actual solution exists.[2] Nevertheless, there is no sharp dividing line between a real and an apparent solution. We are acquainted with all possible intermediate stages.[3]

As the colloids lose many of their characteristic properties by various agencies, so, also, the albumins are easily deprived of their colloidal nature. The process is called a "coagulation." It is irreversible. As we generally deal with the coagulated products in our investigations of the albumins, we shall devote a little space to discussing the ordinary methods employed in effecting coagulation. One of the most important characteristics of albumin solutions is that of coagulating on heating. One of the most important factors in the phenomenon of coagulation is the amount of salt held in solution. We can heat an albumin solution, which has been very carefully freed from salt by dialysis, and it will not coagulate. If salt is then carefully added, albumin separates

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[1] T. Graham: Philosophical Trans. **151,** Part 1, 183, (1861).

[2] J. Sjöqvist: Skand. Arch. Physiol. **5,** 277 (1895). S. Bugarsky and L. Liebermann: Pflüger's Arch. **72,** 51 (1898).

[3] We have suspensions, colloidal solutions, and true solutions. It is easy to distinguish between the end members of the series, but no sharp distinction is drawn between these three classes. — TRANSLATORS.

out. The reaction of the liquid is of the greatest importance. Complete precipitation of the albumin can only occur when the solution is *faintly* acid. Any excess of acid will hold some albumin in solution. Yet a coagulation will take place on heating. The coagulated albumin has combined with the acid. A so-called "acid-albumin" results. The presence of alkali will prevent the coagulation of albumin for the same reason. "Alkali-albuminates" are formed. The coagulated albumins are insoluble in water and in neutral salt solutions. The readily soluble combinations of albumins with alkali and acids can be precipitated by salts.

The coagulating temperature of the different albumins varies. Efforts have often been made to utilize this fact in separating the various albumins. The method is inefficient. For one thing the albumins in solution have different effects upon one another, and then, again, the coagulating temperature varies considerably with the composition of the solvent.

We are also acquainted with other methods of effecting coagulation besides that of heating. A number of albuminous bodies, for instance, globin, myosin, and fibrinogen, will go over into the gel form simply on standing. Many precipitants, like alcohol, acetone, metallic-salt solutions, etc., will also produce the same result. The time required to effect coagulation varies considerably. For instance, the albumins are not immediately changed to their insoluble forms, on being salted-out. They can be filtered, put into solution again, and in this way purified, provided that these processes are carried out in a short time. According to Ramsden,[1] coagulation has even been accomplished simply by shaking.

The most varied albuminous bodies assume very analogous physical, and even chemical, properties after being coagulated. They all form an amorphous powder, which is insoluble in water and salt solutions. We can easily appreciate the fact that such material when used as a starting-point for our investigations of the albumins gives little guarantee of purity or homogeneity. We know that the colloids possess the property of carrying down other substances from solution when they themselves are precipitated. This is also the case with the albumins. They also contain appreciable amounts of ash. It is not yet certain whether ash is an essential constituent of albumin or not; we do know that the amount can be decidedly diminished by dialysis or by other methods.

As a rule, when we are investigating the composition of a new substance, and finally its constitution, the utmost precautions are used to insure purity. To do this we usually resort to crystallization. By re-crystallizing, and fractional crystallization, adhering substances are removed. This accomplished, we analyse the substance in order to determine its composition. The size of the molecule is obtained by a molecular weight determination; and then by preparing a series of deriva-

[1] Arch. Anat. Physiol. **1894**, 517.

tives, decomposing the substance, and by other means, we arrive at the constitution of the body in question. We consider that the constitution of a substance is definitely settled only when, by synthesis, we succeed in reproducing the same substance. We must attempt to follow this same line of procedure in our investigations with the albumins. We now turn to the question of crystallizing the albuminous bodies. Crystals of albumins have been known for a long time. T. Hartig, in 1850,[1] noticed crystalline substances in gluten meal, which are called *aleuron* grains "protein granules," or "plant crystalloids." The albuminous nature of these crystals was established by Radlkofer.[2] They have been observed in many seeds; for instance, in pumpkin seeds, hemp seeds, ricinus seeds, and especially in the Brazil nut. A beautiful example of this kind of crystallization is presented by parasitical plants of the order *Orobanchaceæ*, the tooth-wort,[3] *Lathræa squamaria*. The cell kernels contain protein crystals. A vigorous discussion has arisen as to whether these substances are real crystals, or whether they only possess a crystalline appearance. They possess characteristics which do not correspond with those of true crystals. In the first place, these crystalline-appearing substances swell up under the influence of water, and also of dilute alkali. The refractive index of the crystal then diminishes. The crystalline form also changes, because it does not expand uniformly along its various axes. They are also partially soluble in glycerin. A solid homogeneous residue remains, which retains the form of the original crystal. There has been much discussion about this phenomenon. Fr. N. Schulz [4] has indicated an interesting analogous example of an inorganic crystalline formation. If human urine is allowed to stand for 24–48 hours with dicalcium phosphate, and then filtered, a precipitate of crystals, one-half mm. in size, appears when the liquid is allowed to evaporate of itself. The crystals are like honestone with ragged points, and they are strongly refractive (in polarized light, doubly refractive). If these crystals are treated with dilute acetic acid a part is dissolved. A crystal remains, however, which has the form of the original. It is now singly refractive towards polarized light, and has lost its former high refractive index. The dissolved portion is calcium phosphate; the remainder, calcium sulphate. It is possible that the protein crystals mentioned possess analogous characteristics. There is, however, nothing further known at present to warrant these substances being classed

[1] T. Hartig: Bot. Zeit. **50**, 881 (1850).

[2] L. Radlkofer: Ueber Kristalle proteinartiger Körper pflanzlichen und tierischen Ursprungs, W. Engelmann, Leipzig, **1859**.

[3] A. F. W. Schimper: Diss. Strassburg, **1878**; Z. Kristal. **1880**. F. N. Schulz: Die Kristallisation von Eiweissstoffen u. ihre. Bedeutung f. d. Eiweisschemie, G. Fischer, Jena, 1901.

[4] Fr. N. Schulz: *loc. cit.*, p. 4.

with normal crystals. Such products have also been observed in animal tissues. Thus, six-sided plates have been noticed in the intestinal epithelium of the meal-worm, *Tenebrio molitor*.[1] R. List [2] states that he has observed rhombohedrons and hexahedrons in the pigment cells of the radial nerves of *Sphærechinus granularas*, which gave the albumin reactions. The small yolk plates, as well as other rectangular and quadrangular plates obtained from the eggs of fishes and amphibora, also belong to this class. Such products have also been observed in the eggs of the roe [3] as well as in the epithelium of the testes in man.[4]

These discoveries do not lead to any decision regarding the crystalline qualities of the albumins. The fact that some of the products observed gave albumin reactions does not prove that they were albumins. Small impurities of albumin might have caused them. We would be but little benefited even if the fact should be established that these crystalline substances were albumins. The only value of crystals lies in possibility of recrystallization and purification.

As a matter of fact it has been found possible not only to obtain many of the albumins in crystalline form but many of them have also been recrystallized. Maschke [5] was the first to interest himself in this direction. By evaporating a saturated solution of *Bertholletia* (Brazil nuts), he obtained aleuron crystals in six-sided, tabular prisms.

Schmiedeberg [6] continued this work. The protein crystals were isolated from the Brazil nuts by washing them out with petroleum ether. They were then dissolved in distilled water at 30–35 degrees, and precipitated by passing carbon dioxide into the solution. The precipitate was redissolved by treating it with an excess of magnesium oxide at 30–35 degrees. By careful concentrating the solution, small crystals, of the size of poppy seeds, settled out. These contained 1.4 per cent MgO. Drechsel [7] improved this method considerably. Instead of evaporating he removed the water by dialysis with absolute alcohol.

After grinding a large number of seeds and removing the fat, octahedral crystals have been obtained by means of a five per cent salt solution at 60 degrees. They can be redissolved and again precipitated. Such crystals were obtained from cotton seed, hemp seed, and sun-flower seeds.

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[1] J. Frentzel: Arch. mik. Anat. **26,** 287; Berl. entomol. Zeit. **26,** 1882. W. Biedermann: Pflüger's Arch. **72,** 105 (1898).

[2] R. List: Anat. Anzeiger, **7,** 185 (1897).

[3] V. v. Ebner: Sitzungsber. Akad. Wissensch. zu Wien. **110,** part 3 (1901).

[4] Lubarsch: Virchow's Arch. **145,** 317 and 362 (1896).

[5] O. Maschke: J. prakt. Chem. **74,** 436 (1858).

[6] O. Schmiedeberg: Z. physiol. Chem. **1,** 205 (1877).

[7] E. Drechsel: J. prakt. Chem. **19,** 331 (1879).

It is a matter of great importance that albuminous bodies have been crystallized which do not exist in the crystalline form in nature. Hofmeister [1] succeeded in crystallizing egg-albumin by treating a given volume of egg-white with an equal volume of a cold, saturated solution of ammonium sulphate. The precipitate consisted of globulins, while the solution contained the albumins. By concentrating the filtrate from the globulins at the usual temperature, beautiful microscopic needles were obtained, which could be redissolved in a dilute ammonium sulphate solution, and obtained again by evaporation. The precipitation of these crystals can be greatly accelerated by making the solution faintly acid, by adding either dilute acetic, sulphuric, or hydrochloric acids.[2] Serum albumin has been crystallized in the same manner.[3] Albumin from horse-blood serum has also been obtained in crystalline form. We will mention the fact here, that other albuminous substances are said to have been crystallized, such as casein, lactalbumin, etc. We do not need to dwell longer on this subject, for the investigations are not very convincing.

We are acquainted with a protein which has itself not yet been obtained in crystalline form, although one of its compounds which occurs in nature can be crystallized easily. We refer to the coloring matter of the blood, which is a compound of the albumin globin, and another substance, hematin, which is not of an albuminous nature. Hünefeld [4] noticed a crystalline separation when blood was dried between two glass plates. Reichert,[5] however, is credited with being the true discoverer of oxyhemoglobin crystals. He observed them on the placenta of a nearly mature guinea-pig fœtus and also on the mucous membrane of the uterus of the mother. Oxyhemoglobin may be prepared in various ways. The most satisfactory method consists in centrifugalizing defibrinated horse-blood, pouring off the serum, and washing the paste of blood corpuscles with isotonic salt solution until perfectly freed from serum. The blood corpuscles are mixed with 2–3 times their volume of water at 30–35 degrees and the solution strained. In order to remove the stromata of blood corpuscles, the solution is cooled to 0 degrees, shaken with ether and one-quarter of the total volume of absolute alcohol, also at

[1] F. Hofmeister: Z. physiol. Chem. **14,** 165 (1889); **16,** 187 (1891).

[2] F. G. Hopkins and S. N. Pinkus: J. Physiol. **23,** 130 (1898). H. T. Krieger: Diss. Strassburg, **1899.**

[3] A. Gürber: Sitzungsber. physikal-med. Gesellsch. zu Würzburg, **1894,** 143. A. Michel: Verhandl. d. physikal-med. Gesel. zu Würzburg, **29,** 28, No. 3 (1895), and Diss, Würzburg, **1895.**

[4] F. L. Hünefeld: Der Chemismus in der tierischen Oxydation, F. A. Brockhaus, Leipzig, **1840.**

[5] B. Reichert: Arch. Anat. Physiol. **1849,** p. 197; **1852,** p. 71. Cf. Fr. N. Schulz: *loc. cit.* p. 23.

0 degrees.[1] The crystalline separation of oxyhemoglobin suddenly occurs after standing for some time on ice. As the solubility of the oxyhemoglobins varies according to their animal origin, it is necessary to use varying quantities of water for dissolving them. Thus, in order to obtain the oxyhemoglobin of the cat, it has been found convenient to use an equal volume of water in dissolving the blood corpuscles.[2] Crystals may also be obtained by salting out with ammonium sulphate, and dialysis with alcohol. Hemoglobin[3] and methemoglobin[4] can also be obtained in crystalline form.

Oxyhemoglobin can be redissolved in water at 37–40 degrees, and recrystallized by the addition of alcohol in the manner previously described. When obtained from different animals it crystallizes in various forms: thus the crystals obtained from the squirrel are hexagonal; those from the horse are orthorhombic.

Crystals from insect blood have also been described. H. Landois[5] has obtained crystals from the blood of caterpillars, *Pupidæ*, beetles, and wasps, simply by evaporation. It is questionable whether these were albumin crystals. Crystals have also been isolated from the red sea-algæ, *Rhodophyceæ*, or *Florideæ*. The *Cyanophyceæ* give a crystalline coloring matter called phycocyan. More exact knowledge regarding the components of these albumins is not yet at hand.[6]

Not only are all the external appearances of these crystals identical with those of real crystals, but crystallographical investigations have shown no differences. With the exception of those albuminous "plant crystals" which belong, in part, to the regular system, they are all doubly refractive towards polarized light. As a whole, however, only a few exact optical investigations of albumin crystals have been made.

We have intentionally devoted considerable space to the discussion of these individual crystals. It is a matter of the greatest importance to us in our investigations into the chemistry of albumins. Are we justified, or not, in characterizing a protein substance, as pure? Let us see what conclusions we can draw from the crystallizability of the proteins. The attempt has been made to decide whether a protein was homogeneous by means of an elmentary analysis. We have already shown that it is out of the question to decide the question in this way. It is a well-known

[1] Cf. F. Hoppe-Seyler: Med.-chem. Untersuch. Vol. 2, p. 181, 1867. O. Zinoffsky: Z. physiol. Chem. **10**, 16 (1885).

[2] E. Abderhalden: Z. physiol. Chem. **24**, 545 (1898). Cf. also Fr. Krüger: Z. Biol. **26**, 469 (1890), and Z. physiol. Chem. **25**, 256 (1898).

[3] Cf. G. Hüfner: *ibid.* **4**, 382 (1880).

[4] G. Hüfner and J. Otto: *ibid.* **7**, 65 (1882); **8**, 366 (1884). A. Jäderholm: Z. Biol. **20**, 419 (1884).

[5] Z. wiss. Zool. **14**, 55 (1864).

[6] Cf. H. Molisch: Bot. Zeit. **1894**, 177; **1895**, 131.

fact that the most varied albuminous substances have very similar elementary compositions. It is impossible to recognize the presence of any foreign albumin in a mixture by any such procedure. There is also the added objection, that the same mixture will invariably be obtained by following out a prescribed method.

It is very instructive in this direction that oxyhemoglobin crystals showing no indication of any admixture under the microscope, may, nevertheless, be impregnated with foreign protein.[1] This fact has been established through the discovery that glycocoll was present in a globulin from serum, whereas oxyhemoglobin does not show the least trace of this amino-acid. It has also been shown that glycocoll appears in the oxyhemoglobin of horse-blood, after one crystallization. Another recrystallization gives us a preparation entirely free from glycocoll. In this connection we would refer to the various descriptions of albumins containing carbohydrates, even when the observations were made with crystalline preparations.[2]

The crystallization of albumins does not correspond with the ordinary formation of crystals from other sources. Most of the albumin crystals are obtained by withdrawing the solvent. The production of these crystals does not gradually follow the removal of the solvent. The crystallization is, on the contrary, a very sudden one. This can be best illustrated by salting out with ammonium sulphate. A very slight excess is sufficient to throw out large quantities of crystals from an otherwise clear solution. It is certainly a matter of great importance that no albumin as such has yet definitely been isolated in a crystalline state. A possible exception would be that of the globulins, generally called *edestin*, separated from plant seeds. They contain a considerable amount of sodium chloride. It is impossible to remove this entirely, and still retain the crystalline form. Although we are still in the dark, regarding the influence of sodium chloride on the crystallization of edestin we do know that the egg-albumin and serum-albumin do not themselves crystallize, whereas their sulphates do, as was shown by K. A. H. Mörner.[3] The crystallization of the globin in hemoglobin depends on the presence of hematin.

When we remember the extreme difficulty experienced in obtaining absolutely pure crystals of substances of even low molecular weight, we can hardly expect to obtain really pure products through any methods which in themselves can give no guarantee of efficiency. Although we thoroughly appreciate the advantage of possessing the albuminous body in a crystalline condition, we likewise find it necessary to state that not the least confidence can be placed in the method of obtaining the crystals,

[1] E. Abderhalden: Z. physiol. Chem. **37**, 484 (1903).

[2] E. Abderhalden, P. Bergell and T. Dörpinghans: Z. physiol. Chem. **41**, 530 (1904).

[3] K. A. H. Mörner: Z. physiol. Chem. **34**, 207 (1901).

nor even the system of crystallization itself, as a guarantee of the purity of the individual substance. The only advantage that crystallization possesses is that it gives us a means of separating one product from the mixture, and possibly increasing its purity.

The fact that we have so far not succeeded in obtaining a single absolutely pure, individual, albuminous body, places the whole subject of albumin investigation in a very uncertain light. At every turn we meet this same unfortunate condition. We emphasize this point because a very large number of investigations in the domain of albumin chemistry have but little value for this reason.

This is especially true of the molecular weight determinations of albumin.[1] These have been carried out in various ways. The elementary composition has been investigated to see if this would establish anything, considerable attention having been given to the sulphur content. If albumin considered as "pure," contains one per cent of sulphur, then the molecular weight of the substance must be at least 3200 times that of hydrogen. This method gives us only the minimum value, as we have no means of knowing that only one atom of sulphur is present in the molecule. The amount of sulphur in the various albumins differs greatly. The following calculations have been made:[2]

	Sulphur in Per cent.	Molecular Weight: (with the assumption that each albumin molecule contains one atom of sulphur).
Edestin (crystallized)	0.87	3680
Oxyhemoglobin (horse)	0.43	7440
Serum-albumin (crystallized, horse)	1.89	1700
Egg-albumin (crystallized)	1.3	2460
Globulin	1.38	2320

Taking into consideration the fact that the albumin may contain more than one atom of sulphur, Fr. N. Schulz[3] has estimated the molecular weight of serum-albumin to be 5100, egg-albumin 4900, oxyhemoglobin 14,800, globulin 4,600, edestin 7,300.

The substituted albumins, especially hemoglobin, give us another method for estimating the molecular weight. Oxyhemoglobin contains, besides the albumin globin, a substance containing iron, called hematin. About 0.4–0.5 per cent iron is present in oxyhemoglobin. Every hematin molecule contains one atom of iron. It is generally considered that oxy-

[1] Cf. Fr. N. Schulz: Die Grösse d. Eiweissmoleküls, G. Fischer, Jena, 1903.

[2] Fr. N. Schulz: *loc. cit.* p. 17.

[3] Fr. N. Schulz: *loc. cit.* p. 29.

hemoglobin contains one hematin molecule and one molecule of globin, an assumption for which adequate proof is lacking. A percentage of iron of 0.4–0.5 per cent indicates a molecule of 14,000–11,200; a sulphur content of 0.43–0.67 per cent gives a molecule of 14,899–9500; and 4–5 per cent of hematin [1] one of 14,800–11,800.

Another method for estimating the molecular weight of proteins depends on the formation of metallic compounds.

Harnack [2] has shown that many proteins can be precipitated from solution by means of copper sulphate. We obtain a precipitate containing copper, called copper albuminate. Harnack obtained the following amounts of copper in the precipitates from egg-albumin: (I) 1.34–1.37 per cent Cu, and (II) 2.48–2.73 per cent Cu. Two different copper albuminates were formed therefore. We are not acquainted with the conditions governing the formation of one or the other compound. Copper albuminate (I) would have a molecular weight of 4700, while the second compound probably has the same value, if the assumption of Harnack is correct, that both albuminates represent the same protein substance, differing only in the fact that the first possesses one atom, while the second has two atoms of copper in the molecule.

Other metallic albuminates, such as those with silver, calcium, etc., have been prepared. It is difficult to state whether these are salt-like compounds, or not. Recent investigations on the colloids have indicated the necessity of being extremely cautious in passing judgment on such compounds. Zsigmondy [3] has called attention to a peculiar property of a colloidal gold solution, in the presence of albumin. A pure gold solution is coagulated by an addition of electrolytes; for instance, sodium chloride. If, however, albumin is present, the precipitation does not occur. The albumin protects the colloidal gold. Fr. N. Schulz and Zsigmondy [4] have found it possible to express the degree with which each individual albumin protects the colloidal gold numerically. Thus globulin, under certain conditions, can protect twenty times its weight of gold. If an albumin solution, mixed with a gold solution, is precipitated, the gold is dragged down. A homogeneous red precipitate is obtained. If the globulin be redissolved, the gold will likewise go into solution. It can easily be seen how such a behavior might lead one to believe that there is a compound of albumin and gold, and thus to erroneous conclusions. It is of great interest to know that crystallized egg-albumin also takes up gold, and that the mixture can then be recrystallized. Copper, iron, calcium oxide, etc., can also be held in colloidal solution by means of albumin.

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[1] Fr. N. Schulz: Z. physiol. Chem. **24**, 449 (1898).

[2] E. Harnack: Z. physiol. Chem. **5**, 198 (1881).

[3] R. Zsigmondy: Z. anal. Chem. **40**, 597 (1901).

[4] Hofmeister's Beiträge, **3**, 137 (1902).

These statements are sufficient to show how little value should be attached to the molecular weight determinations of such "compounds." In individual cases we are not able to decide at present whether there is an actual chemical combination, or whether the metal is merely held in solution. No better results have been obtained by using halogen substitution products for the molecular weights. We are still without the necessary knowledge and foundation for such work.

It might be thought that the cleavage-products could be utilized for determining the molecular weights. Unfortunately, we have not yet sufficiently perfected our methods to utilize any individual, characteristic, cleavage-product for such a determination. We must temporarily content ourselves with approximations.

If we take all the known facts concerning the size of the albumin molecule into consideration, and critically examine them, we must conclude that no definite statement can be made. It is, of course, possible that the molecular weight is as large as has been computed; on the other hand, it may be much smaller. It is, therefore, practically useless to assign definite formulæ to individual proteins as long as the methods of molecular weight determinations are still so indirect, and based upon so many unknown factors.

Direct determinations of the molecular weights of proteins have so far been unsuccessful. The raising of the boiling-point method is not applicable, because most of the albumins undergo changes on heating. Determinations made up to the present by means of the lowering of the freezing-point have not taken sufficiently into consideration the amount of ash in the proteins. They are, therefore, practically worthless.

Before discussing the decomposition products of the albumins, or considering the question of the constitution of the albumins, we will now devote a little attention to the various kinds of proteins as far as they can be characterized by our present methods. It is impossible now to classify the large number of known proteins in accordance with purely chemical principles. We are still forced to follow the old grouping. This classification is, however, only accepted as a matter of necessity. The farther we proceed into the chemistry of the albumins, the more we learn of properties which can be utilized to identify individual proteins. We can even indicate a prospective classification of the proteins according to their constituents in an objective and satisfactory manner. We shall presently see that they are essentially composed of amino acids. These are of very different kinds. We distinguish between the mono-amino and the di-amino acids. The relative amounts of these two groups of amino acids vary considerably in the different proteins. We know of proteins like silk, elastin, etc., which are mainly composed of mono-amino acids, the di-amino acids being of little importance. On the other hand, we have the prot-

amines, which are almost entirely built up of di-amino acids. There are many intermediate stages between these two groups; thus, the histons contain more di-amino acids than the above substances, which are rich in mono-amino acids, although less than the protamines. The common proteins, albumin, globulin, etc., occupy an intermediate position between the silk, elastin, etc., group and the histons. In this way we may classify the proteins as follows:

1. Proteins with less than 10 per cent of di-amino acids — elastin, silk, etc.
2. Proteins with about 10 to 15 per cent of di-amino acids — serum-albumin, serum-globulin, casein, etc.
3. Proteins with from, say, 20 to 30 per cent of di-amino acids — histon from the thymus.
4. Proteins with larger amounts of di-amino acids (sometimes 80 per cent or more) (protamines: salmine, clupein, etc.).

Our present knowledge is too inadequate for us to classify all proteins in this way. The boundaries of the different groups are not sharply defined, and we observe all sorts of intermediate stages between them. Furthermore, there is no doubt but that a member of one group may be transformed while in the tissues so that it belongs to a different group. F. Miescher [1] has called our attention to an interesting example of such a transformation. It is well known that, as the spawning season approaches, the salmon journeys from the ocean into fresh-water streams. During the entire period of several months in which it remains in the fresh water, the fish eats nothing. On leaving the salt water it is a powerfully muscular fish. These muscles are required for the stemming of strong currents. Its sexual organs — testes and ovaries — are immaturely developed. Gradually, however, the large lateral-dorsal muscle becomes smaller, while the sexual organs assume large dimensions. There can be no doubt but that the latter develop at the expense of muscular tissue. The mature testis contains a protein rich in di-amino acids, known as salmine. There is but little of this substance present in the immature organ. It then consists chiefly of a histon-like substance. Histons, as a rule, do not occur in any considerable amount in the muscles. It is very probable that the protein in the muscles of the salmon loses di-amino acids, thus increasing the proportion of di-amino acids in the protein, eventually producing protamine with the intermediate formation of a histon. It is highly interesting for the development of our knowledge concerning the metabolism of proteins that we should study such relations further.

There is no question but that we shall shortly be able to classify the proteins according to chemical principles. We must determine in the

[1] Die histochemischen und physiologischen Arbeiten von Friedrich Miescher.

first place what amino acids take part in their formation, and then eventually stereochemical studies will decide the question. We have to depend at present chiefly upon physical differences. Although many proteins may be fairly well characterized in this way, by their solubility in water, in salt solutions, etc., there are many others in which this is not the case.

The proteins, as a whole, may be first of all separated into two main groups: — (1) The Simple Proteins, and (2) The Compound Proteins, or Proteids. The first group is subdivided into the *true albumins* and the so-called *albuminoids*. The true proteins comprise (*a*) the albumins (serum-albumin, egg-albumin, and lactalbumin, etc.); (*b*) the globulins (serum-globulin, egg-globulin, lactoglobulin, and cell-globulin); (*c*) the plant-globulins and plant-vitellins; (*d*) fibrinogen; (*e*) myosin; (*f*) phosphorized proteins, or the so-called nucleo-albumins (casein, vitellin, nucleo-albumins of cell-protoplasm); (*g*) histons; (*h*) protamines. While these groups are fairly well distinguishable, as we shall soon see, the following group, which likewise belongs to that of the simple proteins, is characterized more from a morphological point of view. The albuminoids include (*a*) collagen; (*b*) ceratin (from hair, feathers, horn, etc.); (*c*) elastin; (*d*) fibroin (from silk); (*e*) spongin and conchiolin; (*f*) amyloid; (*g*) albumoid and perhaps the melanins.

To the compound proteins, or proteids, belong nucleoproteids, hemoglobin, and glucoproteids.

In the following pages we shall merely devote space enough to such description of the individual proteins as seems absolutely necessary for later discussion. Those interested are referred to the special works on the subject.[1]

We will first take up the simplest albuminous substances, the simple proteins. The albumins and globulins comprise a well-characterized group. They are generally found together; for example, in blood-serum, milk, and the whites of eggs. The albumins are soluble in pure water. If blood-serum is dialyzed against distilled water, a precipitate will form on standing. This is globulin, which had been held in solution by neutral salts. Precipitation follows as the latter diffuse. The albumins, on the other hand, remain dissolved. They are also soluble in dilute salt solutions, as

[1] An exhaustive description of the individual proteins is found in Otto Cohnheim's Chemie der Eiweisskörper, and in Gustave Mann's The Chemistry of the Proteids, which is based upon the former. We shall follow, as a rule, Cohnheim's classification. Even to-day the chemistry of the amino acids might be used as a basis for a classification, but in order to prevent misunderstandings we will here adhere to the older method.

The following works are instructive: Viktor Griessmayer: Die Proteïde der Getreidearten, etc. (1897). Leo Morochowetz: Das Globulin des Blutfarbstoffes, etc. Le Physiologiste Russe, **41–47** (1903), and **48–60** (1904). F. Hofmeister: Ueber Bau und Gruppierung der Eiweisskörper, Ergeb. Physiol. (Asher and Spiro), **1**, 759 (1902).

well as in acids and alkalies. Solutions of pure albumins are neutral. The albumins also differ from the globulins in their behavior on "salting-out." They are not precipitated when their neutral solution is saturated with sodium chloride. Even saturating the solution with magnesium sulphate solution does not produce precipitation. They are not precipitated by a half-saturated ammonium sulphate solution. In acid solutions they are, however, precipitated by saturating with sodium chloride or magnesium sulphate. The albumins, as previously stated, have been obtained in crystalline form.

The globulins are insoluble in pure water and in dilute acids, but are dissolved by dilute alkalies and neutral salt solutions. They can be precipitated from solution by the addition of water or acids. Passing carbon dioxide through their solution is sufficient to precipitate them. The globulins can, consequently, be easily coagulated. They may be redissolved, only when freshly precipitated. The globulins act like acids, and turn blue litmus red. They are precipitated by a half-saturated ammonium sulphate solution. They are very widely distributed. The serum-, milk-, and egg-globulins are best known. There are, undoubtedly, other groups of closely allied proteins, which are, at present, classified separately. Albuminous bodies, very much like the globulins, have been isolated from various animal organs. Thyreo-globulin[1] is assigned to this class. It contains a large percentage of iodine.

The globulin-like proteins present in plant seeds are grouped separately. They act as reserve material for the seeds, often occurring in large masses, and are easily obtainable.[2] They are occasionally found in crystalline form, as has been mentioned, and can often be crystallized. Edestin, the best known of these, occurs in various seeds, and can be dissolved in a five per cent sodium chloride solution at sixty degrees, and recrystallized therefrom. These vegetable proteins all react acid and are insoluble in water; they, however, all dissolve in salt solutions, and can be recovered from these by diluting or acidifying.

The phytovitellins, also called "vegetable-casein," which have been but little investigated, are proteins obtained from plants, and are provisionally placed in this class. Some of them contain phosphorus, and should, therefore, preferably, be included with the nucleo-albumins. It has not yet been decided definitely whether this phosphorus is actually a part of the protein molecule, or only an impurity. The latter assumption seems justified, as the method of preparation is crude, and very little effort has been made to purify them. The reserve proteins stored in the seeds of various cereals belong to this class. These substances are partially soluble in alcohol. The gluten-casein of wheat, legumin of the legumes,

[1] A. Oswald: Z. physiol. Chem. **27**, 14 (1899); **32**, 121 (1901).

[2] Cf. H. Ritthausen: Die Eiweisskörper der Getreidearten, etc., Bonn, **1872.**

conglutin of the lupins, almonds, nuts, etc., are insoluble; while gliadin, found in wheat, rye, barley, and oats, is soluble. Zein, a protein obtained from corn, is soluble in alcohol. This group of alcohol-soluble albuminous bodies from plant seeds is also characterized by the absence of lysine, in their composition, whereas almost all of the other proteins yield this as a cleavage-product.

The group of fibrinogens and fibrin is better defined. We shall dwell more in detail on these proteins [1] later on. They have, in common with casein and myosin, the faculty of being clotted, i.e., changed to a solid state, by a ferment. This curdling is not identical with coagulation. Although the curdled proteins are no longer soluble in water, they can still be coagulated, i.e., completely denaturized by heating or by treating them with alcohol. Fibrinogen is found in the blood-plasma of all invertebrates. It is changed into fibrin by the action of a ferment. The coagulation of blood, which normally occurs only when the blood has left its containing vessels, is dependent on this action. We shall see later that this process is an extremely complicated one which has not yet been explained entirely.

Myosin acts very much like fibrinogen, and is found in the fibres of striated muscle in a soluble form. Its curdling causes *rigor mortis*. The cause of the curdling of this muscle material is not understood. A ferment action, analogous to that of fibrin formation, has been offered in explanation, although such a ferment has not yet been proved definitely to exist. We must also mention the fact that besides myosin other albuminous substances, among these myogen,[2] have been described. It is difficult to determine whether these proteins are distinctively-characterized albuminous substances, or, more probably, the same myosin in different forms. We have seen that many proteins, including myosin, can be very easily denaturized. These products, therefore, have entirely different properties, and easily give one the impression of containing a protein of a peculiar nature. We cannot go far astray if we confine ourselves, at least for the present, to calling them simply, " muscle-albumins." We also find these same substances in the smooth muscles. Analogous proteins must be present in other organs, as they also show the same *rigor mortis* phenomenon. This, however, gradually disappears. No satisfactory explanation of this process has as yet been presented.

We will now discuss a group of proteins whose distinctive characteristic is the presence of phosphorus. This group of nucleo-albumins includes a very heterogeneous collection of proteins. They also possess another lesser characteristic in being largely liquefied by pepsin-hydrochloric acid digestion, the complex containing phosphorus being split off and finally

[1] Cf. the Lecture on Blood.

[2] O. von Fürth: Ergeb. Physiol. (Asher and Spiro) **1**, 110 (1902).

appearing as an insoluble precipitate. Later on this goes into solution. This complex has been called paranuclein by Kossel,[1] and pseudonuclein by Hammarsten.[2] It is at present entirely arbitrary to include the nucleo-albumins among the simple proteins. Didactic considerations were mainly responsible for placing them in this class. The nucleo-albumins have often been classified with the nucleoproteids on account of their common phosphorus content. The latter, however, are sharply distinguishable from the former by the fact that purine bases, pyrimidine derivatives, and pentoses enter into their composition. O. Cohnheim[3] proposes instead of nucleo-albumin the name of *phospho-globulin* to prevent such confusion. This group includes casein, vitellin, and a series of cell-neucleo-albumins. It is possible that legumin and the so-called vegetable-casein belongs to this class. The nucleo-albumins are invariably distinct acids. When pure they are insoluble in water. Their salts, on the other hand, are easily soluble in alkalies and ammonia. They are precipitated by acids. Boiling a solution of their salts is not sufficient to coagulate them.

We shall consider casein and its digestion more in detail later. Here its occurrence in milk will be merely mentioned. Vitellin occurs in the yolk of eggs. It has never been prepared in a pure condition. Nucleo-albumins are supposed to occur in all cells. With the exception of casein, no other member of this group has so far been isolated in a pure condition. This group already shows the total inadequacy of our methods of preparation. As soon as we are compelled to attempt the isolation of a given protein from a mixture of albuminous material by precipitation and certain questionable reactions, we meet with unsurmountable difficulties. The names of the various proteins are here largely derived from their morphological source. The physical properties of the albuminous substances are naturally dependent on the medium in which they are found. That these substances are largely influenced by extraneous conditions, can be easily shown by studying the behavior of the same protein when dissolved in various solvents.[4] The exceptionally large amounts of admixed salts necessarily have an effect on the other physical properties of any individually precipitated protein. Although we are undoubtedly right in considering the albumins and the globulins as distinct individuals, this is not the case with those others just mentioned. Many are unquestionably mixtures.

We shall now consider the proteins which are relatively rich in di-amino acids. As a result of their composition they are of a more or less basic

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[1] A. Kossel: Arch. Anat. Physiol. **1891,** 181.

[2] O. Hammarsten: Z. physiol. Chem. **19,** 19 (1893).

[3] O. Cohnheim: *loc. cit.* p. 190.

[4] Cf. E. Abderhalden and O. Rostoski: Z. physiol. Chem. **46,** 125 (1905); E. Abderhalden: Z. exp. Path. Therap. **2,** 642, 1905.

character. They are, for this reason, precipitated by alkalies, although redissolved by an excess. They are readily soluble in acids.

The histons belong to this class. They belong just as much to the simple proteins as to the more complicated ones. They do not occur as such in nature. They are always linked with some other radical, and must be separated from it when prepared for study. The first histon, in the narrower sense, was isolated by Kossel [1] from the blood corpuscles of a goose. The histon obtained from the leucocytes of the thymus-glands has been most carefully studied.[2] The histons are very widely distributed. They are found in the spermatozoa of fishes, and can be shown to occur as antecedents of the protamines; for instance, in the immature testes of the salmon.[3] Many authors place globin, the protein component of hemoglobin, in this class. It is very basic in its nature, although it otherwise behaves differently from the other histons. It really occupies an intermediate position between the histons and the simple proteins.

The histons have been very carefully examined by Ivar Bang.[4] He mentions the following as characteristic reactions: — They are precipitated from their water solutions by ammonia, but are redissolved by an excess. The histons are only coagulated by boiling in the presence of salts. They form a precipitate with nitric acid in the cold, redissolve on heating, but again settle out on cooling. Neutral solutions of histons give precipitates with solutions of ovalbumin, casein, or serum-albumin, which contain but little admixed salts. This is considered a very characteristic reaction. These precipitates contain one part histon and two of casein, two of serum-albumin or one of ovalbumin. These reactions do not apply to all histons. The various members of the histon group differ greatly from one another. Their chief characteristic is the large amount of bases present.

The protamines, discovered by Fr. Miescher [5] in the mature spermatozoa of the salmon, are closely related to the histons. A. Kossel [6] has greatly

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[1] A. Kossel: Z. physiol. Chem. **8,** 511 (1883–84).

[2] L. Lilienfeld: *ibid.* **18,** 473 (1894).

[3] Cf. F. Miescher (O. Schmiedeberg): Arch. exp. Path. Pharmak. **37,** 100 (1896). One experiment showed only about 40 per cent of bases in a product obtained in the beginning of October from the testes of the salmon. This was evidently a mixture of histon and protamine. A second preparation showed about 60 per cent of bases, while the protamine obtained from mature testes showed as much as 80 per cent bases.

[4] Ivar Bang: Z. physiol. Chem. **27,** 463 (1897); **30,** 508 (1900). Hofmeister's Beiträge, **4,** 115, 331, and 362 (1903).

[5] Fr. Miescher: Ver. Naturfors. Gesellsch. Basel, **6,** 138 (1874). J. Piccard: Ber. 7, 1714 (1874), and Fr. Miescher's complete works, *loc. cit.*

[6] A. Kossel: Z. physiol. Chem. **22,** 176 (1896); **25,** 165 (1898); **26,** 558 (1899); Ber. **34,** 3214 (1901); Z. physiol. Chem. **40,** 311 (1903–04); also A. Kossel and A. Mathews: *ibid.* **25,** 191 (1898). A. Kossel and F. Kutscher: *ibid.* **31,** 165 (1900). A. Kossel and H. D. Dakin: *ibid.* **40,** 565 (1903–04).

extended our knowledge of them; so much so, that most of their fundamental constituents are already well established. Kossel and his students have also found protamines in the spermatozoa of other fishes. They resemble one another very closely, although they are not identical, as is indicated by their percentages of mono- and di-amino acids. The protamine group is very well defined. They contain, above all, a very large amount of bases. Arginine is the main cleavage-product of the protamines. The amount varies between 58–84 per cent according to the origin of the protamine. They also contain mono-amino, as well as the di-amino, acids, as we shall see later. To assign to the protamines a particular position among the albuminous substances would certainly be arbitrary and unjustifiable at present. They are closely related to all the remaining proteins, and are formed from them. There is also no justification for considering them as the simplest proteins. Although one of the cleavage-products of the protamines, arginine, is found in large amount, it must not be forgotten that other amino acids are also present, and that the constitution of the protamines may be just as complicated as that of other proteins.

The protamines can be purified, which is of great advantage in their preparation. The free protamines are obtained pure only with difficulty. They are best obtained in the form of sulphates, and then changed over into chlorides. The latter can be precipitated from methyl alcohol solution by means of platinum chloride. M. Goto [1] has analyzed these platinum salts, and obtained the following values:

Variety.	C	H	N	Pt	Cl	O
Salmine (from salmon)	22.96	4.32	14.83	24.73	26.56	6.70
Clupein (from herring)	22.81	4.30	12.59	24.64	26.57	9.09
Scombrine (from mackerel)	23.49	4.75	13.57	24.09	25.99	8.11
Sturine (from sturgeon)	24.32	4.49	14.20	23.10	25.42	8.47

Sulphur has not yet been discovered in the protamines, and is probably absent. We are not at present aware of any reason why a lower molecular weight should be assigned to them than to the other proteins. The protamines are not coagulated by heat. While the ordinary proteins are precipitated by the alkaloid reagents (for instance, phospho-tungstic acid) only in acid solution, and the histons in neutral solution, the protamines will be even thrown out in alkaline solution. The protamines can be salted-out by ammonium sulphate and sodium chloride.

The protamines have toxic properties; [2] 15–18 mg. of scombrine, salmine,

[1] M. Goto: Z. physiol. Chem. **37**, 94 (1902).

[2] W. H. Thompson: Z. physiol. Chem. **29**, 1 (1900).

or clupein, and 20–25 milligrams of sturine, per kilo weight of the animal, are sufficient to kill a dog. It is still undecided whether this poisoning is due to the protamines or to some admixture.

Besides the protamines mentioned, cyclopterine[1] from the lump-fish, (*Cyclopterus lumpus*), and acipenserine, from the testes of the sturgeon (*Acipenser stellatus*),[2] are also described as protamines. Two other protamines may be mentioned: α- and β-cyprinine,[3] which are obtained from the sperm of the carp (*Ciprinus carpio*). The sperm of the brook-trout (*Salmo fario*), the white snapper (*Coregonus oxyrhynchus*), the sheath-fish (*Silurus glanus*), and the pike (*Esox lucius*),[4] also contain protamines. Protamines have not been isolated positively from any other representatives of the animal kingdom. The significance of the protamines has not yet been established.

Related to the proteins just described is a group of albuminous bodies, whose biological significance differs materially from that of any so far mentioned. Their common properties have united the heterogeneous substances into one group, called the "albuminoids." They constitute the frame-work of the animal tissues. They are not found in the cell protoplasm, nor in the tissue fluids. We shall see later, that their significance also corresponds to their entire composition. They are not to be considered as nutrient materials in the narrower sense, and participate but little in the intermediate metabolism. Incidentally they are difficultly digestible; in fact, being somewhat resistant to the digestive ferments. The albuminoids are to the animal body what the higher carbohydrates (for instance, cellulose) are to the vegetable. They are all insoluble in water and salt solutions. They are only slightly attacked by acids and alkalies. It is practically useless to attempt to purify the albuminoids. They can only be studied in the manner in which they occur in nature. It is an entirely arbitrary assumption that they exist as a chemical entity.

Collagen occupies a special position among the albuminoids. It constitutes the foundation of the bones and cartilages, and constructs the fibrils of the connective tissues. It may be extracted from these tissues by boiling with water. The product which goes into solution is called glue, glutin, or gelatin. In contra-distinction to the other proteins, collagen is soluble in warm water, and solidifies on cooling. Numerous investigations indicate that it is not an individual substance, undoubtedly differing according to the animal, or organ, from which it is obtained. The nature of the change underlying the formation of gelatin has

[1] N. Markowin: Z. physiol. Chem. **28**, 313 (1899).
[2] D. Kurajeff: Z. physiol. Chem. **32**, 197 (1901).
[3] A. Kossel and H. D. Dakin: *ibid.* **40**, 565 (1903), *loc. cit.*
[4] A. Kossel: *ibid.* **22**, 176 (1896), *loc cit.*

been but little studied. It may possibly be due to a hydrolytic decomposition.

Another group of albuminoids is included under the name of *keratins*. They comprise the so-called "horn substance," and are found, as such, in the hair, feathers, nails, hoofs, horns, etc. *Neuro-keratin*, which forms a part of the sheath of the medullary nerves, belongs to this class. The *keratins* are noted for their insolubility in water, dilute acids and alkalies. They have a high percentage of sulphur. *Ovokeratin*, in the membranes surrounding the eggs of many animals, is closely related to the keratins. *Gorgonin*, constituting the foundation of the axial skeleton of coral (*Gorgonia cavolini*), is also included. It is especially noted for its high percentage of iodine.

The *elastin* bodies form a group by themselves. They predominate in the formation of the elastic tissues. Elastin is generally prepared from the *Ligamentum nuchæ* of the ox.

Fibroin, obtained from the threads of silk-worms, is the best known albuminoid. It is characterized by a very low percentage of di-amino acids. We shall return to this substance later.

Spongin, forming the framework of sponges, and *conchiolin*, the basis of the skeleton of the mussel, are included among the albumoids. Amyloid, to whose content of chondroitin-sulphuric acid we recently referred, is also assigned to this group. It occurs only under pathological conditions. It is found, on the one hand, in the form of small kernels in the brain (the so-called *Corpora amylacea*), and then, again, as large deposits in the parenchyma of many organs. This is called an *amyloid degeneration*. The cause of its formation is but little known.

We must finally consider the group of the albumoids. It includes the most varied albuminous substances, about whose constitution nothing is at present known. To this class belong the *Membranæ propriæ* of many glands, sarcolemma, osseo-albumoid, chondro-albumoid, the albumoid of lentils, the elementary matter of *Chorda dorsalis*, ichthylepidin (occurring in the scales of fishes), the horny layer in the crop of birds, reticulin (which composes the reticular tissues of the intestinal mucosa), and many other analogous substances.

It is impossible, at present, to give any further exact account of the various representatives of the albumoids. The fact that they are obtained with difficulty, and, more especially, that it is almost impossible to purify them, has made any exact study of the class up to the present time entirely futile. Observers have concerned themselves chiefly with an investigation of various physical properties of individual proteins of this group, and especially to the action of ferments upon them. Their practical indigestibility, has made it possible to remove the ordinary proteins from them. The great predominance of albuminous substances, not

only in the cells, but also in all the fundamental and basic elements, distinctively separates the animal from the vegetable world. As the plants are capable of utilizing the very diverse carbohydrates in building up the various polysaccharides, so the animal organism is able to assimilate the simple proteins (those of milk, for example) for its nourishment, producing the large number of albuminous substances found in the cells and tissues. The albuminoid and albumoid groups are good illustrations of how the animal organism utilizes the proteins for its varied functions, and how it adjusts the composition to the function.

We shall now discuss the proteids, or compound proteins. We can do this rather rapidly, because the non-albuminous component of the proteids is the more interesting, and will be considered elsewhere. The large number of nucleoproteids belong to this group. They are composed of albumin and nucleic acid. The former may consist of certain of the protein groups previously mentioned. We are acquainted with compounds of nucleic acids with histons, as well as with protamines. It is an open question as to whether other proteins do not likewise participate in the formation of nucleoproteids. It must be admitted, however, that the very existence of the nucleoproteids has been questioned.[1] Nucleic acid has the property of precipitating protein from solution. In preparing the nucleoproteids we only extract the various organs with, for instance, water. On adding acid, generally acetic acid, the nucleoproteids are precipitated from the extract. We can imagine that the nucleic acid is present in the extract perhaps in the form of its sodium salt, together with the albumin. As a matter of fact, by acidifying, the nucleic acid is freed, which precipitates the soluble albumin. We can, by adding nucleic acid to albuminous substances, obtain precipitates which are very much like nucleoproteids. Nucleoproteids have, however, also been obtained by "salting-out."[2] It is, therefore, very probable that the nucleoproteids are present, as such, in the various organs.

We do not, at present, know the manner of linking between the nucleic acid and the protein. There are apparently various kinds of combination. For instance, 0.8 per cent of hydrochloric acid is sufficient to separate histon from the nucleo-histon obtained from the thymus gland; on the other hand the pancreas-proteid is decomposed into nuclein and albumin, even in neutral solution on boiling. The composition is not as simple as the name nucleoproteid might indicate. There seems to be more than a mere combination of nucleic acid and protein. If a nucleoproteid is decomposed, we, of course, obtain a protein, for instance histon. The

[1] I. Bang: Z. physiol. Chem. **30,** 508 (1900). T. B. Osborne and I. F. Harris: *ibid.* **38,** 85 (1902).

[2] F. Malengreau: La Cellule, **17,** 339 (1900). W. Huiskamp: Z. physiol. Chem. **32,** 145 (1901); **39,** 55 (1903).

other compound is not a nucleic acid, but a combination of this with albumin. This is called *nuclein*. On further decomposition, it breaks down into albumin and nucleic acid.

The nucleoproteids are all soluble in water and salt solutions. They are very soluble in alkalies. They are distinctly acid in their characteristics. They are precipitated by acids, although redissolved by an excess. The nucleoproteids can be "salted-out" and coagulated by heat and other means. When nucleoproteids are digested with pepsin-hydrochloric acid, nuclein settles out, while the albuminous cleavage-product is dissolved by the ferment in the usual manner. Fr. Miescher,[1] the discoverer of the nucleins, noticed this characteristic property. The nucleins themselves are but little affected by pepsin-hydrochloric acid, although more so by trypsin. It is very difficult to purify them; in fact, it is doubtful whether they have ever been isolated in a pure condition.

The nucleoproteids often contain iron, and it is very probable that the main supply of this element in the system occurs in these, and in hemoglobin. They are present in every cell, and are found in the nucleus. Miescher first noticed them in the little pus cells. They were isolated shortly afterwards also from the blood corpuscles of birds and snakes. We must also state that efforts have been made to place the ferments themselves in the group of nucleoproteids. By the discovery of nuclein and the nucleoproteids, Fr. Miescher has substantially advanced our knowledge of the constituents of the nuclei. The fact that all nuclei — whether plant or animal — contain nucleoproteids is another link between these two great kingdoms. We must not, however, forget that we know practically nothing about the biological significance of these substances. They have interested us greatly, especially on account of their predominance in the nuclear material, and the ease of obtaining them. The importance assigned to them may, however, be entirely unwarranted. Our knowledge of the other constituents of the nuclei is far too meager to tell us much concerning the functions of the nucleus.

Nucleoproteids have been isolated from almost every organ. So, also, from the spermatozoa-masses. Those of fishes contain up to 96 per cent of nucleic-acid-protamine, or -histon. Fr. Miescher and Schmiedeberg give the following composition to salmon spawn: 60.5 per cent nucleic acid, 35.56 per cent protamine. A nucleic-acid-histon has been obtained from the sea-urchin (*Arbacia pustulosa*).[2] The spermatozoa of bulls also contain a nucleoproteid, which, however, does not contain protamine nor histon, but some other kind of protein.[3] Nucleoproteids have also been isolated from the thymus gland, from the red corpuscles of birds

[1] Fr. Miescher: Hoppe-Seyler's Med.-chem. Untersuchungen, p. 441 (1871).

[2] A. Mathews: Z. physiol. Chem. **23**, 399 (1897).

[3] Fr. Miescher: Arc. exp. Path. Pharm. **37**, 100 (1896).

and reptiles, from the pancreas, from the gastric juice, from the thyroid gland, from suprarenal glands, and from muscles. Nucleoproteids have also been found in tumors. The nucleoproteid of yeast has been much studied on account of the ease with which its nucleic acid is obtained. We must also call attention to the presence of nucleoproteids in the vegetable kingdom.

Oxy-hemoglobin likewise belongs to the group of proteids. It is composed of globin and hematin. We have met the former while discussing the histons. Oxy-hemoglobin contains, according to the investigations of Fr. N. Schulz,[1] about 4–5 per cent hematin. We do not, at present, know whether different kinds of animals possess different kinds of hemoglobin; in fact, we are not at all certain that one and the same species of animal has a uniform hemoglobin. The crystalline form of the hemoglobin is of little value. The investigations of the globin portion have also been of little service. The hemoglobin from the horse has been most thoroughly studied. The decomposition of the hemoglobin from the dog gave corrresponding amounts of amino acids. The second component, hematin, seems to have constant properties, irrespective of the animal from which it is obtained. We shall devote more attention to hematin when we discuss the composition of blood. We shall also have to consider then the part hemoglobin plays as a carrier of oxygen and carbon dioxide.

The glucoproteids form the third subdivision of the proteid group. They are composed of a protein and a carbohydrate complex. We have already met them in our discussion of the carbohydrates,[2] and have seen that glucosamine is produced by the hydrolysis of the ordinary mucins, while galactosamine is obtained from the mucin of frog-spawn. These are the only carbohydrates that have been positively isolated from the glucoproteids. It is, indeed, very questionable whether the carbohydrates mentioned pre-exist as such. The whole group of glucoproteids is still very indefinite. While it is comparatively easy to decompose the nucleoproteids and hemoglobin into their albuminous and non-albuminous constituents, this does not hold with the glucoproteids. The carbohydrate group is only split off by boiling with mineral acids or by the action of alkalies. It is, of course, possible that the glucoproteids are true albuminous bodies, differing only from the other proteins, in that more carbohydrates take part in their formation. Certain of the ordinary albumins may indeed really belong to this group. If this be true, we would then have all shades of proteins, some with considerable carbohydrates others with less, and some which do not contain any carbohydrate at all in the molecule.

[1] Fr. N. Schulz: Z. physiol. Chem. **22**, 449 (1898).

[2] Cf. pp. 20 and 25.

It would be much more correct if we, for the present, entirely drop the name glucoproteid until we were certain that the carbohydrates occupy the same relative position to the protein in the molecule, as, for example, hematin does to hemoglobin. We will, therefore, classify the glucoproteids as true proteins, in the largest sense, and deal with the carbohydrate cleavage-products in the same manner as with the other protein components. It is certainly a matter of considerable interest to know that amido-carbohydrates — that is, sugars which are intermediate between the amines and the true carbohydrates — take part in the building up of these proteins.

To this group belongs a series of albuminous substances whose physical appearance is sufficient to identify them as a class. They are called *mucins* and *mucoids*. They may be recognized even by their elementary composition. The occurrence of the carbohydrate groups rich in oxygen lowers the percentages of carbon and nitrogen. The amount of carbohydrate present is a very variable one, ranging from 3–37 per cent, according to the substance in question. It is very difficult to purify them even approximately. They are not coagulated by heat, — a property which distinguishes them from the ordinary proteins. They may, nevertheless, be easily denaturized. They can be "salted-out." The mucins and mucoids are distinctively acid, and can be precipitated by acids. They are readily soluble in alkalies, alkaline carbonates, and ammonia.

The mucins are very widely distributed. They constitute the slimy material of many secretions, and are eliminated by the respiratory [1] and digestive tracts, sometimes from individual cells (goblet cells) and again from larger glands — such as the salivary glands. There are also mucous-producing cells in the bile ducts and the urinary passages. The mucins are also produced to a considerable extent by invertebrates, e.g., slime of snails. The mucin from the respiratory passages [2] and from the submaxillary glands,[3] has been studied most. Mucin from the invertebrates does not seem to be excreted as such, but is only produced secondarily from a substance called ***mucinogen***.

Proteins closely related to the mucins have been observed in ovarian cysts, which are peculiar tumorous formations of the ovaries. They are called *para-* and *pseudo-mucin*.[4] The latter differs from the ordinary mucins in that it is not precipitated from its solutions by acetic or nitric acids. Paramucin is occasionally found in gelatinous masses in cysts. It resembles mucin in being precipitated by acids.

The *mucoids* are closely related to the mucins. They are found to

[1] F. Müller: Z. Biol. **42**, 468 (1901).
[2] O. Hammarsten: Z. physiol. Chem. **12**, 163 (1887).
[3] O. Hammarsten: Pflüger's Arch. **36**, 373 (1883).
[4] O. Hammarsten: Z. physiol. Chem. **6**, 194 (1882).

some extent dissolved, and to some extent they participate in the construction of tissue. Their classification is based upon principles morphological rather than chemical-physical. They are sometimes included with the mucins, and sometimes classed as an independent group. We shall mention only the most important of them. Deserving of mention are the mucoids prepared from tendons, bones, and cartilages. The last named has been thoroughly investigated. *Chondro-mucoid* in conjunction with collagen constitutes the elementary material of cartilage. It contains considerable sulphur and a reducing sugar. When hydrolyzed we obtain a protein and a carbohydrate-ethereal sulphuric acid, the so-called *chondroitin-sulphuric acid*.[1] This is a colloidal substance, and has been investigated by Schmiedeberg,[2] and later by A. Orgler and C. Newberg.[3] By boiling with acids for a short time it decomposes into sulphuric acid and a residue, *chondroitin*, free from sulphuric acid. Further treatment with acid produces an amido-polysaccharide, whose exact nature has not yet been determined. Chondroitin-sulphuric acid is found not only in cartilages, but also in bones in *ligamentum nuchæ*, and in the mucous membrane of the pig. It occurs especially in amyloid, a protein which is found in the tissues under certain pathological conditions. Mörner [4] found chondroitin-sulphuric acid repeatedly in the urine to the extent of 0.05 per cent.

The proteins found in the vitreous humor, in the cornea, and in the umbilical cord, also belong to the group of mucoids, as does the *ovomucoid* obtained from the white of an egg. The latter may be isolated by coagulating the globulin and albumin, and adding alcohol to the filtrate. A reducing substance, gluocsamine, can be split off from it. Steudel [5] obtained 29.4 grams glucosamine from 100 grams ovomucoid. Blood serum also contains a mucoid body. Mörner describes another substance belonging to this group, which he obtained from the urine. An analogous product also occurs in the ascitic fluid.

We must admit that a large amount of uncertainty attaches to this group of albuminous substances. Secure foundations are lacking. It is practically impossible to purify these products, beyond getting rid of gross impurities. The properties of the various members of this group are such, that it is very difficult to work with them. It is difficult to obtain them in large quantities. We must, therefore, look with skepticism upon all new mucins and mucoids, and should await the time when purely chemical investigations will have sufficiently classified the mem-

[1] C. T. Mörner: Skand. Arch. f. Physiol. **1**, 210 (1889). Cf. Lecture III, p. 49.
[2] O. Schmiedeberg: Arch. exp. Path. Pharm. **28**, 355 (1891).
[3] A. Orgler and C. Neuberg: Z. physiol. Chem. **37**, 399 (1903).
[4] K. A. H. Mörner: Skand. Arch. Physiol. **6**, 332 (1895).
[5] H. Steudel: Z. physiol. Chem. **34**, 353 (1901-02).

bers of this group, at least to the extent of recognizing the nature of the individual decomposition products.

Hammarsten [1] has isolated a peculiar substance from the albuminous gland of the Roman snail (*Helix pomatia*), which is possibly a protein, difficult to classify, or, more probably, a proteid. It contains a lævo-rotary carbohydrate, which yields a dextro-rotary substance on boiling with acids. This material also contains phosphorus. It does not belong to the nucleo-proteids, because it possesses no xanthin bases. To this group, called " phospho-glucoproteids," is assigned the substance *ichtulin*, which has been obtained from fish eggs.

The proteins and proteids already mentioned do not, by any means, exhaust the list. We have mentioned only those which have been fairly well characterized, and which are starting points for further investigation. The albuminous substances, and especially some of the albuminoids, isolated from plant seeds and the animal fluids, are undoubtedly best known. We frankly admit that our knowledge is very vague concerning the albumins which participate in the construction of the various organs. It is very probable that the number of proteins and proteids occurring in the tissues is extremely large as is indicated by the description of the various tissue-albumins. On the other hand, we must remember that when proteins are attacked but slightly their properties are changed completely so that they may be mistaken for new kinds of protein. It is also probable that the tissues, and especially the cell proteins, are continually undergoing changes. It is still undecided whether the albuminous constituents of the tissues are to be considered as stable substances, to a certain degree, or as undergoing continual decomposition and reconstruction. From this question arises the extremely important problem of the whole subject of protein metabolism, about which we are still in the dark.

We have, so far, neglected to mention a group of nitrogenous compounds, which are closely related to the proteins. These are the *melanins*, which are very widely distributed in the animal kingdom. They are found in the very large number of pigments occurring in the hair, feathers, choroidea of the eyes, skin, etc. Their presence in tumors is very interesting; and their unusually large occurrence in the *melano-sarkoma* of horses — especially of white horses — has drawn much attention to them. In the muscles of these animals (for instance, the glutæi), there are often embedded very large tumors, which appear, according to age, as very black, firm masses, or like a cyst, containing an inky, finely granulated fluid. It is certainly significant that these masses of pigment are obtained in animals whose hair has no pigments. The pure white

[1] O. Hammarsten: *loc. cit.* Pflüger's Ann. **36**, 373 (1883).

horse produces, therefore, a pigment, but it is not utilized. This pigment, called *hippomelanin*, has been very exhaustively investigated.[1] It occurs as a very finely divided, brownish-black powder. No individual constituent of melanin has so far been definitely isolated. The constituents of the other melanins, prepared from the hair, choroidea, etc., has also never been cleared up. The melanins are, undoubtedly, not simple substances. It is difficult to obtain any exact knowledge about these substances, because they are not readily purified. They are extremely resistant to acids and alkalies, and to oxidation and reduction processes. Some will dissolve in alkali; others will not. Some contain iron; in others, this element is lacking. It has been suggested that, because some of the melanins contain iron, they are related to the coloring matter of the blood. It is possible that some of these pigments may trace their origin back to hematin, although no proof has been presented to substantiate this hypothesis. The melanins are characterized by a high carbon and a low hydrogen content. Many of them have considerable sulphur in their composition.

By the hydrolysis of almost all the albuminous bodies with acids, products are obtained which very much resemble the naturally-occurring melanins. These black products are called *humin substances*. They are supposed to be related to the natural melanins, and the suggestion has been made that glucosamine, tryptophane, tyrosine, and lysine participate in the formation of pigment. We know nothing definite[2] about the constituents of these humin substances, and are unable to decide whether there is any distinct relationship between these and the melanins. We will particularly emphasize the fact that the hypothesis just mentioned to the effect that the humin substances are built up of the fundamental constituents of the albumins, is entirely without empirical justification.

We have now mentioned the most important classes of the remarkably diversified group of the proteins, or albuminous substances. The unsatisfactoriness of the whole system of classification is evident from our presentation of the subject. It only serves as a temporary basis for further orientation. It is highly important that we should sharply and distinctly emphasize how slight the proofs are in the case of most of these substances that we are dealing with simple substances rather than with mixtures, and how extraordinarily cautious we must be for this reason in passing judgment upon the results, which must eventually be referred to more physico-chemical investigations.

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[1] J. Berdez and M. Nencki: Arch. exp. Path. Pharm. **20**, 346 (1885). M. Nencki and N. Sieber: *ibid.* **24**, 17 (1887).

[2] Cf. also Fr. Samuely: Hofmeister's Beit. **2**, 355 (1902)

LECTURE VIII.

ALBUMINS OR PROTEINS.

II.

The Components of Protein.

Although the number and distribution of the proteins in Nature are very great, still they show great similarity in the way they are constructed. Various methods have been tried for effecting the cleavage of proteins. Up to the present time, hydrolysis, whether brought about by the action of acids, of alkalies, or of ferments, has alone been productive of results. Experiments performed in the attempt to obtain known products by oxidation have thus far been unfruitful.[1] It is, moreover, hardly to be expected that in the case of such a complicated substance as albumin we shall obtain much idea of its chemical constitution by means of oxidation or reduction processes. We shall limit ourselves here, therefore, to a consideration of those investigations which have been of service in the further development of the entire chemistry of the proteins. We shall first of all take up those substances which are formed by the hydrolysis brought about by the action of acids or alkalies. If a protein is boiled for some time with fuming hydrochloric acid, or with twenty-five per cent sulphuric acid, its character is completely changed. It is broken down into numerous, simpler cleavage-products. These are of various kinds. They possess, however, certain common characteristics. As far as we know, they nearly all crystallize well, and contain nitrogen, hydrogen, and oxygen. These cleavage-products are known in general as *amino acids*. They can be easily identified and prepared in a pure condition. Besides these amino acids we find varying amounts of ammonia, and frequently humin substances. We shall presently see that we are acquainted with a large number of the cleavage-products of the proteins, although an appreciable part of the molecule is still unexplained. We shall also soon learn that all proteins, as far as our present knowledge goes, contain these same amino acids. Occasionally one or another amino acid is missing, although, as a whole, there is a very general agreement in the qualitative composition. The following amino acids have been isolated up to the present time:

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[1] Cf. Otto von Fürth: Hofmeister's Beiträge, **6**, 296 (1905). The older literature is carefully compiled in this article.

I. ALIPHATIC SERIES.

1. Mono-amino-mono-carboxylic acids. { Glycocoll. / Alanine. / Amino-isovaleric acid. / Leucine. / Isoleucine.
2. Mono-amino-hydroxy-mono-carboxylic acids: Serine.
3. Mono-amino-di-carboxylic acids . . { Aspartic acid. / Glutamic acid.
4. Di-amino-carboxylic acids . . . . . { Lysine. / Arginine
5. Di-amino-hydroxy-mono-carboxylic acids. Di-amino-tri-hydroxy-dodecylic acid.
6. Amino acids containing Sulphur: Cysteine and Cystine.

II. AROMATIC SERIES.

Mono-amino-mono-carboxylic acids: Phenylalanine.
Mono-amino-hydroxy-mono-carboxylic acids: Tyrosine.

III. HETERO-CYCLIC COMPOUNDS.

Mono-α-amino-mono-carboxylic acids . { Pyrrolidine-carboxylic acid. / Tryptophane.
Hydroxy-mono-amino-mono-carboxylic acids: pyrrolidine-carboxylic acid.
Histidine.

The carbohydrate group should also be included. These occupy a peculiar position, because they are absent from a large number of the proteins; in others their occurrence is questioned; while in still another group of proteins they appear in larger amount, but only in part as a direct constituent of the albumin molecule. Many authors classify all these proteins containing carbohydrates as compound albumins. As previously indicated, we do not consider this as justifiable. We shall subsequently return to this carbohydrate group.

Let us turn to the individual amino acids. We shall consider their distribution when we return to the composition of the individual proteins. For the moment we will only classify them according to their constitution, as this is necessary in order to understand their biological significance.

The mono-amino-mono-carboxylic acids can be derived from the normal fatty acid series: $C_nH_{2n}O_2$. The simplest member of this series is glycocoll, also called glycine or amino-acetic acid:

$$\begin{array}{l} CH_2(NH_2)^1 \\ | \\ COOH \end{array}$$

[1] We shall use this formula, but the following is also possible:

$$\begin{array}{l} C\ O\ O \\ |\ \ | \\ CH_2-NH_2. \end{array}$$

Glycocoll was one of the first-known cleavage-products of proteins. As early as 1820 Braconnot[1] obtained it, in conjunction with leucine, on boiling glue with dilute acid or alkali. It is also obtained, as such, from the muscles of the scallop, *Pecten irradians*.

Alanine is the next homologue of glycocoll. It is an α-amino-propionic acid: $CH_3 . \overset{*}{C}H(NH_2). COOH$. It contains an asymmetric carbon atom, *, and is consequently optically active, as are most of the other acid cleavage-products of albumin, which itself rotates the plane of polarized light. Alanine, as it occurs in nature, is dextro-rotary.

An amino-butyric acid has been described as a cleavage-product from proteins. Later investigations, however, have not shown its presence in the protein molecule. On the other hand, its next homologue, amino-valeric acid, has been obtained very often. The amino-valeric acid so far isolated from the proteins does not have a normal chain, but a branching one. It is an α-amino-isovaleric acid: $\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CH . \overset{*}{C}H(NH_2)COOH$. It is dextro-rotary.

Leucine also has a branching chain, and is an α-amino-isobutyl-acetic acid: $\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CH . CH_2 . \overset{*}{C}H(NH_2)COOH$. The leucine, usually obtained by the cleavage of proteins, is *l*-leucine. In this form it occurs in many plants and in invertebrates. *Penicillium glaucum* produces *d*-leucine from the inactive leucine. The constitution of leucine has been proved by E. Schulze and A. Lickiernik.[2]

Felix Ehrlich [3] has recently separated an isomer of leucine from molasses sludge, and, soon after, also showed its presence in many plant, and animal proteins. It is an α-amino-methyl-ethyl-propionic acid:

$$\begin{matrix}CH_3\\C_2H_5\end{matrix}\!>\!CH . \overset{*}{C}HNH_2 . COOH.$$

Its constitution has been proved by its synthesis, and also that it is decomposed by pure-culture yeast into *d*-amyl-alcohol. The relations of iso-leucine to *d*-amyl-alcohol are shown as follows. We will com-

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[1] H. Braconnot: Ann. Chim. Phys. **13,** 113 (1820).

[2] Ber. **24,** 669 (1891), and Z. physiol. Chem. **17,** 513 (1893).

[3] Felix Ehrlich: Ber. **37,** 1809 (1904); Z. Ver. Zuckerind, **1904,** 975; **55,** 592 (1905). We shall dwell upon the syntheses and decomposition of the amino acids, only as much as is necessary in order to understand biological processes. We may also add, that this form of synthesis has often been utilized in the reactions between ammonia and the halogen fatty acids. Cf. E. Fischer: Ber. **39,** 530 (1906).

pare at the same time the formation of ordinary leucine from iso-amyl-alcohol:

$\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CH\,.\,CH_2\,.\,CH_2OH$

iso-amyl-alcohol
$+ O$
$- H_2O$

$\begin{matrix}CH_3\\C_2H_5\end{matrix}\!>\!\overset{*}{C}H\,.\,CH_2\,.\,OH$

d-amyl-alcohol
$+ O$
$- H_2O$

$\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CH\,.\,CH_2\,.\,CHO$

iso-valeraldehyde
$+ HCN + NH_3$
$- H_2O$

$\begin{matrix}CH_3\\C_2H_5\end{matrix}\!>\!\overset{*}{C}H\,.\,CHO$

d-valeraldehyde
$+ HCN + NH_3$
$- H_2O$

$\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CH\,.\,CH_2\,.\,\overset{*}{C}HNH_2\,.\,CN$

iso-valero-amino-nitril
$+ 2\,H_2O$
$- NH_3$

$\begin{matrix}CH_3\\C_2H_5\end{matrix}\!>\!\overset{*}{C}H\,.\,\overset{*}{C}HNH_2\,.\,CN$

d-valero-amino-nitril
$+ 2\,H_2O$
$- NH_3$

$\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CH\,.\,CH_2\,.\,\overset{*}{C}HNH_2\,.\,COOH$

α-amino-isobutyl-acetic acid

= leucine

$\begin{matrix}CH_3\\C_2H_5\end{matrix}\!>\!\overset{*}{C}H\,.\,\overset{*}{C}HNH_2\,.\,COOH$

α-amino-methyl-ethyl-propionic acid

isoleucine

Serine is closely related to alanine. It was isolated in 1865, by Cramer,[1] from silk glue (or sericin). It is an α-amino-β-oxypropionic acid: $CH_2(OH).\ \overset{*}{C}H(NH_2).\ COOH$.(?). Serine as it occurs in nature is lævo-rotary.[3]

Of the di-basic amino acids only two are known: *α-aspartic acid* and *glutamic acid*. The former is an α-amino-succinic acid.

$$\begin{matrix}COOH\\|\\*CH(NH_2)\\|\\CH_2\\|\\COOH.\end{matrix}$$

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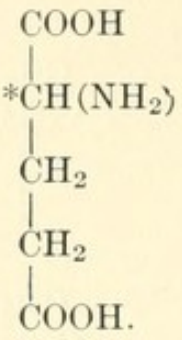
[1] E. Cramer: J. prakt. Chem. **96**, 76 (1865).

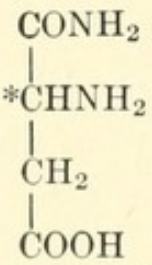
[2] For the synthesis from ammonia, hydrocyanic acid, glycolaldehyde, cf. E. Fischer and H. Leuchs: Sitzber. Akad. Wiss. Berlin, **1902**, and Ber. **35**, 3787 (1902).

[3] Emil Fischer: Ber. **40**, 1501 (1907).

The latter is the next higher homologue, α-amino-glutaric acid:



Although the amino acids previously mentioned which possess both carboxyl and amino groups are not distinct acids nor bases, but possess their combined characteristics, these dicarboxylic acids have a distinctively acid character. The natural aspartic acid is the lævo-rotary. Glutamic acid rotates polarized light to the right. Both dicarboxylic acids occur widely distributed in the vegetable kingdom as amides. Thus *asparagine* is an amide of amino-succinic acid:



Asparagine.

It was first found in asparagus sprouts. Soon afterwards it was found that the asparagine collects in the embryo, which are kept in the dark. Asparagine seems to play an important part during germination. E. Schulze,[1] whom we have to thank for very exhaustive investigations on the accumulation of asparagine in embryo, gives the following values:

Age of embryo in days . .	4	7	10	12	15	16
% asparagine of the dry substance of the embryo . .	3.3	11.2	17.3	22.3	25.0	25.7
% asparagine of the dry substance of the seeds .	3.12	9.78	15.24	18.22	19.43	. . .

As regards the distribution of asparagine in various portions of the embryo, it is worthy of note that Schulze found 31.81 per cent of asparagine in the dry substance of the axillary organs of lupines, while the cotyledons gave only 7.62 per cent. Asparagine occurs in plants in the dextro- and lævo-rotating varieties. The latter occurs more abundantly.

[1] E. Schulze: Landwirtsch. Jahrb. **1878,** 411.

The two varieties can be easily recognized by their crystalline structure. One is left-handed hemihedral, and the other right-handed. The *d*-variety tastes sweet, while the other is insipid.

Glutamine, the amide of amino-glutaric acid, has been found in germinating pumpkin seeds, by Schulze and Barbieri.[1] Its occurrence is similar to that of asparagine.

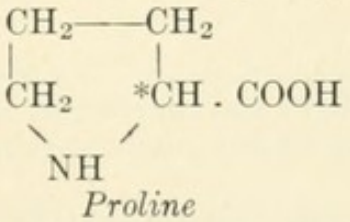
As far as we know, these two amides do not occur in the animal organism. We shall later on consider their value as a food material.

Before discussing the properties of the di-amino acids, we shall devote a little attention to the remaining mono-amino acids. *Phenylalanine*, first discovered by Schulze and Barbieri [2] in the embryo of lupines, has recently been recognized as an invariable constituent of all the albumins so far investigated. Its constitution is that of phenyl-amino-propionic acid: $C_6H_5 . CH . \overset{*}{C}HNH_2COOH$. It occurs in nature as the *l*-variety.

Another aromatic amino acid, which has been known for a long time as a constituent of albumin, is *tyrosine*, which can be easily isolated on account of its difficult solubility in water. It is *p*-hydroxy-phenyl-amino-propionic acid: $C_6H_4OH . CH_2 . \overset{*}{C}H(NH_2) . COOH$. It occurs in nature in both modifications, although mainly in the lævo form.

Tyrosine gives several color reactions, of which that of Hoffmann is the most important. It is known under the name of *Millon's reaction*. If tyrosine is boiled with a nitric acid solution of mercuric oxide, containing a little nitrous acid, the liquid becomes colored, and the resulting precipitate is rose-colored or a dark brownish red. This reaction is not confined to tyrosine. It is given by all benzene derivatives, in which a hydrogen atom has been substituted by a hydroxyl group. This reaction has become of great importance on account of the fact that all proteins which contain tyrosine will give it. Millon's reaction is, therefore, a test for proteins.

We now come to the *heterocyclic* compounds. Two representatives of this class have been discovered by Emil Fischer:[3] *α-pyrrolidine-carboxylic* acid, also called proline, and *hydroxy-pyrrolidine-carboxylic* acid; the latter being probably an *hydroxy-α-pyrrolidine-carboxylic* acid:



Proline

and $C_5H_9NO_3$.

Hydroxy-proline

[1] E. Schulze and J. Barbieri: Ber. **10**, 199 (1877).

[2] *Ibid.* **14**, 1785 (1881); Z. f. physiol. Chem. **12**, 405 (1888).

[3] E. Fischer: Z. physiol. Chem. **33**, 151 (1901); Ber. **35**, 2660 (1902). Cf. H. Leuchs: *Ibid.* **38**, 1937 (1905).

Both proline and oxy-proline have been found in the decomposition products of most albumins. The first is invariably present. It is, of course, a question whether we are dealing with primary products. It is possible that they are produced secondarily from another substance by ring formation. Sorensen [1] has suggested that the primary substance in the production of proline may be α-amino-δ-hydroxyvaleric acid. We have not yet succeeded in isolating the amino-hydroxyvaleric acid, and it must be remembered that α-proline is obtained by alkali hydrolysis, as well as by acid.[2] A small amount of proline has also been obtained by peptic [3] and tryptic [4] digestion. We have nothing at hand at present to warrant us in excluding α-proline and oxy-proline as primary albumin decomposition products.

Tryptophane also belongs to this class of amino acids. It had been known for a long time, that especially in tryptic digestion mixtures [5] some substance was present which was characterized by certain color reactions.

In an acetic acid solution it gives with chlorine or bromine water a violet color. Also, if we insert a pine splinter, which has been previously dipped into hydrochloric acid and then rinsed off, into a concentrated tryptophane solution, the stick will turn purple on drying. This is the so-called *pyrrole reaction.* It was soon shown that many of the reactions characteristic of the albumins were due to the presence of tryptophane. If we add a little glyoxylic acid, and then concentrated sulphuric acid, to a water solution of albumin, a beautiful blue-violet coloration appears. O. Neubauer and Rohde [6] have indicated recently a new reaction for tryptophane in albumin. If we add to a water solution, or suspension, of albumin, 5 to 10 drops of a 5 per cent solution of *p*-dimethyl-amino-benzaldehyde containing 10 per cent of sulphuric acid, and then, while shaking, cautiously pour into the solution a little concentrated sulphuric acid, a reddish-violet coloration appears, which soon takes on a beautiful reddish violet shade. The absorption spectrum shows a wide, faint band in the orange (λ 615–670), and a second, obscure, band in the green (λ 555–540).

F. G. Hopkins and Cole [7] were the first to prepare tryptophane in a pure

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[1] S. P. L. Sörensen: Compt. rend. trav. Lab. Carlsberg, **6,** 137 (1905); Z. physiol. Chem. **44,** 448 (1905).

[2] E. Fischer: Z. physiol. Chem. **35,** 227 (1902).

[3] Commercial pepsin was used in these experiments. The possibility, therefore, remains that other tissue ferments might have acted in conjunction with the pepsin, for the reason that the commercial preparation is obtained by extracting the mucous lining of the stomach.

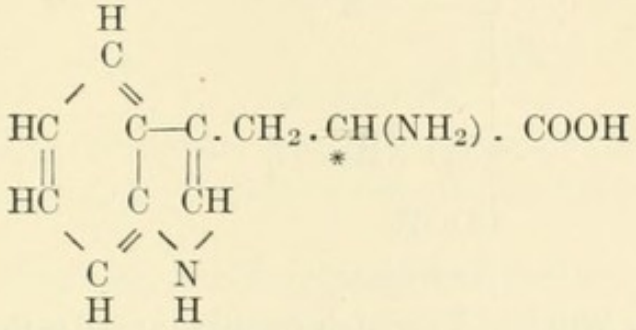
[4] E. Fischer and E. Abderhalden: Z. physiol. Chem. **40,** 215 (1903).

[5] Cf. E. Stadelmann: Z. Biol. **26,** 491 (1890). R. Neumeister: *ibid.* **26,** 324 (1890). M. Nencki: Ber. **28,** 560 (1895).

[6] E. Rohde: Z. physiol. Chem. **44,** 161 (1905).

[7] F. G. Hopkins and S. W. Cole: J. Physiol. **27,** 418 (1901); **29,** 451 (1903).

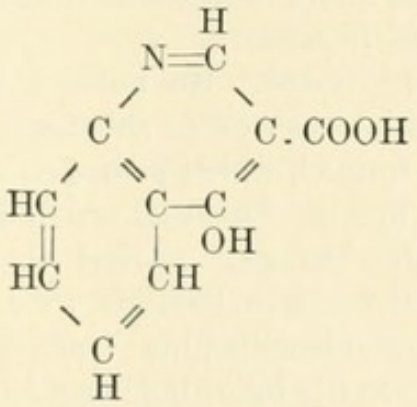
state. They permitted bacteria to act on tryptophane, and obtained indole, skatole, skatole-carboxylic acid, and skatole-acetic acid. The bacillus of anthrax, and *Bacterium coli,* in a strong anaërobic solution, produce skatole-acetic acid, while putrefactive bacteria, on the other hand, give skatole-carboxylic acid in conjunction with indole and skatole. The constitution of tryptophane has been recently established by the synthesis of Ellinger and Flamand.[1] It is indole-α-aminopropionic acid.



Tryptophane

The common form is dextrorotary both in alkaline and acid solutions.

It may be mentioned that tryptophane is related to a peculiar metabolic product of the dog, namely, kynurenic acid, which is a γ-hydroxy-β-quinolin-carboxylic acid of the following constitution:[2]



Kynurenic acid

The transformation of tryptophane into this acid is not yet understood. Possibly some idea of the process may be obtained from the fact that a compound rich in oxygen which can be converted into quinolin is found together with tryptophane. At present, however, we do not know whether this *oxy-tryptophane* corresponds to a primary decomposition product from protein, or whether it is formed secondarily from tryptophane.

To the group of heterocyclic building material of protein histidine also

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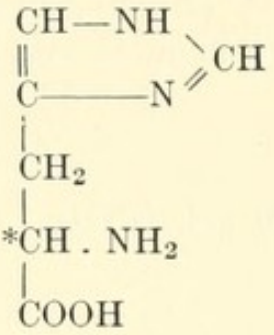
[1] A. Ellinger and C. Flamand, Ber. **40**, 3029 (1907).

[2] E. Abderhalden and M. Kempe; Z. physiol. Chem. **52**, 207 (1907).

belongs. We are indebted to A. Kossel [1] for its discovery among the cleavage-products of the protamine, sturine. It was for a long time assigned to the di-amino acids, also called hexon bases. Pauly [2] has recently succeeded in throwing some light on its constitution. He gives it the formula of α-amino-β-imidazol-propionic acid:

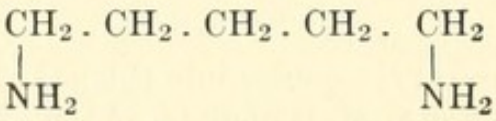


Histidine

F. Knoop and A. Windaus,[3] through further investigations, have shown this constitution to be correct. The compound is lævorotary and has an alkaline reaction.

Only three di-amino acids are, as yet, known. These are *lysine*, *arginine*, and *di-amino-tri-hydroxydodecoic* acid. The constitution of the latter is not yet determined. It was separated from casein by Emil Fischer and Emil Abderhalden.[4] There are many indications that it, or other closely allied substances, occurs in other proteins.

Drechsel [5] first isolated lysine. He noticed in the decomposition of casein by hydrochloric acid that other substances besides ammonia and mono-amino acids were formed, which possessed a strong basic character. Among these lysine was found. Drechsel concluded that it was probably a di-amino-caproic acid. Ellinger [6] proved that this supposition was correct. He allowed putrefactive bacteria to act on lysine, and after a while obtained pentamethylenediamine (cadaverine). Ladenburg has shown [7] that cadaverine has the following constitution:



Cadaverine

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[1] A. Kossel: Z. physiol. Chem. **22**, 177 (1896–97); Sitzber. Akad. Wiss. Berlin, **1896.**

[2] H. Pauly: Z. physiol. Chem. **42**, 508 (1904).

[3] F. Knoop and A. Windaus: Hofmeister's Beitr. **7**, 144 (1905); **8**, 407 (1906).

[4] Fischer and Abderhalden: Z. physiol. Chem. **42**, 540 (1904).

[5] E. Drechsel: Ber. **21**, 117 (1889); Arch. Anat. Physiol. **1891**, 254. E. Schulze and E. Winterstein: Ergebnisse Physiol. (Asher and Spiro), **1**, 32 (1902).

[6] A. Ellinger: Z. physiol. Chem. **29**, 334 (1902). Cf. Ber. **31**, 3183 (1899); **32**, 3542 (1900).

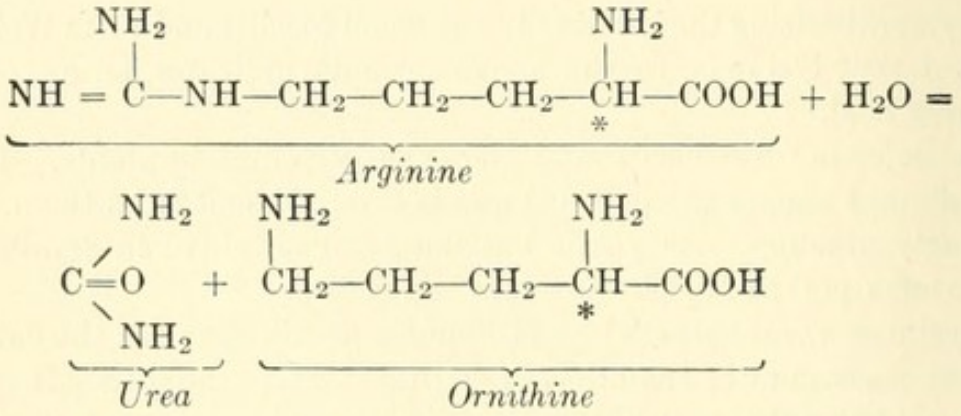
[7] Ladenburg: *ibid.* **19**, 780 (1886).

We can assume that cadaverine is formed by splitting off carbon dioxide from lysine, which has the empirical formula $C_6H_{14}N_2O_2$:

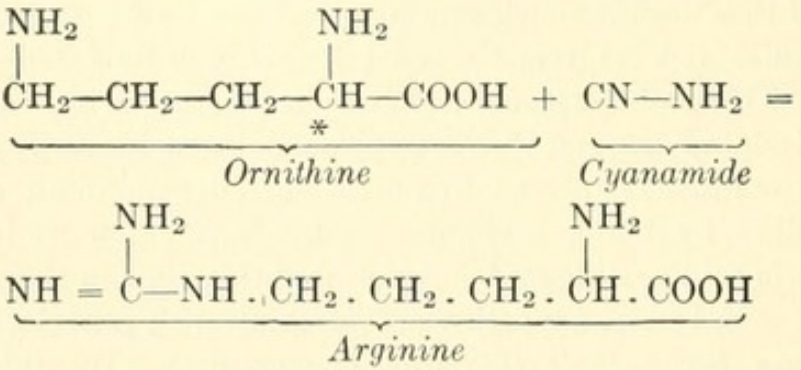
$$\begin{array}{l} \mathrm{CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot \overset{*}{C}H \cdot COOH} \\ \quad\quad\quad\

The position of the carboxyl group had not been determined. Emil Fischer [1] finally cleared up the constitution of ornithine by its synthesis. By this it was definitely determined that ornithine was an α-, δ-, diamino-valeric acid.

Thus one of the decomposition products of arginine, the ornithine, was identified. It was next to be decided in what form the urea which was obtained from arginine occurred in it. E. Schulze and E. Winterstein suggested that a guanidine derivative was united to the ornithine. The separation of urea and ornithine from such a compound would proceed in the following manner:



Schulze and Winterstein finally succeeded in synthesizing arginine from ornithine and cyanamide, thereby establishing the constitution of arginine.



B. Bénech and F. Kutscher [3] succeeded in obtaining guanidine from arginine, by oxidation with barium permanganate.

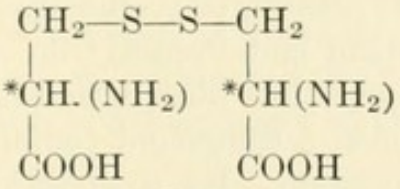
We have only one group of the remaining protein decomposition products to consider; that is, those containing sulphur. We have previously mentioned that sulphur is one of the essential constituents of albumin. It is only absent from the protamines. The occurrence of sulphur in proteins was recognized at an early date. It had been noted that on boiling albuminous material with alkalies considerable amounts

[1] E. Fischer: Sitzber. Akad. Wiss. Berlin, **1900**; Ber. **34**, 454 (1901).
[2] E. Schulze and E. Winterstein: Z. physiol. Chem. **34**, 128 (1901).
[3] E. Bénéch and Fr. Kutscher: *ibid.* **32**, 278 (1901).

of alkaline sulphides were split off.[1] Fleitmann [2] was the first to observe that only a part of the sulphur was split off by the action of alkalies, while another portion remained unacted upon. From these observations he differentiated between oxidized and un-oxidized sulphur in albumin. The latter, only, was susceptible of being split off. This distinction later on caused much misunderstanding among investigators of the sulphur components of albumin. A. Krüger [3] is entitled to much credit for having shown the futility of this classification. He makes a distinction between "loosely-" and "firmly-" combined sulphur. Fr. N. Schulz [4] proved that such a distinction was more justifiable, by showing that one of the sulphur cleavage-products of albumin, cystine, only gave off part of its sulphur on boiling with alkali; in fact, little more than half. Various albuminous substances, such as keratin (from the horn of cattle, and human hair), serum-albumin, and serum-globulin, acted in the same manner as cystine. Mörner [5] then succeeded in obtaining large quantities of cystine from the above-mentioned proteins, and showed that this amino acid is probably the only sulphur compound present. Other proteins certainly contain other sulphur compounds besides cystine.

Among the decomposition products containing sulphur, *cystine* is the only one that has been positively identified. Wollaston,[6] in 1810, first isolated this from a renal calculus. Since then it has been separated also from organs. Külz [7] first isolated it from digestive solutions of fibrin, and Emmerling [8] found it in horn. The wide distribution of cystine as a decomposition product of albumin is now generally acknowledged.

The constitution of cystine has only recently been established; it is an α-diamino-β-dithio-dilactylic acid:



Cystine

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[1] The sulphur content of the proteins was at one time a factor of great importance in the views prevailing concerning their constitution. Cf. E. Friedmann: Ergeb. Physiol. (Asher and Spiro) **1**, 15 (1902). E. Abderhalden: Biochem. Zentr. **2**, 257 (1904).

[2] T. Fleitmann: Ann. **61**, 121 (1847); **66**, 380 (1848).

[3] A. Krüger: Pflüger's Arch. **43**, 244 (1888).

[4] Fr. N. Schulz: Z. physiol. Chem. **25**, 16 (1898).

[5] K. A. H. Mörner: *ibid.* **28**, 595 (1899); **34**, 207 (1901–02).

[6] Wollaston: Phil. Trans. **1810**, 220.

[7] E. Külz: Z. Biol. **27**, 415 (1890).

[8] O. Emmerling: Verh. Ges. Naturforsch Aerzte. **2**, 391 (1894).

By reduction, we obtain *cysteine* from it, which is an α-amino-β-thio-propionic acid:

$$\begin{array}{l} CH_2 \,.\, SH \\ | \\ CH \,.\, (NH_2) \\ | \\ COOH \end{array}$$

Cysteine

Cysteine, therefore, is closely related to alanine and serine. Friedmann [1] oxidized cysteine and obtained cysteic acid:

$$\begin{array}{l} CH_2 \,.\, SO_2 \,.\, OH \\ | \\ CH(NH_2) \\ | \\ COOH \end{array}$$

Cysteic acid

from which, by splitting off carbonic acid, we obtain taurine:

$$\begin{array}{l} CH_2 \,.\, SO_2 \,.\, OH \\ | \\ CH_2(NH_2) \end{array}$$

Taurine

This indicated an important relationship between a product related to taurocholic acid and cystine. The correctness of the formula of cystine as presented by E. Friedmann, and soon after by C. Neuberg,[2] has recently been strengthened by the synthesis of cystine by Erlenmeyer.[3]

Observations by Baumann and Preusse [4] do not harmonize with the above formula. They found that when brombenzene was fed to a dog, the excreted urine contained a compound containing bromine, nitrogen, and sulphur in its composition. Its composition was $C_{11}H_{12}BrNSO_3$. Baumann and Preusse designated this compound: Bromphenyl mercapturic acid. From this, acetic acid and a compound, $C_9H_{10}BrNSO_2$, are obtained by hydrolysis. The latter formula corresponds to the empirical formula of cysteine, if the bromphenyl residue is replaced by a hydrogen atom. The compound formed together with acetic acid was therefore considered as bromphenyl cysteine. From the resulting cleavage-

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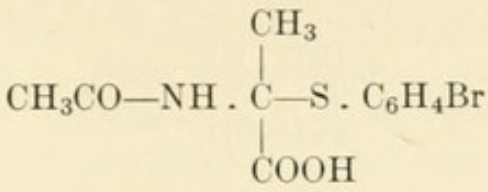
[1] E. Friedmann: Hofmeister's Beitr. **3**, 1 (1902).

[2] C. Neuberg: Ber. **35**, 3161 (1902). Cf. K. A. H. Mörner: Z. physiol. Chem. **42**, 349 (1904).

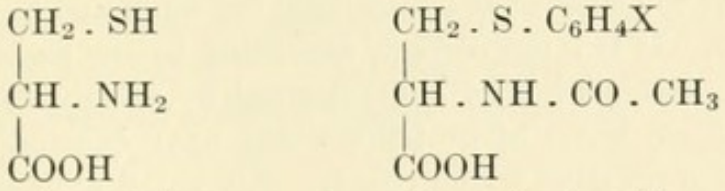
[3] Erlenmeyer: Jr. Ber. **36**, 2720 (1903).

[4] E. Baumann and C. Preusse: *ibid.* **12**, 806 (1879); Z. physiol. Chem. **5**, 309 (1881).

products, Baumann and Preusse proposed the following formula of mercapturic acid:



If this formula be correct, then it must correspond to a differently constituted cysteine than the above one. We might expect that cystine could occur in various modifications. E. Friedmann,[1] proceeding from this hypothesis, undertook to prove the constitution of mercapturic acid, and showed that such an assumption was unnecessary, for he found that mercapturic acid and cysteine have their amino and thio groups analogously situated:



Albumin-cysteine *Mercapturic acid*

Recent investigations have also shown that probably only one cysteine exists.[2] Patten[3] has shown that only cystine and not cysteine occurs in the original albumin molecule.

It is necessary to mention at this point that all the compounds so far discussed, with the exception of phenylalanine, can also be obtained by the hydrolytic action of ferments upon the albumins. Phenylalanine, as already indicated, is found as such in plant seeds. As fermentation hydrolysis undoubtedly furnishes the mildest possible form of decomposition, we are justified in concluding that the cleavage-products of protein which have been just mentioned exist as such in the albumin molecule.

While discussing the proteids, we mentioned the so-called "glucoproteids" of the albumins, which are characterized by a high percentage of glucosamine, and, possibly, other carbohydrates. On account of the firmness with which these groups are attached to the albumin molecule, it is at present considered more correct to place them with the simpler proteins rather than with the complex varieties. We can easily imagine that glucosamine is held in combination in a manner analogous to that which unites the amino acids to one another. We can also easily indicate

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[1] E. Friedmann: Hofmeister's Beitr. **4**, 486 (1903).

[2] E. Fischer and U. Suzuki: Z. physiol. Chem. **45**, 405 (1905).

[3] A. J. Patten: *ibid.* **39** 350 (1903).

the close relationship of glucosamine to the amino acids as well as to the carbohydrates. This is very evident from the following comparison:

$CH_2 . OH$	$CH_2 . OH$	$CH_2 . NH_2$
$\vert$	$\vert$	$\vert$
$CH . OH$	$CH . OH$	CH_2
$\vert$	$\vert$	$\vert$
$CH . OH$	$CH . OH$	CH_2
$\vert$	$\vert$	$\vert$
$CH . OH$	$CH . OH$	CH_2
$\vert$	$\vert$	$\vert$
$CH . OH$	$CH . NH_2$	$CH . NH_2$
$\vert$	$\vert$	$\vert$
$CH : O$	$CH : O$	$COOH$
Glucose	*Glucosamine*	*Lysine*

It is not right to consider as exceptional the albumins containing carbohydrate groups. It is more correct, according to our present knowledge, to speak of albuminous bodies characterized by a high content of glucosamine, just as we know of proteins which have considerable glycocoll. Just as there are proteins which contain only small amounts of glycocoll, and some with none at all, so we also recognize proteins which possess small quantities of glucosamine, as well as those which have none of this hexose base. The fact that other amino sugars probably participate in the constitution of proteins does not affect this conception. It is doubtful whether nitrogen-free sugars occur in albumin. It must also not be forgotten that the presence of glucosamine, as a primary cleavage-product, is doubted. A complex carbohydrate has been assumed to be the antecedent of the glucosamine. The results at present are not sufficiently exact to settle the question.[1] The conception that the " carbohydrate-group " is a constituent of the albumin molecule in the same sense as the amino acids, is rendered somewhat improbable by the fact that various observers have obtained entirely different carbohydrate values in the analysis of one and the same substance. It has even been suggested that specific proteins, e.g., serum-albumin, unite with the sugars, conduct them to the tissues, and finally give them up to the latter. Such an assumption would be comprehensible if it were known that the carbohydrate groups were loosely bound to the albumin molecule. This, however, is not the case. A more satisfactory explanation would be that the varying carbohydrate content of the proteins was due to the albumins investigated not being identical.

As yet we know but little about the quantities of glucosamine present in the mucins, — the proteins richest in carbohydrates. It is only known

[1] L. Langstein: Ergebnisse der Physiologie (Asher and Spiro), **1**, 63 (1902); **3**, 453 (1904).

that the mucins and their related substances can contain as much as 30 per cent glucosamine. It is not at all unreasonable to expect varying results from the same mucin, because it is absolutely impossible to purify these compounds thoroughly. It is more striking that egg-albumin, which is so easily crystallized, does not give concordant results on the amount of glucosamine. It must also be remembered that egg-albumin contains, besides albumin and globulin, ovi-mucoid, which contains about 30 per cent glucosamine. By the recent investigations of Fr. N. Schulz and Zsigmondy,[1] it was shown how extremely difficult it is to free egg-albumin from colloidal substances, even after sixfold crystallization. In recrystallized egg-albumin, values varying from 16 to less than one per cent of glucosamine were found.[2] We do not err, in assigning the fluctuations of the carbohydrate content, which far exceed the analytical error, to this cause of varying purity of the substance under investigation. It is, therefore, not yet determined whether egg-albumin is entitled to a "carbohydrate group." The same holds true regarding serum albumin. This, also, invariably incloses some serum-mucoid,[3] which is relatively rich in glucosamine. In fact, serum-globulin is distinctly different, in that, aside from a trace of glucosamine, it also splits off grape-sugar. Now serum-globulin is obtained by precipitation with ammonium sulphate solution. To purify this, to the extent possible with the crystallizable proteins, is entirely impossible. Besides grape-sugar, serum also contains small quantities of other carbohydrates of unknown constitution. It is very probable that the precipitated serum-globulin contains such a carbohydrate mixed with it. Up to the present time, we have had no investigation which would warrant us in assigning any nitrogen-free carbohydrates to the albumin molecule.

If we sum up what we know about the "carbohydrate groups" of the proteins, we will conclude that the mucins and mucoids contain such groups; while although the remaining proteins may contain carbohydrates, their presence has not been proved positively.

It is very important that absolute clearness should prevail in regard to this question. We shall see later, that many facts make it seem probable that carbohydrates are formed from albumin. The assumption that, according to our present knowledge, the "carbohydrate group" of the

[1] Fr. N. Schulz and Zsigmondy: *loc. cit.*

[2] E. Abderhalden, P. Bergell, and T. Dörpinghaus: Z. physiol. Chem. **41**, 530 (1904). Direct experiment showed that repeated recrystallization reduced the quantity of glucosamine present in albumin. Albumin crystallized once gave 7 per cent, three times gave 4 per cent, while the seventh time showed only 2.5 per cent glucosamine. That the values in this case are higher than in the work just mentioned, is due to the fact that the crude osazone was weighed, while in the former the analytically pure osazone was used as a basis for the calculation.

[3] C. N. Zanetti: Ann. Chim. Farmac. **12**, 1897.

proteins is the source of the sugar formation from albumin, is without justification, as we have just said. If sugars are formed from albumin, then undoubtedly the amino acids are to be considered as their immediate antecedents. It may be mentioned in addition that glucosamine especially is apparently not utilized at all by the organism for the production of glycogen.

It is certainly not without significance that the mucins and mucoids, proteins which are also widely distributed among the invertebrates, should contain glucosamine — an amino-hexose — which is known to be the basis for the formation of chitin.

The presence of carbohydrates in the albumins is indicated by certain color reactions. If we add a few drops of an alcoholic solution of α-naphthol to a solution of albumin, and allow a layer of concentrated sulphuric acid to flow beneath this, a violet ring appears at the junction of the two fluids. On shaking, the whole solution takes on a violet tinge. On adding alcohol, ether, or caustic potash to this, the coloration becomes yellow. If we use thymol instead of the α-naphthol, the coloration produced is carmine-red. It turns green on dilution. These reactions — called the "Molisch sugar tests"[1] — depend on the formation of furfurol from the carbohydrates present, by the action of sulphuric acid.

Another test which has been supposed to indicate the presence of a carbohydrate group in proteins is the violet to deep blue color obtained by boiling with fuming hydrochloric acid, when they have previously been hydrolyzed. This is known as "Liebermann's reaction," but it is not certain that this albumin reaction is due to carbohydrates.

In this connection we must call attention to two other albumin reactions. If we add strong nitric acid to an aqueous solution of albumin, a yellow coloration appears, very often in the cold, although generally only on boiling. If we add an excess of caustic soda to this, the solution becomes reddish brown; while if ammonia be used, an orange color results. This is called the "xantho-proteic reaction," and depends on the formation of nitro-derivatives, and, according to Salkowski,[2] requires the presence of aromatic groups.

All the reactions which have now been mentioned for albumin, require the presence of specific groups, and only apply to such proteins as contain them. The blackening, which results when a protein is heated with caustic alkali and a lead salt, is characteristic of a group containing sulphur. It depends on the formation of lead sulphide. Millon's reagent indicates the tyrosine group; the glyoxylic acid characterizes the tryptophane combination. The carbohydrate group is detected by the Molisch reaction, and also, possibly, by the Liebermann reaction. The xantho-

[1] Molisch: Monatsh. **7**, 198 (1888).

[2] E. Salkowski: Z. physiol. Chem. **12**, 211 (1887).

proteic reaction indicates the presence of aromatic groups. We are also acquainted with another important color reaction, which does not properly characterize any group as such. This is the so-called " Biuret-reaction." If we freely add caustic soda or potash to an albumin solution, and then carefully, drop by drop, a dilute solution of copper sulphate, a blue to rose-violet coloration appears, which goes over into a blue on the addition of more copper sulphate. The higher decomposition products of albumin, the peptones, give a red coloration.

The cleavage-products of protein just mentioned, have been obtained by the hydrolytic action of acids and of alkalies. We can easily imagine that in these cases secondary decompositions take place. A number of scientists doubted the occurrence of so many amino acids, and preferred to assume that the proteins contained groups which gave rise to the formation of these various amino acids during the hydrolysis brought about by the reagents.[1] It were conceivable that ornithine, proline, and amino-valeric acid originate from the same atomic grouping; also lysine and leucine, on the one hand, and tyrosine and phenylalanine on the other. Such a conclusion does not harmonize with our present knowledge of the actions of acids and alkalies, because they invariably yield the individual amino acids in the same quantities. That these amino acids occur in the proteins is very evident from their appearance, as such, in sprouting plants, and even in the animal organism under specific conditions. The most important proof of their original occurrence is indicated by their appearance during digestion. The albumins are broken down by the hydrolytic action of ferments, especially by trypsin, into amino acids. The decomposition by fermentation is the mildest imaginable. It takes place at 37° C. All the known amino acids have been obtained from digestion mixtures except phenylalanine and diamino-trihydroxy-dodecylic acid. The latter has never been looked for, while the former does not appear in a state of combination which is accessible to the proteolytic ferments.

The albumin as it reaches the digestive organs of an animal is subjected to the action of two proteolytic ferments, pepsin and trypsin. Later on we shall go more into detail regarding the behavior of the albumins during the process of natural digestion. We shall, at present, devote our attention to the subject of artificial digestion, i.e., the digestion of the protein outside of the alimentary tract. We must say that the results obtained in these investigations do not harmonize in matters of detail. This is mainly due to the different methods employed in using these ferments. Until recently, the physiological chemist utilized extracts of organs whether of the stomach or of the pancreatic gland, and the extirpated organs

[1] O. Loew: Hofmeister's Beitr. **1**, 567 (1900).

themselves. We are, however, aware that many ferments occur in the tissues, which are far more energetic in metabolic processes, and act in another direction, than the digestive ferments. Many of the results obtained are undoubtedly due to the interaction of these tissue-ferments. We are now able to obtain the digestive fluids in purest form, thanks to the excellent methods originated by Pawlow [1] and his students. It is possible, on the one hand, to prepare a small special stomach, that is, a pouch obtained by tying up a part of the walls of the stomach so as to form a blind-sack, from which the pure gastric juice may be obtained without the least admixture of any food residues. On the other hand, we can obtain absolutely pure pancreatic juice, clear as water, by making a pancreatic fistula, i.e., by grafting into the abdominal wall that part of the mucous membrane of the duodenum at which the pancreatic duct enters. As we shall see later, this fluid is inactive when the piece of intestinal mucous membrane carrying the papilla is cut away. It must first be made active preferably by the addition of intestinal juice. It is only possible to obtain absolutely correct results by utilizing such ferment solutions.

Amino acids do not immediately appear when the proteins — edestin, for example — are undergoing digestion. We observe first of all that the albumin is dissolved.[2] We notice at the same time that the digestion mixture contains dialyzable substances which are not amino acids. The digesting mixture may even be boiled without causing coagulation. It has been held that the protein molecule by hydrolytic cleavage is decomposed into products with lower molecular weights; the higher of these are known as *albumoses*, from which in turn *peptones* are formed. There is no sharp distinction between these two classes. Strictly speaking, the conception of albumoses and peptones is not a chemical, but a biological one, and we shall treat of them here as forming one class, and drop the term "albumose." It represents, instead of certain chemical individuals, a group of compounds which exist temporarily in a similar condition. For the present, these names do not signify much to us. Not content with this distinction of the two groups of substances, scientists have classified them according to their solubility relations, — according to the extent to which they may be precipitated, etc., — thereby designating them with new names. It has also been found that the peptones obtained from different proteins are not identical, so that they have been named according to the protein from which they are formed.

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[1] J. P. Pawlow: Ergebnisse d. Physiol. (Asher and Spiro) **1**, 246 (1902).

[2] The earliest observations on tryptic digestion were made by Corvisart: Gaz. Hebdom. Nos. 15, 16, 19 (1857). W. Kühne: Virchow's Arch. **39**, 130 (1867). Cf. also E. Abderhalden: Z. physiol. Chem. **44**, 17 (1905).

Thus we speak of globuloses, vitelloses, etc. Undoubtedly, we shall eventually find that a great deal of this difference in behavior is due to the different amino acids, which are contained in the different proteins, and their arrangement in the molecule, so that before long we shall be able to replace this purely biological conception by a chemical one. For the present the investigations have gone beyond our actual knowledge, and have led to certain results, which do not yet rest upon a firm foundation. For this reason we shall not attempt to describe any of the numerous special albumoses and peptones, but simply content ourselves with the conception itself. The albumoses are in general characterized by the fact that they are precipitated when their solutions are saturated with ammonium sulphate, while the peptones then remain in solution. By means of the behavior of a digesting mixture towards ammonium sulphate, we can determine how far the digestion has already gone.[1]

Up to this point the changes produced upon the protein molecule by the pepsin-hydrochloric acid of the stomach and the trypsin of the pancreas are apparently quite similar. In both cases albumoses and peptones are formed. Of course the action of pepsin may nevertheless be entirely different from that of trypsin in spite of this external similarity. It may be that a different place in the protein molecule is attacked. Unquestionably even in gastric digestion a large quantity of products are obtained which represent lower products than the peptones, and some of these do not even give the biuret reaction. Simple amino acids, however, with the exception of traces of tyrosine, have not been found here.[2]

Tryptic digestion goes much farther. We quickly observe crystalline depositions on the walls of the vessel in which the digestive mixture is placed. This is tyrosine, which separates on account of its difficult solubility. It is very quickly split off from the albumin molecule. In 48 hours, and even in less time, the entire tyrosine content of the albumin can be isolated as such.[3] In the digestion of edestin from cotton-seeds, for example, the following observations were made:[4]

PERCENTAGE OF TYROSINE OF THE TOTAL AMOUNT OCCURRING IN EDESTIN.

Time of digestion	1 day.	2 days.	3 days.	8 days.
	78.4	97.6	97.6	100

[1] See page 188.

[2] Emil Abderhalden and Otto Rostoski: Z. physiol. Chem. **44**, 265 (1905).

[3] E. Abderhalden and B. Reinbold: Z. physiol. Chem. **44**, 284 (1905).

[4] *Ibid.* **46**, 159 (1905).

Tryptophane, as well as cystine, can be obtained just as quickly as tyrosine; the former being easily recognized by the violet color which is formed when bromine water and acetic acid are added to the digesting liquid. The remaining amino acids are obtained later on. This has been successfully proved in the case of glutamic acid. The following percentages of the total amount of this amino acid occurring in edestin, were obtained:

Time of digestion	1 day.	2 days.	3 days.	8 days.	16 days.
	4.3	7.4	10.9	31.1	60.2

Alanine, leucine, amino-valeric acid, and aspartic acid, acted in the same manner; while α-proline and phenylalanine could, in no case, be separated from a digesting fluid.

The following observations have given us an explanation of this peculiar behavior:[1] If we digest casein, edestin, serum-globulin, egg-albumin, hemoglobin, or fibrin with pancreatin,[2] or even with pancreatic juice, we obtain all the mono-amino and di-amino acids in the digesting mixture, with the exception of proline and phenylalanine. These amino acids do not occur, or, if so, only in minute quantities, even when tryptic digestion precedes that of the pepsin-hydrochloric acid.[3] Now we can precipitate even from a greatly diluted digesting mixture, by means of phosphotungstic acid, a product which apparently is a mixture of highly complicated compounds. It sometimes gives the biuret reaction; then again, no result is obtained — according to the time of digestion. No free amino acids can be isolated from this product, although we can obtain such by hydrolysis with fuming hydrochloric or 25 per cent sulphuric acid. In the presence of small amounts of alanine, leucine, aspartic acid, and glutamic acid, we obtain large amounts of α-proline and phenylalanine; and, in those proteins containing glycocoll, even this amino acid, in amounts approximating those contained in the protein in question. The proteins evidently contain groups which resist the action of ferments. Of especial interest is the fact that here also the rate at which the individual amino acids are separated varies.

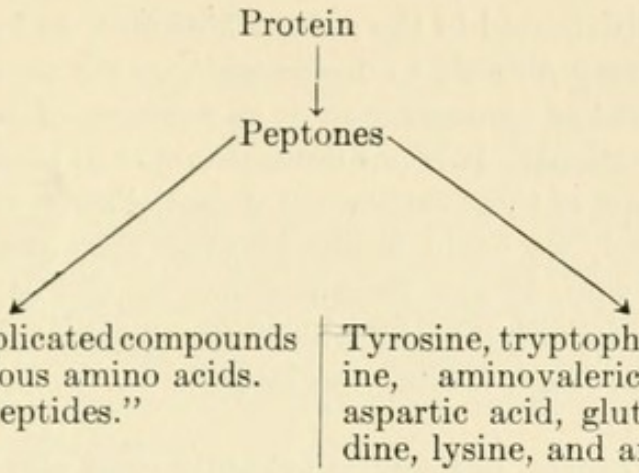
From these investigations it is clear that fermentative decomposition is a progressive one. An immediate disruption of the protein does not occur.

[1] E. Fischer and E. Abderhalden: Z. physiol. Chem. **39**, 81 (1903).

[2] A commercial preparation of pepsin was used in this experiment, consequently impure. It is very probable that the latter disintegrated albumin more than the gastric juice alone would do.

[3] E. Fischer and E. Abderhalden: Z. physiol. Chem. **40**, 215 (1903).

The following diagram will give an idea of the hydrolysis brought about by means of the pancreatic ferment, trypsin:



The product consisting of amino acids still combined with one another, and which may for the present be designated as "polypeptides," is different in the case of different proteins. In the case of edestin the amount is smaller than in the case of casein, and that obtained from the latter is less than from serum-globulin.

From the investigations of Abderhalden and Reinbold,[1] it has been clearly shown that, even the peptones, which still give the characteristic red biuret reaction, do not immediately break down into amino acids. There are certainly intermediate products between the peptones and the amino acids. Here, again, the progressive decomposition is clearly evident. Doubtless the simpler peptides, which we will shortly discuss, also appear as intermediate products. We shall return to this shortly.

The most important result obtained from these investigations is, that the amino acids, which split off from albumin by the action of alkalies and acids, are already formed in the albumin molecule and are not formed by a secondary process; and, further, that in spite of the early appearance of crystalline cleavage-products, the fermentative decomposition need not necessarily be far advanced. All the tyrosine occurring in a digesting mixture can be detected, even if, for instance, only seven per cent of the amount of glutamic acid occurring in the albumin has been set free.

Besides pepsin and trypsin, we have to consider *erepsin*, which occurs in the alimentary tract as an albumin-splitting ferment, and has been isolated by O. Cohnheim.[2] It does not act on the proteins themselves, but only on their decomposition products, the peptones. The only exceptions to this rule are casein, protamines, and histons; these are attacked

[1] *Loc. cit.*

[2] O. Cohnheim: Z. physiol. Chem. **33**, 451 (1901); **35**, 134 (1902). Cf. also S. Salaskin: *ibid.* **35**, 419 (1902).

by erepsin. The decomposition products are the same as those produced by trypsin. At present it is difficult to pass judgment on the actual existence of this ferment. According to the investigations of Vernon,[1] it occurs widely distributed in the animal kingdom, and is to be found in all tissues. It is very difficult to decide whether the proteolytic ferments should be considered as homogeneous or as mixtures of ferments of different individual functions. It is not unreasonable [2] to assume that for each protein, or for a class of these substances, a special series of ferments exists. On the other hand, we could easily imagine that one ferment follows another, step by step, in the decomposition, in the same manner as is true with the carbohydrates, in which case diastase decomposes them only to the maltose stage, leaving the latter to be further acted upon by maltase.[3]

As a matter of course, proteolytic ferments must also be active in the tissues and cells, and many observations indicate that the action is analogous to that of trypsin. This applies not only to the animal cells, but also to those of plants. Especially noteworthy is the ferment *papayotin* occurring in the milk of the melon, *Carica papaya*. It quickly dissolves albumin. Its action appears to be similar to that of trypsin.[4] Other active ferments have also been isolated from various plants, as, for instance, from the sap of the fig-tree, *Ficus carica*, and *macrocarpa*. Other plants, like the banana, are credited with possessing a ferment analogous to pepsin.

Especial interest attaches to those plants which also secrete ferments externally which correspond to the digestive fluids characteristic of the animal organism. They constitute the large group of carnivorous plants. We will mention merely the *Drosera* and *Pinguicula*, growing in peat bogs; and the varieties of *Utricularia* inhabiting brooks and stagnant pools. The *Nepente* species, *Dionæa muscipula*, act on a larger scale. It is still a question whether the action of the ferment produced is analogous to that of pepsin or to that of trypsin. It has even been suggested that the fermentative action of *Nepente* is due to bacteria.

In the cryptogams the proteolytic ferments are also widely distributed, and in many cases have been detected.

Before discussing the manner in which the fundamental constituents of the albumins are combined, we must devote a little attention to several other substances which occur among the cleavage-products of the proteins,

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[1] H. M. Vernon: J. Physiol. **32,** 33 (1904); **33,** 81 (1905).

[2] Cf. Lecture on Ferments.

[3] Cf. W. M. Bayliss and E. H. Starling: J. Physiol. **29,** 174 (1903). K. Mays: Z. physiol. Chem. **38,** 428 (1903). L. Pollak: Hofmeister's Beitr. **6,** 95 (1904). K. Kiesel: Pflüger's Arch. **108,** 334 (1905).

[4] O. Emmerling: Ber. **35,** 695 (1902).

but which, nevertheless, probably do not occur as a constituent of the original molecule.

Amongst these is *leucinimide*. It is an anhydride of leucine, and is 3–6, di-isobutyl, 2–5, di-acipiperazine:

$$\begin{array}{l} C_4H_9 \cdot CH \cdot NH \cdot CO \\ \qquad\quad | \qquad\quad | \\ \qquad CO \cdot NH \cdot CH \cdot C_4H_9 \end{array}$$

Ritthausen[1] first observed this in an acid hydrolysis. Cohn[2] also described it. Salaskin and Kowalewsky[3] recently even separated it, although only in minute quantity, from peptic and tryptic digestion.[4] It has not yet been decided whether leucinimide occurs as such in the albumin molecule. It is possible that it is formed by a secondary process, perhaps from a leucyl-leucine.

Pyroracemic acid, $CH_3 \cdot CO \cdot COOH$, discovered by Mörner,[5] is unquestionably formed by a secondary reaction. It is probably produced from alanine, serine, or cystine. The origin of α-thiolactic acid, discovered by Suter,[6] is problematical. It may possibly be derived from cystine, although this has the thio group in the β position. Ornithine, which is certainly a secondary decomposition product, is derived from arginine.

The albumins quickly undergo putrefaction.[7] They are also decomposed by bacteria in the intestines. It is necessary to become acquainted with the compounds formed in this manner. They are all related to the amino acids already mentioned. The bacteria decompose the albumin in the same manner as do the proteolytic ferments, especially trypsin. Peptones, and finally amino acids, are produced.

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[1] Ritthausen: Die Eiweisskörper der Getreidearten, Bonn, **1872.**

[2] R. Cohn: Z. physiol. Chem. **22,** 153 (1896–97); **29,** 283 (1900).

[3] S. Salaskin and K. Kowalewsky: *ibid.* **38,** 567 (1903).

[4] The author has himself also tried to isolate leucinimide from peptic and tryptic digestion products, but in vain. Something went into solution in the acetic ether. Its easy solubility in dilute hydrochloric acid showed that it was not leucinimide. He, however, succeeded in obtaining about one per cent of leucinimide by hydrolyzing casein with 25 per cent sulphuric acid.

[5] K. A. H. Mörner: Z. physiol. Chem. **42,** 121 (1904).

[6] Suter: *ibid.* **20,** 564 and 577 (1895); Hofmeister's Beitr. **3,** 184 (1902). K. A. H. Mörner: Z. physiol. Chem. **42,** 365 (1904).

[7] E. and H. Salkowski: Z. physiol. Chem. **8,** 417 (1884); *ibid.* **9,** 8 (1884); **9,** 491 (1885); **27,** 302 (1899). N. Nencki: Ber. **7,** 1593 (1874); **8,** 336 (1875); **10,** 1032 (1877); J. pr. Chem. **26,** 47 (1882); Z. physiol. Chem. **4,** 371 (1880); Z. med. Wiss. **1878,** 47. Nencki: Opera omnia, vol. i, pp. 92, 113, 144, 244, 246, 674, 537, 354, 418, etc. E. Baumann: Ber. **12,** 1450 (1879); Z. physiol. Chem. **4,** 304 (1880); **6,** 183 (1882); **7,** 282 and 553 (1895). E. Baumann and L. Brieger: *ibid.* **3,** 149 and 284 (1879). L. Brieger: J. pr. Chem. **17,** 124 (1877); Ber. **10,** 1027 (1877); **12,** 1986 (1879); Z. physiol. Chem. **2,** 241 (1878); **3,** 134 (1879); **4,** 414 (1880); **5,** 366 (1881). Cf. also L. Brieger: Die Ptomaine, Berlin, **1886.**

The decomposition, however, does not end here. The bacteria break these down in two directions. On one hand they split off the amino acids. Simple acids remain: acetic acid is produced from glycocoll; propionic acid from alanine; valeric acid from amino-valeric acid; etc. The δ-amino-valeric acid, found in putrefying mixtures, may be produced from ornithine, or by splitting off the pyrrole ring from α-pyrrolidine-carboxylic acid. Tartaric acid, phenyl-propionic acid, *p*-hydroxyphenyl-propionic acid, and skatole-acetic acid are also found. On the other hand, carbon dioxide is split off from the amino acids by bacterial action. This process gives us pentamethylenediamine (cadaverine) from lysine; while tetramethylenediamine (putrescine) results from arginine and ornithine.

From phenylalanine we obtain phenylethylamine $C_6H_5 . CH_2 . CH_2NH_2$, with evolution of carbon dioxide; and from tyrosine, oxyphenylethylamine. As a rule the decomposition does not stop at these products. They are oxidized. We can indicate the further destruction of tyrosine (*p*-hydroxyphenyl-amino-propionic acid) as follows:

p-hydroxyphenyl-amino-propionic acid	$C_6H_4 . OH . CH_2 . CH.NH_2.COOH$
p-hydroxyphenyl-propionic acid	$C_6H_4 . OH . CH_2 . CH_2.COOH$
p-hydroxyphenyl-acetic acid	$C_6H_4 . OH . CH_2 . COOH$
p-hydroxymandelic acid	$C_6H_4 . OH . CH(OH) . COOH$
p-cresol	$C_6H_4 . OH . CH_3$
phenol	$C_6H_5 . OH$

In an analogous manner phenylalanine (phenyl-amino-propionic acid) breaks down through phenyl-propionic acid and phenyl-acetic acid. Tryptophane (skatole-amino-acetic acid) gives skatole-acetic acid, skatole-carbonic acid, skatole and indole. We shall meet with phenol, skatole, and indole in the animal organism. They are produced in intestinal putrefaction, and appear in the urine combined with sulphuric acid. Sulphureted hydrogen is liberated from cystine.

LECTURE IX.

ALBUMINS OR PROTEINS.

III.

Composition of Individual Proteins. Constitution.

In the formation of proteins, the amino acids alone participate, as far as we know, with the single exception of the amino-hexose, glucosamine, which occurs in many varieties of albumin. The number of these amino acids already discovered is very large. It includes the following: glycocoll, alanine, amino-valeric acid, leucine, isoleucine, α-pyrrolidine-carboxylic acid (proline), oxypyrrolidine-carboxylic acid (oxyproline), serine, phenyl-alanine, glutamic acid, aspartic acid, tyrosine, cystine, tryptophane, lysine, histidine, arginine, and diaminotrihydroxydodecoic acid. It is of chief interest to learn whether the proteins, at present known, contain the same fundamental substances, or whether specific groups of proteins are characterized by their content of individual amino acids. Another matter of considerable importance is the relative quantity of the different amino acids, occurring in the proteins. It is possible that the differences between the various proteins are due to varying relations of the quantities of individual amino acids present. On the other hand, it is a matter of the greatest importance to know the quantitative amounts of these constituents of the albumins for use in further work on this subject. We should like to know how great a portion of the whole albumin molecule is already understood. Unfortunately, we have no quantitative method for estimating the amino acids. True, we can estimate very exactly some of the cleavage-products, like tyrosine and glutamic acid, but for the remainder of the amino acids we can only estimate the approximate amounts. Our knowledge concerning protein formation was, until recently, very limited.

Although various amino acids had been isolated, and the quantitative relations of lysine, arginine, and histidine in different albumin molecules had been established, through the researches of A. Kossel, investigators as a rule attempted only to prepare the proteins in as pure a form as possible, and to classify them according to their elementary composition. A turning-point in the whole chemistry of the albumins was reached when E. Fischer [1] introduced a new method for isolating the amino acids. Briefly, the process consists in forming the esters of the mono-amino acids, and

[1] E. Fischer: Z. physiol. Chem. **33**, 151 (1901).

separating them by fractional distillation. The amino acids are then recovered by saponifying the amino acid esters. On account of considerable differences in the boiling-points of these esters, it is possible to obtain by mere distillation a fairly satisfactory complete separation of the amino acids. With the help of this method a considerable number of protein substances have been carefully examined. We will give in the following summary the results thus arranged according to the classification previously given. It may be said that the amounts of amino acids indicated, represent the minimum values. As the various proteins were all analyzed under the same conditions, it is, therefore, possible to compare the individual proteins according to their percentages of monoamino acids. The values given are all based on 100 grams of ash-free material, dried at 100 degrees.

1. THE ALBUMIN GROUP.

	Serum-albumin.[1]	Egg-albumin.[2]
Glycocoll	0.	0.
Alanine	2.7	8.1
Leucine	20.0	7.1
α-Proline	1.0	2.25
Phenylalanine	3.1	4.4
Glutamic acid	7.7	8.0
Aspartic acid	3.1	1.5
Cystine	2.3	0.2
Serine	0.6	..
Tyrosine	2.1	1.1
Tryptophane	present	present

[1] E. Abderhalden: *ibid.* **37,** 495 (1903).

[2] E. Abderhalden and F. Pregyl: *ibid.* **46,** 24 (1905).

2. THE GLOBULIN GROUP.

	Serum-globulin.[1]	Edestin from hemp-seed.[3]	Edestin from cotton-seed.[4]	Edestin from sunflower seed.[5]
Glycocoll	3.5	3.8	1.2	2.5
Alanine	2.2	3.6	4.5	4.5
Aminovaleric acid	present	present	present	0.6
Leucine	18.7	20.9	15.5	12.9
α-Proline	2.8	1.7	2.3	2.8
Phenylalanine	3.8	2.4	3.9	4.0
Glutamic acid	8.5[2]	6.3	17.2	13.0
Aspartic acid	2.5	4.5	2.0	3.2
Cystine	0.7	0.25	...	...
Serine	...	0.33	0.4	0.2
Tyrosine	2.5	2.1	2.3	2.0
Tryptophane	present	present	present	present
Oxyproline	...	2.0	...	...
Lysine	...	1.0	...	...
Arginine	...	1.7	...	...
Histidine	...	11.1	...	...

[1] E. Abderhalden: Z. physiol. Chem. **44,** 17 (1905).

[2] E. Abderhalden and F. Samuely: *ibid.* **46,** 193 (1905).

[3] E. Abderhalden: *ibid.* **37,** 499 (1903); **40,** 249 (1903).

[4] E. Abderhalden and O. Rostoski: *ibid.* **44,** 265 (1905).

[5] E. Abderhalden and B. Reinhold: *ibid.* **44,** 284 (1905).

3. GROUP OF THE PLANT-CASEINS, PHYTOVITELLINS, LEGUMINS, ETC.

	Proteins soluble in alcohol.		Conglutin from Lupinus seeds.[5]	Legumin.[7]	Albumin from Pine seeds.[8]
	Gliadin[1] from wheat flour.	Zein.[2]			
Glycocoll	0.9	not determined	0.8	1.0	0.6
Alanine	2.7	0.5	2.5	2.8	1.8
Aminovaleric acid	0.33	present	1.1	1.0	present
Leucine	6.0	11.2	6.75	8.2	6.2
α-Proline	2.4	1.5	2.6	2.3	2.8
Phenylalanine	2.6	7.0	3.1	2.0	1.2
Glutamic acid	37.5	11.8	19.5	16.3	7.8
Aspartic acid	1.3	1.0	3.0	4.0	1.8
Serine	0.12	...	present	...	present
Tyrosine	2.4	10.1[3]	2.1	2.8	1.7
Tryptophane	about 1.0	...	present	...	present
Lysine	0.	0.	2.1	5.5	0.25
Arginine	3.4 [4]	1.82 [6]	6.6 [6]	4.6 [6]	10.9 [6]
Histidine	1.7	0.81	0.65	1.1	0.62

[1] E. Abderhalden and F. Samuely: Z. physiol. Chem. **44,** 276 (1905)
[2] L. Langstein: *ibid.* **37,** 508 (1903).
[3] F. Kutscher: *ibid.* **38,** 111 (1903).
[4] A. Kossel and F. Kutscher: *ibid.* **35,** 165 (1900).
[5] E. Abderhalden and J. B. Herrick: *ibid.* **45,** 479 (1905).
[6] E. Schulze and E. Winterstein: *ibid.* **33,** 547 (1901).
[7] E. Abderhalden and B. Babkin: *ibid.* **47** (1906).
[8] Abderhalden and Teruüchi: *ibid.* **45,** 473 (1905).

4. GROUP OF FIBRINOGENS AND FIBRINS.[1]

Glycocoll	3.0
Alanine	3.6
Aminovaleric acid	1.0
Leucine	15.0
α-Proline	3.6
Phenylalanine	2.5
Glutamic acid	10.4
Aspartic acid	2.0
Serine	0.8
Tyrosine	3.5
Tryptophane	present

[1] Abderhalden and Voitinovici: Z. physiol. Chem. **52,** 368 (1907). Cf. also A. Bruner: Diss. Berlin, 1905.

5. GROUP OF NUCLEO-ALBUMINS.

	Casein from cow's milk.[1]	Casein from goat's milk.[6]
Glycocoll	0.	0.
Alanine	0.9	1.5
Aminovaleric acid	1.0	...
Leucine	10.5	7.4
α-Proline	3.1	4.6
Phenylalanine	3.2	2.75
Glutamic acid	11.0	12.0
Aspartic acid	1.2	1.2
Cystine	0.065[2]	...
Serine	0.23[3]	...
Tyrosine	4.5	4.95
Tryptophane	1.5	present
Diaminotrihydroxydodecoic acid	0.75[4]	present
Hydroxyproline	0.25[3]	...
Lysine	5.80	...
Arginine	4.84 [5]	...
Histidine	2.59	...

[1] Cf. also E. Abderhalden: *loc. cit.* and E. Fischer: Z. physiol. Chem. **33**, 151 (1901).
[2] K. A. H. Mörner: *ibid.* **34**, 207 (1901–02).
[3] E. Fischer: *ibid.* **39**, 155 (1903).
[4] E. Fischer and E. Abderhalden: *ibid.* **42**, 540 (1904).
[5] E. Hart: *ibid.* **23**, 347 (1901).
[6] E. Abderhalden and A. Schittenhelm: *ibid.* **47**, 1906.

6. GROUP OF THE HISTONS.

	Histon from the thymoid gland.[1]	Globin from Oxyhemoglobin of the horse.[2]
Glycocoll	0.5	0.
Alanine	3.5	4.2
Leucine	11.8	29.0
α-Proline	1.5	2.3
Phenylalanine	2.2	4.2
Glutamic acid	0.5	1.7
Aspartic acid	not found	4.4
Cystine	...	0.3
Serine	...	0.6
Tryptophane	...	present
Tyrosine	5.2	1.5
Hydroxyproline	...	1.5
Lysine	6.9	4.3
Arginine	15.5	5.4
Histidine	1.5	11.0

[1] E. Abderhalden and P. Rona: Z. physiol. Chem. **41**, 278 (1904).
[2] E. Abderhalden: *ibid.* **37**, 484 (1903). Cf. also E. Fischer and E. Abderhalden: *ibid.* **36**, 268 (1902).

7. GROUP OF THE PROTAMINES.

The protamines, as we have already mentioned, have been very carefully studied by A. Kossel and his students. They are mainly composed of di-amino acids. It is only recently that mono-amino acids have been detected in the protamines. A. Kossel [1] states that the protamines contain only small quantities of specific mono-amino acids. An observation somewhat at variance with this, was made [2] in a very carefully purified sample of salmine, but this may be explained perhaps by an immature condition of the testes from which the preparation was obtained. We have already mentioned that as a matter of fact the amounts of di-amino acids isolated from the proteins in the testes of the salmon depend on the maturity of the latter. It is very probable that the protamines, which evidently can be traced back to the proteins of the muscles, are formed from histons, which are to be considered as the transition stage in the transformation from the proteins of the muscles to the protamines. The salmine investigated contained alanine, leucine, and α-proline, while phenylalanine and aspartic acid were also, undoubtedly, present.

Kossel and his students give the following amino acids as the constituents of the protamines:

	In 100 grams of Albumin are present			
	Arginine.	Lysine.	Histidine.	Alanine.
	gms.	gms.	gms.	
Salmine	87.4	0.	0.	–
Clupeine	82.2	0.	0.	+
Cyclopterine	62.5	0.	?	–
Scombrine	+	0.	0.	–
Sturine	58.2	12.0	12.9	–
Cyprinine	4.9	28.8	0.	–
Cyprinine II	+	+	0.	–

	In 100 grams of Albumin are present				
	Amino-valeric acid.	α-Proline.	Tyrosine.	Trypto-phane.	Serine.
Salmine	4.3 gms.	11.0 gms.	–	–	7.8
Clupeine	+	–	–	–	+
Cyclopterine	–	–	8.0 gms.	–	–
Scombrine	–	–	–	+	–
Sturine	–	–	–	–	–
Cyprinine	–	–	traces	–	–
Cyprinine II	+	–	–	–	–

[1] Cf. A. Kossel: Z. physiol. Chem. **40**, 311 (1903). A. Kossel and H. D. Dakin: *ibid.* **40**, 565 (1904); **41**, 407 (1904).

[2] E. Abderhalden: *ibid.* **41**, 55 (1904).

8. GROUP OF ALBUMINOIDS.

	Silk fibroin.[1]	Edestin.[4]	Keratin from horn.[7]	Keratin from horse-hair.[9]	Keratin from goose-feathers.[10]
Glycocoll	36.0	25.75	0.34	4.7	2.6
Alanine	21.0	6.6	1.2	1.5	1.8
Aminovaleric acid	0.	1.0	5.7	0.9	0.5
α-Proline	present [2]	1.7	3.6	3.4	3.5
Leucine	1.5	21.4	18.3	7.1	8.0
Phenylalanine	1.5	3.9	3.0	0.0	0.0
Glutamic acid	0.	0.8	3.0	3.7	2.3
Aspartic acid	present	probably present	2.5	0.3	1.1
Cystine	—	—	very much [8]	over 10 per cent [8]	—
Serine	1.6 [3]	—	0.7	0.6	0.4
Tyrosine	10.5	0.34 [5]	4.6	3.2	3.6
Lysine	in small amounts [3]	—	—	—	—
Arginine	1.0	0.3 [6]	2.25	—	—
Histidine	in small amounts	—	—	—	—

	Gelatin.[11]	Silk Gelatin.[12]
Glycocoll	16.5	0.1 — 0.2
Alanine	0.8	5.
Aminovaleric acid	1.0	...
Leucine	2.1	—
α-Proline	5.2	—
Phenylalanine	0.4	—
Glutamic acid	0.88	—
Aspartic acid	0.56	—
Cystine	—	—
Serine	0.4 [13]	6.6
Tyrosine	0	5.0
Tryptophane	0	—
Lysine	2.75	—
Arginine	7.62 [14]	+
Histidine	0.40	4
Hydroxyproline	3.0 [15]	—

[1] E. Fischer and A. Skita: Z. physiol. Chem. **33,** 177 (1901).
[2] E. Fischer: *ibid.* **39,** 155 (1903).
[3] Fischer and Skita: *ibid.* **35,** 221 (1902).
[4] E. Abderhalden and A. Schittenhelm: *ibid.* **41,** 293 (1904).
[5] H. Schwarz: *ibid.* **18,** 487 (1894).
[6] A. Kossel and F. Kutscher: *ibid.* **25,** 551 (1898).
[7] E. Fischer and T. Dörpinghaus: *ibid.* **36,** 462 (1902).
[8] K. A. H. Mörner: **28,** 595 (1899); **34,** 207 (1901–02).
[9] E. Abderhalden and H. G. Wells: *ibid.* **46,** 31 (1905).
[10] E. Abderhalden and E. R. LeCount: *ibid.* **46,** 40 (1905).
[11] E. Fischer, P. A. Levene, and R. H. Aders: *ibid.* **35,** 70 (1902).
[12] E. Fischer: *ibid.* **35,** 221 (1902).
[13] E. Fischer and E. Abderhalden: Z. physiol. Chem. **42,** 540 (1904).
[14] E. Hart: *ibid.* **23,** 347 (1901).
[15] E. Fischer: *ibid.* **35,** 221 (1902).

A glance at the composition of the different kinds of proteins shows that they all, with the exception of the protamines, contain the same fundamental constituents. Occasionally one or another amino acid is absent; thus, glycocoll does not occur in egg- or serum-albumin; lysine is absent in the plant albumins which are soluble in alcohol; tyrosine and tryptophane in gelatin. If we compare the relative amounts of the various amino acids occurring in the individual proteins, we notice appreciable differences. This is especially striking if we compare the individual groups. In the first place we notice the varying proportions of the mono- and di-amino acids that go to make up the individual proteins. The latter are very strongly represented in the protamines, and least so among the albuminoids. Between these two extremes we find the common albumins and the histons. Very noticeable is the predominance of special mono-amino acids, e.g., glutamic acid and leucine in the albumins present in the seeds of plants. It constitutes one-third of gliadin. If we compare the individual groups of proteins among themselves, we find in many cases a very general similarity. Thus, glycocoll is absent in egg-albumin and serum-albumin, whereas the globulins invariably contain it. We are, therefore, in a position to classify chemically at least a portion of the different proteins. The fact that they all contain the same fundamental constituents makes it easier for us to understand their transformations in the animal organism.

Although the complete hydrolysis of itself serves to give us a fairly comprehensive knowledge of the amino acids participating in the construction of the albumins, it, on the other hand, does not give us any idea of the manner in which these substances are united. Until recently it had not been possible to split off complexes from the proteins, and to identify positively individual compounds containing only a part of the total amino acids. We are certainly justified in concluding that the albumoses have a lower molecular weight than the original proteins, and that the peptones are, without doubt, to be considered as still lower cleavage-products. Up to the present time the albumoses and peptones have been grouped almost entirely according to their limits of precipitation and solubility. Only in specific cases have they been characterized by the absence of a definite amino acid, or by an excess of such. Recently M. Siegfried and his students have tried to obtain products by cautious hydrolysis of some of the albuminous substances which would contain only a part of the amino acids occurring in the original protein. Siegfried[1] has described several such products. He calls them *kyrines*. It is at present impossible

[1] Siegfried: Ber. math-physikal. Kl. klg. sächsischen Gesel. Wiss. Leipzig, Sitzung II. III. p. 63, 1903; Z. physiol. Chem. **38,** 259 (1903); **43,** 46 (1904); **43,** 44 (1904). Cf. also C. Bockel: *ibid.* **38,** 289 (1903). T. R. Krüger: *ibid.* **38,** 320 (1903). W. Scheermesser: *ibid.* **41,** 68 (1904). Z. H. Skraup and R. Zwerger: Monatsh. **26,** 1403 (1905).

to say whether they are individual substances or mixtures. Thus far their study has not helped our knowledge regarding the construction of the albumin molecule. There is no doubt that the product obtained by Emil Fischer and Emil Abderhalden by means of tryptic digestion, which did not give the biuret reaction and was designated as "polypeptide," represents a cleavage product of a lower order of magnitude than the peptones. It is very probably a mixture of various decomposition products. The reason why we have not yet succeeded in getting an idea of the structure of albumin by means of partial decomposition, is due to the fact that with the large number of amino acids we should necessarily expect to find a great many different decomposition products. For instance, in a digesting mixture we find, besides peptones and free amino acids, other cleavage-products which do not give the biuret reaction. As it is almost impossible to separate the amino acids already known from such a mixture, it is, therefore, natural to expect, considering our unfamiliarity with the higher complexes, that we can hardly hope to isolate them in a satisfactory manner.

Recognizing this fact, Emil Fischer[1] recently began to investigate the constitution of the albumins from an entirely different standpoint. He chose the synthetic method. By linking the amino acids together, compounds must necessarily result which bear some relation to the albumins. After obtaining a knowledge of the characteristics of these synthetic substances, it should be possible to devise ways and means to produce analogous compounds from the albumins. We may say, at the start, that Emil Fischer's early expectations have already been partially realized. While the constitution of albumin was, until recently, very much in darkness, we can now thank Emil Fischer and his students for their extensive researches toward solving this problem. Emil Fischer's work will, undoubtedly, constitute the foundations of both the chemistry and the biology of the albumins. We must return to it in all phases of the question, and shall, therefore, only briefly outline its fundamental characteristics here.

Emil Fischer started with the assumption that the amino acids in the albumins were combined in the form of an amide-linking. He has shown that the amino acids possess the ability of easily combining among themselves, thereby splitting off water, the amino group of one amino acid reacting with the carboxyl group of another. The simplest representative of this class of compounds, glycyl-glycine, is produced in the following manner:

$$\underbrace{NH_2 . CH_2 . COOH}_{\text{Glycocoll}} + \underbrace{HNH . CH_2 . COOH}_{\text{Glycocoll}} - H_2O$$

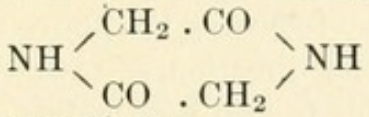
$$= \underbrace{NH_2 . CH_2 . CO . NH . CH_2 . COOH}_{\text{Glycyl-glycine}}$$

[1] Cf. E. Fischer: Ber. **39**, 530 (1906).

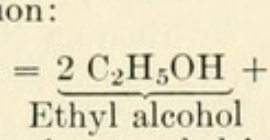
In the same way we can conceive a combination of two alanine molecules forming alanyl-alanine; from two leucine molecules we obtain leucyl-leucine, etc. Emil Fischer has called this whole class of compounds "peptides." Just as the carbohydrates are divided into mono-, di-, tri-, or polysaccharides, so Emil Fischer has classified the peptides according to the number of amino acids participating in the composition of the molecule as mono-, di-, tri-, tetra-, penta-, hexa-, etc. and poly-peptides. He characterizes them according to the amino acids entering into their composition. We can just as successfully unite two or more different amino acids as we can two similar ones in producing peptides. Emil Fischer and his students have already produced a very large number of such chains. As examples of these we mention — Dipeptides: glycyl-alanine, alanyl-glycine, alanyl-leucine, leucyl-alanine, leucyl-glycine, glycyl-*l* tyrosine, glycyl-phenyl-alanin, leucyl-proline, prolyl-leucine, seryl-serine, lysyl-lysine, arginyl-arginine, histidyl-histidine; Tripeptides: leucyl-glycyl-glycine, leucyl-alanyl-alanin; Tetrapeptides: dileucyl-glycyl-glycine, tetra-glycine, dialanyl-cystine, dileucyl-cystine; Penta-peptides: penta-glycine, leucyl-tetraglycine, etc. The number of combinations possible by linking these amino acids together is necessarily very great. If we also take into consideration the fact that all of the amino acids, excepting glycocoll, contain an asymmetric carbon atom (isoleucine has two of them), the possible number of isomeric combinations is still further increased. The number of individual optical isomers, according to van 't Hoff's formula, is represented by 2^n, in which n indicates the number of asymmetric carbon atoms which in this case — if we neglect glycine and isoglycine — is equal to the number of component amino acids.

In order to give a more satisfactory idea of the syntheses of peptides, an example of each important method will be briefly given.

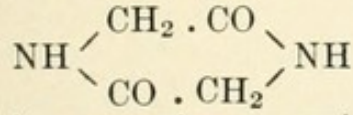
If glycocoll is converted into its ester, $CH_2 . NH_2 . CO . O . C_2H_5$, the latter goes over into glycine anhydride, a diketopiperazine:



according to the following equation:

$$2\ NH_2 . CH_2 . CO . O . C_2H_5 = \underbrace{2\ C_2H_5OH}_{\text{Ethyl alcohol}} + \text{  }$$

From this substance Emil Fischer[1] succeeded in producing the first and simplest of the peptides, by the action of dilute alkali:



$$+ H_2O = \underbrace{NH_2 . CH_2 . CO . NH . CH_2\,COOH.}_{\text{Glycyl-glycine}}$$

[1] Cf. literature E. Fischer: Ber. **39**, 530 (1906).

In the same manner, although with more difficulty, alanine hydride gives us alanyl-alanine; while from leucinimide we get leucyl-leucine.

A second method of coupling the amino acids consists in uniting them with an acid radical containing halogen, and then replacing the halogen by an NH_2 group. The following may act as an example of this form of polypeptide synthesis:

In order, for instance, to produce glycyl-glycine, glycocoll is caused to unite with chloroacetyl chloride. Chloroacetyl-glycine results:

$$\underbrace{Cl \cdot CH_2 \cdot CO \cdot Cl}_{\text{Chloroacetyl chloride}} + \underbrace{NH_2 \cdot CH_2 \cdot COOH}_{\text{Glycocoll}} = \underbrace{Cl \cdot CH_2 \cdot CO \cdot NH \cdot CH_2COOH}_{\text{Chloroacetyl-glycine}} + HCl.$$

If ammonia is allowed to act on chloroacetyl-glycine, we immediately obtain glycyl-glycine:

$$\underbrace{ClCH_2 \cdot CO \cdot NH \cdot CH_2COOH}_{\text{Chloroacetyl-glycine}} + 2\,NH_3 = NH_4Cl + \underbrace{NH_2 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2COOH.}_{\text{Glycyl-glycine}}$$

We can then take this dipeptide, glycyl-glycine, treat it again with chloroacetyl chloride, thus adding another glycyl radical to it. Treating the substance thus formed with ammonia gives us diglycyl-glycine:

$$NH_2 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOH.$$

Naturally, by following out this same method, we can use other acid radicals, thus introducing other amino acids. Should we, for instance, desire to produce alanyl-glycine, we start with glycocoll and bromo-propionyl chloride, forming α-brom-iso-capronyl chloride, which corresponds to leucine.

It will be noticed that, by following the above method, it is only possible to extend the chain in one direction, — towards the amine group. It was, of course, also desirable to add new amino acids to the carboxy side. This was accomplished by chlorinating the amino acids. If phosphorus pentachloride be added to an amino acid under definite conditions, the carboxyl group is changed into the COCl group. The free amino acid also combines at the same time with a molecule of hydrochloric acid. The hydrochloride of the amino-acid-chloride results:

$$\begin{array}{l} R \cdot CH \cdot COCl \\ \quad | \\ NH_2 \cdot HCl. \end{array}$$

The peptides can, naturally, be chlorinated, and the grouping further extended. By this means we quickly obtain long chains.

As an example of the formation of a polypeptide by lengthening the chain at the carboxyl end, we may cite the synthesis of leucyl-glycyl-glycine from brom-iso-capronyl-glycine-chloride and glycine-ethyl-ester. The resulting brom-iso-capronyl-glycyl-glycine-ester is saponified; and the tripeptide, leucyl-glycyl-glycine, results on treating this with ammonia. This, itself, can then be chlorinated, and again united with a peptide-ester, or even with a peptide itself.

We have gone into the subject of the synthesis of the peptides somewhat in detail, owing to the importance of the problem. Synthesis has always been a great factor in biological-chemical knowledge. By this means the constitutions of many substances have been determined, and many debatable questions settled. Synthesis, as we have seen, plays an even more important part in the chemistry of the albumins. With its assistance we hope to determine the constitution of the albumin molecule, and with it, also, we expect to clear up the questions relating to the first decomposition products, — the peptones.

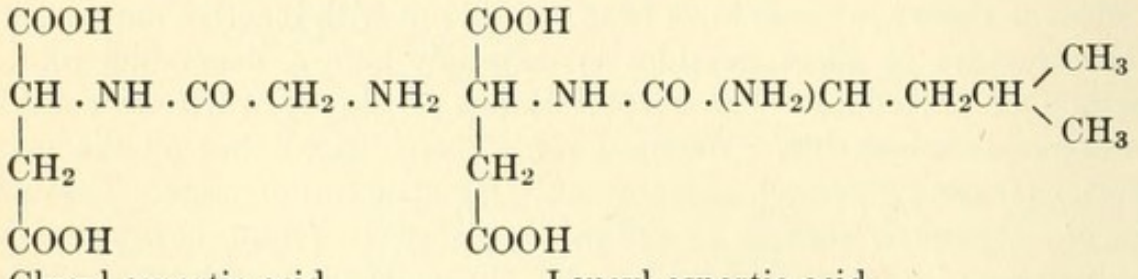
Most of these syntheses have been carried out with inactive amino acids. The structure of these peptides is definitely known, depending on the method of procedure. The subject is not so simple when we consider its stereo-chemical side. We have already mentioned that all the amino acids, excepting glycocoll, contain an asymmetric carbon atom. The number of asymmetric carbons in the polypeptides, therefore, corresponds to the number of amino acids combined in the molecule — with the exception of glycocoll. If, for instance, we have a dipeptide of the following general formula,

$$NH_2 . \underset{*}{CHR} . O . CO . NH . \underset{*}{CHR} . COOH,$$

it is necessary to have, according to van 't Hoff's formula, on account of the two asymmetric carbon atoms, indicated by asterisks, four different active varieties. If we designate the optical antipodes by *d* and *l*, the following forms will be possible: *dd*, *ll*, *dl*, *ld*. Two can produce a racemic compound (*dd-ll*) (*dl-ld*). If we start with the racemic amino acids, as has been very generally done, we necessarily expect to obtain two isomeric inactive compounds. This, in fact, is actually the case in practice. Other complications also arise, as, for instance, when we combine a racemic amino acid with an active one; for example, in preparing leucyl-*l*-tyrosine. Here we have, on the one hand, *dl*-leucine, and on the other *l*-tyrosine. In this case we expect two compounds: a *dl*- and a *ll*-variety. The relations are, of course, much simpler, if we employ only active components in the synthesis. In such a case, we obtain only active peptides; and if we proceed from those optically active forms of amino acids, which occur in nature, we must obtain amino acid chains, which correspond to those occurring in the albumin molecule. For our requirements the optically active polypeptides are naturally

of much more importance than the racemic bodies above mentioned. We have, therefore, referred to them here, only because it will be necessary to dwell on them more in detail later, when we consider more fully[1] the subject of fermentation. It is clear, that we can never tell, *a priori*, whether the polypeptides constructed from racemic amino acids, comprise the modifications existing in the albumin, or not. It is, therefore, of the greatest importance for the whole future investigation, that Emil Fischer, supported by his satisfactory method of chlorinating the amino acids, is producing polypeptides from active materials exclusively, which he obtains by splitting the racemic compounds into their optically active components.

We must not forget to mention that the peptide chains, produced from di-amino acids, and especially the di-carboxylic acids, aspartic and glutamic acids, give much greater opportunities for variation. In the latter cases the amino acids can attach themselves first to the amino groups, and then, again, at the two carboxyls, thus producing branching chains, as can be shown by the following formulæ:



Glycyl-aspartic acid

Leucyl-aspartic acid

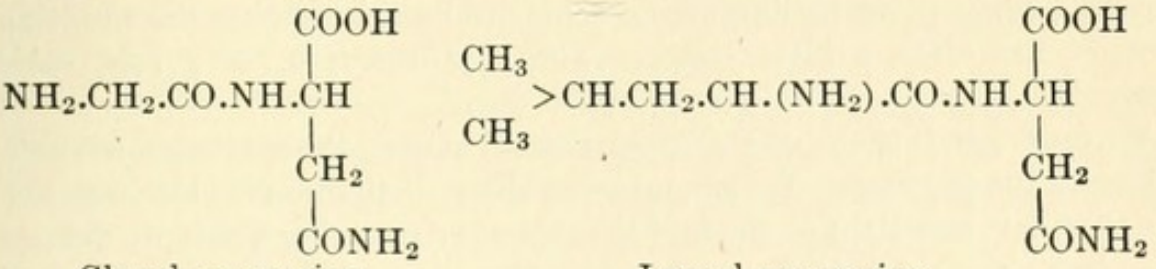
Aspargyl-mono-glycine

Aspargyl-dialanine

These illustrations may suffice to indicate the many possible combinations which may arise by introducing these dibasic amino acids into the peptide chains. We will refer here again to a discovery which we have already touched upon. If we hydrolyze albumin, that is, alter the substance by addition of water, either by acids, alkalies, or ferments, ammonia will

[1] Cf. Lecture on Ferments.

be set free. This is especially true in many of the varieties of albumin from plants. Owing to the vigorous action of the acid or alkali, secondary products, the humin substances are produced. We might imagine that the liberated ammonia stood in some relation to the formation of these substances. This assumption is, however, doubtful, because, on the one hand, the quantity of ammonia set free bears little relation to the amount of humin substance formed; and then again, ammonia is produced in especially large amounts in the hydrolysis by ferments. It would be more nearly correct to assume that acid-amides are present in the albumins, and, possibly, in the following form:[1]



Glycyl-asparagine Leucyl-asparagine

These compounds are of especial interest to us on account of the fact that the reserve albumin, which is stored in plant seeds, is broken down by fermentation when these seeds begin to germinate, while large quantities of asparagine and glutamine appear in its place. We can, of course, also conceive that these acid-amides pre-exist in the albumin. As it is, these problems are still very much in doubt.

As Emil Fischer has pointed out, the simple amide structure is not the only possible conception of the grouping of the amino acids in the protein molecule. There is no reason why piperazine rings should not be present in albumin. The hydroxyacids, like tyrosine and serine, present another possibility of combination. They can go over into esters or ether-groups by intramolecular anhydride formation.

A question of utmost importance to us is: What justification have we for assuming an anhydride system of linkage for the amino acids in the albumin molecule? There are various reactions among the members of the polypeptide group confirming this conception. Many of them give the biuret reaction. It is naturally of some interest that glycyl-glycine and triglycine do not give the biuret reaction, while tetraglycine does so in a very marked degree. It has been known for a long time that the so-called "biuret-base," which has recently been shown to be the ester of triglycyl-glycine, gives a very strong biuret reaction. It is very easily produced when glycine-ester is simply allowed to stand carefully protected against moisture. Dialanyl-cystine shows a very beautiful biuret reaction. The higher polypeptides, containing seven or more amino acids, as leucyl-pentaglycine, give a distinctively red biuret test, whose shade exactly

[1] E. Königs: Diss. Berlin, **1903.**

corresponds with that of the peptones from silk. Many polypeptides are precipitated from dilute solution by phospho-tungstic acid. It is also interesting to note that difficultly soluble amino acids produce polypeptides which are easily soluble, and that difficultly soluble polypeptides often instantaneously become soluble on the introduction of another amino acid. Tetraglycine is difficultly soluble. Leucyl-tetraglycine is easily soluble. As is well known, the peptones are all easily soluble in water, although it is necessary to remember that we are here dealing with mixtures whose components may be capable of keeping each other in solution. The changes which occur in the taste of these substances is also very interesting: for instance, when sweet-tasting amino acids are linked together the resulting product has often a bitter taste. The peptones also, as a rule, taste distinctly bitter.

We must admit that many analogies exist between the synthetic polypeptides and the peptones. We can make no sharp distinction in this direction. We must not lose sight of the fact that we are comparing a sharply defined chemical compound with a mixture. The name "peptone" does not indicate any definite compound; in fact, may not even represent distinctly analogous cleavage-products of protein. It is much better to assume that the peptones represent all stages of decomposition between that of albumoses and the amino acids.

Although we do not expect at present to obtain positive results by direct comparison in this way, excellent progress has been made by biological experiments. It was of the greatest significance that certain polypeptides were decomposed by the pancreatic ferment[1] in the identical manner as the peptones themselves. Thus glycyl-*l*-tyrosine quickly breaks down into its components, glycocoll and *l*-tyrosine. It is also especially interesting to note that the racemic polypeptides are broken down asymmetrically; that is, only half of the racemic substance is attacked.[2] The following example is given to illustrate this clearly. If we prepare the polypeptide, alanyl-leucine, from racemic alanine and leucine, we necessarily expect to obtain, according to the theoretical conceptions previously considered, four combinations, which contain the four active amino acids, *l*- and *d*-alanine and *l*- and *d*-leucine. One racemic compound contains *d*-alanine and *d*-leucine and *l*-alanine and *l*-leucine (*d*-alanine-*d*-leucine + *l*-alanine-*l*-leucine). The second is constructed in the following manner: *d*-alanine-*l*-leucine + *l*-alanine-*d*-leucine. The pancreatic ferment, however, splits only one of these two racemic combinations. Experience has already taught us that optically active amino acids in the albumins are split off; hence, we are justified in concluding, that of the two racemic compounds men-

[1] E. Fischer and P. Bergell: Ber. **36**, 2592 (1903); **37**, 2103 (1904). E. Fischer and E. Abderhalden: Sitzungsber. Akad. Wiss. Berl. **1905**; Z. physiol. Chem. **46**, 52 (1905).

[2] For further details, see Lecture on Ferments.

tioned, the ferment will attack only that specific combination which contains the corresponding optically active amino acid in the albumin. As *d*-alanine and *l*-leucine are formed by the hydrolysis of albumin, the racemic compound necessarily contains the combination, *d*-alanyl-*l*-leucine. Therefore the partially hydrolysed racemic body, obtained by fermentation is *d*-alanyl-*l*-leucine + *l*-alanyl-*d*-leucine. The unchanged portion must be *d*-alanyl-*d*-leucine + *l*-alanyl-*l*-leucine.

We have intentionally taken this example in order to show the selective action of trypsin on the large number of polypeptides, on the one hand, and, on the other, to illustrate the fact that the different behavior of the ferments towards various racemic compounds can be utilized in determining the configuration of the substance in question.

The results obtained by fermentation only become conclusive after all of the different racemic compounds have been subjected to the treatment. We can only then decide whether or not a definite compound is attacked by trypsin. The relations become much more simple when the active peptides are subjected to investigation. Even then, however, if a certain combination of amino acids is not hydrolyzed by trypsin, it does not by any means follow necessarily that such a combination is not present in albumin. We have already seen that in the breaking down of the different proteins by trypsin, different amounts of residues remain which resist digestion strongly. The albumin molecule evidently contains chains which are not broken by trypsin.

We already know, and shall later discuss the subject more in detail,[1] that the ferments, as a rule, work in a specific manner, and are very strongly influenced by differences of configuration. The fact that trypsin splits the synthetic polypeptides is a strong indication for the assumption that such anhydride-linked amino acid chains are present in albumin.

Another important proof, that completely agrees with Emil Fischer's assumptions, is obtained by a study of the behavior of the polypeptides in the animal organism. They are decomposed in the same manner as proteins, even when injected subcutaneously. Glycyl-glycine is hydrolyzed. A small part of it appears in the urine as glycocoll.[2] Dialanyl-cystine and dileucyl-cystine are similarly acted upon, and the cystine is consumed in the same manner as if it were introduced as such into the animal organism. Glycyl-*l*-tyrosin is likewise completely consumed.[3] Finally, it has been definitely proved for glycyl-glycine, tri-glycine, and alanyl-alanine,[4] that the decomposition of these peptides proceeds in the same manner as if the individual components alone had been introduced.

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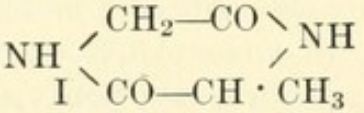
[1] E. Abderhalden and P. Bergell: Z. physiol. Chem. **39**, 9 (1903).
[2] E. Abderhalden and F. Samuely: *ibid.* **46**, 187 (1905).
[3] E. Abderhalden and P. Rona: *ibid.* **46**, 176 (1905).
[4] E. Abderhalden and Y. Teruuchi: *ibid.* **47**, 159 (1906).

Glycine anhydride and alanine anhydride are likewise utilized. They may possibly be decomposed in the intestine, and converted first of all into polypeptides. Leucyl-leucine is utilized in the same manner as leucine.[1]

The keystone of the whole proof must be regarded as certainly reached if we can obtain from albumin itself products analogous to the polypeptides. This has been accomplished. Its accomplishment was due entirely to applying the knowledge gained concerning the synthetic polypeptides to the study of the decomposition of proteins. It was found possible to act upon proteins in such a way that they did not break down entirely into their simplest components, — the amino acids, — and, on the other hand, to carry the decomposition beyond the point of the most complicated cleavage-products. Such a partial decomposition could be most easily accomplished by acting on those albumins on which the usual reagents, acids and alkalies, and, above all, the proteolytic ferments, have least effect. We have succeeded in obtaining from silk-fibroin, by a preliminary action of acid in the cold and a subsequent digestion by pancreatic juice, a polypeptide in the form of its anhydride.[2] All its characteristics, and especially its cleavage into *d*-alanine and glycocoll, as well as its conversion into the polypeptide, proved that a compound was present which corresponded to the polypeptide composed of *d*-alanine and glycine. We do not err in designating it as glycyl-*d*-alanine. The compound was isolated as a polypeptide-ester and converted into its anhydride by the action of alcoholic ammonia. By splitting the glycyl-alanine anhydride we naturally obtain two products depending on the portion of the piperazine ring attacked; thus, glycyl-*d*-alanine or *d*-alanyl-glycine result, as is indicated by the following formula:

II



If the ring is ruptured at I, we obtain, by the addition of water: glycyl-alanine, $NH_2 . CH_2 . CO . NH . CH . CH_3 . COOH$. If at II, we get alanyl-glycine, $NH . CH . CH_3 . CO . NH . CH_2 . COOH$.

The anhydride itself does not, therefore, give us the answer regarding the form of polypeptide from which it was produced. We do, however, know that alanyl-glycine is easily separated into its constituents by trypsin, whereas glycyl-alanine is not acted upon. We may, therefore, conclude that the compound isolated was glycyl-*d*-alanine. Then, again, this same cleavage-product is formed by acid action — whether by concentrated hydrochloric, or 70 per cent sulphuric acid. Under these conditions, i.e.

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[1] E. Abderhalden and F. Samuely: Z. physiol. Chem. **47** (1906).

[2] E. Fischer and E. Abderhalden: Ber. **39**, 752 (1906).

in the absence of proteolytic action, a second polypeptide could be obtained in the form of its anhydride; namely, glycyl-*l*-tyrosine and *l*-tyrosyl-glycine. Finally it has been found possible to isolate from elastin, glycyl-*l*-leucine anhydride[1] and *d*-alanyl-leucine anhydride;[2] furthermore, glycyl-*d*-alanine has been isolated directly from silk,[2] and *l*-leucyl-*d*-glutamic acid prepared from gliadin.[2] The most important discovery in this field is undoubtedly the fact that the tetrapeptide[2] obtained from silk-fibroin, consisting of two glycine molecules, one of alanine, and one of tyrosine, shows properties with which many albumoses correspond. This proves that the albumoses are not closely related to the proteins; i.e., they are not very complicated compounds. In fact, the properties of the so-called *albumoses* result from the nature of the amino acids of which they are composed. In the above case the properties of *l*-tyrosine are evident. It will be well, in the future, to drop the name *albumose*, and for the present speak only of peptones which are precipitated by ammonium sulphate and of those which are not. The more complicated cleavage-products are not precipitated by ammonium sulphate, while the simpler ones, e.g. tyrosine or cystine, are salted out by this reagent.

There is no doubt that other dipeptides, and especially those with longer amino acid chains, will shortly be discovered in the same manner. The train of thought suggested by Emil Fischer's difficult researches concerning the constitution of the albumins has thereby received complete justification. Where formerly all was darkness, a bright light has suddenly appeared. It is no longer difficult to picture the whole subject of albumin decomposition. A whole array of new problems is immediately suggested by Fischer's investigations. While his successes in developing the chemistry of carbohydrates and purines were of tremendous value in advancing both fields from a biological standpoint, it is doubtless true that his new efforts, which are of far greater biological importance, will result in great changes concerning our conceptions of the entire biology of the proteins. Much darkness, however, still surrounds many questions.

We are still incapable of interpreting the significance of the albumins as food for the animal organism. We anxiously await the moment when the fetters will be loosened, which for decades have restricted the progress of the whole subject of biology. We are deeply interested in all problems in connection with albumin. Here stands a large group of ferments — conceptions with no tangible support. The same applies to the tremendous number of toxins, anti-toxins, and allied substances. All investigators of these various subjects are anxiously awaiting the solution of the

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[1] E. Abderhalden and F. Samuely: Z. physiol. Chem. **47** (1906).

[2] E. Fischer and E. Abderhalden: Ber. **39**, 752 (1906).

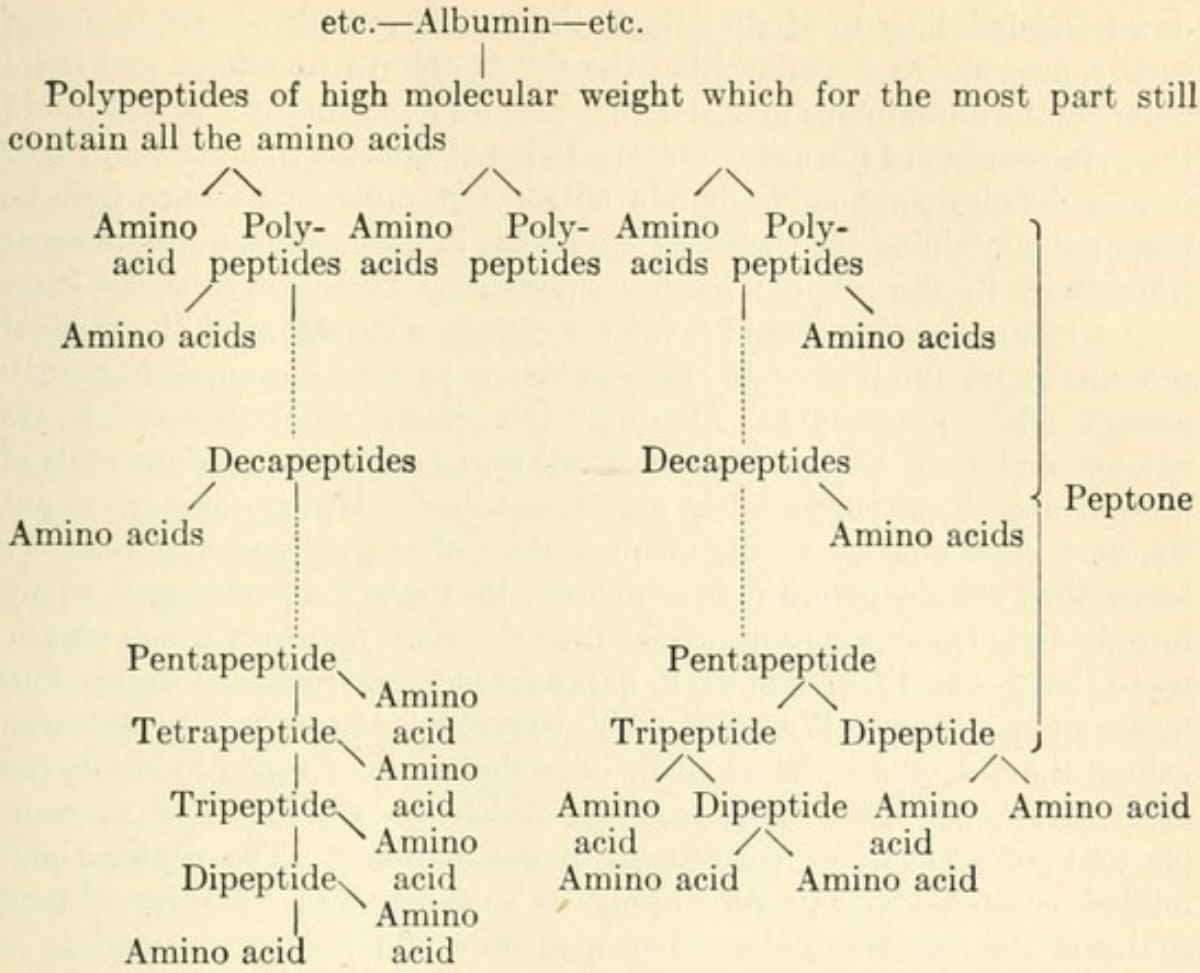
problem of the constitution of albumin! They all anticipate new impulses therefrom — new developments; and, above all, new methods. Although many dreams will not come true and many hopes may be unfulfilled, the biology of the albumins will, undoubtedly, especially in the narrower sense, open new fields of effort, and, when placed upon a satisfactory foundation, will show great progress.

Let us see now what assumptions concerning decomposition of the albumins by ferments can be based upon the above observations. We have seen that the first cleavage-products of albumin are the peptones. We can easily imagine that the albumin molecule breaks down in the first place into a series of long chains of amino acids. Even these may be much differentiated among themselves. It is not necessary that each of these chains should contain all the amino acids present in the albumin. These chains then break down into cleavage-products containing a smaller number of amino acids. We can imagine hereby that a complicated cleavage-product breaks down into several simpler ones, each containing more than one amino acid. Many observations indicate, however, that the amino acids themselves appear at an early stage.

It is of interest that practically simultaneously with the appearance of tyrosine, cystine, tryptophane, etc., in the digesting liquid, those products called albumoses diminish in amount, and finally disappear. This corresponds to the observations made in the breaking down of tetrapeptides. As soon as the tyrosine is removed by trypsin, the albumose character disappears. Tyrosine can be detected within a few hours after the beginning of digestion. Subsequent decomposition takes place with the constant production of more amino acid. Smaller chains are produced from the peptones with larger amounts of amino acids, until finally the greater part of the amino acid chains are decomposed into their constituents. The peptones are therefore to be considered as a large mixture of various kinds of polypeptides. The best distinction that we can make is that only those polypeptides belong to the peptone class which will give the biuret reaction. Unquestionably, the term *peptone* will gradually disappear, and we shall eventually deal only with chemical individuals.

We shall here refer, as we now do in the synthetic chemistry of albumins, to di-, tri-, tetra-, and polypeptides. The biuret reaction is only used as a convenience in indicating the limit of the branching compounds to be included in the peptone class. The polypeptides which give this reaction gradually pass over into those which no longer do so. Between the peptones of the longest chains and the simple amino acids there are continuous transitory stages.

The decomposition of the proteids by means of trypsin can be illustrated in the following manner:



This is, of course, only a scheme, and we are frank in stating that future investigations alone can establish its validity. Many other combinations are possible. We can easily imagine that the amino acid chains also break down in such a manner that the amino acids are not produced immediately, but that chains are formed, containing only a part of the original amino acids occurring in the polypeptide. In this connection we are reminded of dialanylaspartic acid. If we assume that the chain is lengthened from the alanine group, one of the chains could very easily be split off, leaving an " aspartic-acid-mono-polypeptide." We must also remember that there are polypeptides which are evidently not affected by the digestive ferments. We can very easily imagine such combination as a result of our investigations concerning the behavior of the synthetical polypeptides to the pancreatic ferments. It is not without interest, that the mixture of polypeptides observed in the tryptic digestion of albumin contained large amounts of phenylalanine and proline, the very acids from which synthetic peptides were formed that resisted the action of ferments.

If, departing from the plan of our lectures, we attempt here to unravel a problem which, according to the experimental knowledge at hand, is not yet fully ripe for discussion, this is done partly because many discoveries give important support to these views, and largely because only upon such a foundation are we able to obtain a clearer understanding of the breaking

down and building up of albumin in the animal organism. By means of such a progressive decomposition the cell is able to transform and build up anew the albuminous material that reaches it, so that it is suited for all the requirements of the cell-content. It is not difficult to understand how from a definite compound-albumin all sorts of different proteins may be prepared containing the various amino acids in proportions quite different from those in the original mother substance. It is not necessary that such a transformation should involve a complete reduction of the original protein to its fundamental constituents; a partial decomposition may answer all requirements. Although the details of fermentation are not yet definitely known, we can, however, consider the fundamentals of protein decomposition as fairly well established. We can also point out the very close analogy to the disintegration of the polysaccharides. We know that starch, before it is converted into dextrose, undergoes many intermediate transformations, concerning the exact nature of which we are, up to the present time, as much in darkness as we are in regard to the albumoses and peptones. We are only able to recognize the former as mixtures, calling them *dextrins*. The first definite chemical cleavage-product is the disaccharide maltose. The dextrins, which we still consider as complicated polysaccharides, correspond to the peptones. The dextrins and related compounds may very easily be considered as mixtures of long chains of dextrose molecules. The maltose would then correspond to a dipeptide. The conditions in the carbohydrates are comparatively simple, because starch is considered as composed of a series of only one kind of molecular combination, i.e., dextrose, — whereas, with the albumins, there are many different fundamental substances. On the other hand, we are also acquainted with proteins — like the protamine, salmin — which are of simple construction, being mainly composed of arginine, while we also know of polysaccharides in the vegetable world which are not far behind the proteins as regards complexity. As an example of a " mixed " disaccharide, we have cane-sugar, which breaks down into one molecule of dextrose and one of lævulose, and also to mannorhamnose, which splits into one molecule of mannose and one of rhamnose.

We are also acquainted with mixed trisaccharides. On hydrolyzing rhamninose, a glucoside occurring in the fruit of *Rhamnus infectoria*, two molecules of rhamnose and one of *d*-glucose are obtained. Gentianose, from varieties of *Gentiana*, contains two molecules of glucose and one molecule of fructose. We know of a large number of polysaccharides, in whose constitution many sugar varieties participate: pentoses, methylpentoses, hexoses, etc. We only mention these examples to illustrate the analogy between the polysaccharides and the proteins. A very large number of combinations are possible by using many different constituents. The proteins predominate in the animal organism. They

are characteristic of the individual tissues. The secret of the individuality of the various cells undoubtedly depends on their configuration. Every species, every variety,— in fact, every individual,— has its own " albumin." According to this conception, the carbohydrates are of less significance to the animal organism. They are essentially food materials, and are necessarily but a small factor in the production of animal tissues. It is entirely different when we consider the vegetable kingdom. The carbohydrates predominate here. They construct the plant tissues, and all the numerous living processes are dependent on their presence. Hence their variety, and their production from the heterogeneous elements. Carbohydrates, as regards their entire physiological significance and their composition, are to the vegetable world what the protein substances are to the animal organism.

The greater the number of amino acids participating in the composition of a protein, the wider the uses to which that protein can be put. On the other hand, the simpler the function of the protein, the more dominant becomes one or the other of the amino acids. Fibroin from silk, for instance, contains 36 per cent glycocoll, and over 20 per cent alanine; elastin gives us 26 per cent glycocoll, and over 10 per cent leucine; gliadin, a " reserve albumin " of plants, contains over 30 per cent glutamic acid; while in the protamines we often find over 80 per cent arginine. It would, of course, be wrong to compare these albuminous bodies with others, and say that they were simple in composition. They are simply more homogeneous. Whether the amino acids grouped together are all of a kind or much diversified, has but little bearing on the question of their constitution or configuration.

The observation that the pancreatic ferment does not attack some of the synthetic polypeptides, and the discovery that during the digestion of proteins many complicated cleavage-products remain, which still contain large percentages of glycocoll, phenylalanine, and *l*-proline, leads us to conclude that the animal organism utilizes such groups, or similar ones, as a foundation, or back-bone, for building up new albuminous substances. It is certainly of some significance that elastin contains so much glycocoll and leucine. The combination leucyl-glycine, on the other hand, is obviously unacted upon by trypsin. Silk also contains such compounds, as is shown by the discovery of glycyl-*d*-alanine in it. The cell can protect itself by forming just such combinations. The fact that most vigorous fermentation processes are continually taking place in the cell, and that in spite of this the cell retains its own constituents — its amour — intact, becomes much more comprehensible to us from such considerations.

The significance of Emil Fischer's synthetic polypeptides lies, moreover, in still another direction. Up to the present time it has not been possible to test the proteolytic ferments as to their homogeneity. We have to

content ourselves with a knowledge of their methods of action. There is no doubt that with the assistance of these synthetic polypeptides, new questions will arise in this connection and will probably be solved. We shall be able to determine whether the various kinds of animals possess the same kinds of proteolytic ferments, or those which act differently. We are inclined to the latter belief, at least in special cases. We know that the feathers of birds and the hair of the *Mammalia* are subjected to the inroads of parasites, being eaten up by them. These minute animals must possess much more vigorous proteolytic ferments than have been vouchsafed to us, because the keratins are, so far as we know, entirely indigestible by vertebrates. We also hope to obtain a complete explanation of the differences between the action of trypsin and of pepsin by studying the relations of these proteolytic ferments toward the polypeptides.[1] Up to the present time none of the synthetic peptides have been acted upon by pepsin.[2] It is possible that the amino acid chains utilized were not long enough. In fact, pepsin-hydrochloric acid seems to decompose albumin in a manner entirely different from that characteristic of trypsin.[3] Evidently peptones, and other cleavage-products which do not give the biuret reaction, are produced. There are, however, no amino acids formed.[1] The significance of gastric digestion is still quite obscure. It may possibly be that it causes a preparatory cleavage of the albumins, so that the trypsin has more opportunity to act. It can also be shown experimentally that tryptic digestion is hastened and proceeds much farther, if a pepsin-hydrochloric acid one precedes it.

With the assistance of the polypeptides we also hope to get an insight into cell metabolism. It has already been demonstrated that the tissues, especially the liver, contain proteolytic ferments, which are capable of dissolving bonds between amino acids that are unattacked by trypsin. Thus, an extract from the liver will split glycyl-glycine completely into its components.[4] By extending these investigations to include the various organs, we will ultimately succeed in finding those which are the most important factors in the decomposition of the albumins.[5] The polypeptides will also be of great service to us for comparative purposes. It will be of the greatest interest to learn whether the representatives of the various animal classes will disintegrate the individual polypeptides in the same manner, or whether there will be differences in the decomposition products.

From all these problems it is at once obvious how important is the synthetic linking together of the amino acids into the polypeptides for all branches of biological science.

[1] E. Abderhalden and P. Rona: Z. physiol. Chem. **47** (1906).

[2] E. Fischer and E. Abderhalden: *ibid.* **46,** 52 (1905).

[3] E. Fischer and E. Abderhalden: *ibid.* **40,** 215 (1903). F. Obermeyer and E. P. Pick: Hofmeister's Beitr. **7,** 331 (1905).

[4] E. Abderhalden and O. Rostoski: *ibid.* **44,** 265 (1905).

[5] E. Abderhalden and Y. Teruuchi: Z. physiol. Chem. **47,** 1906.

LECTURE X.

ALBUMINS OR PROTEINS.

IV.

Degradation and Formation of Protein in the Animal and Vegetable Organisms.

It will be necessary to learn something about the origin of the proteins in our food, before proceeding to discuss the behavior of such substances when taken into the animal organism, their decomposition in the alimentary tract, their absorption and assimilation, and the end-products resulting from their combustion. The animal organism, as we shall see later, is only capable of synthesizing its albumins from the same material, or from its immediate decomposition products. It is incapable of utilizing inorganic nitrogenous compounds to produce its albumins, and similarly the animal cells cannot synthesize the albumins from organic nitrogenous substances, unless these are related directly to the albumins themselves. The animal organism is entirely dependent on the vegetable kingdom for its albumin requirements. The plants prepare the proteins for it.

When living material, whether it be vegetable or animal, decays, its organic constituents undergo putrefaction. Ammonia, in large amount, is finally produced from nitrogenous compounds. This is changed into nitric acid in the soil, nitrates resulting. The formation of saltpeter in the soil is a process which has been known for a long time. Even H. Davy [1] was aware of the production of nitrates at the expense of ammonia and atmospheric oxygen. It was eventually discovered that the process of forming saltpeter, also called "nitrification," was due to the vital activity of microbes. The pure culture of these organisms followed much later.[2] This was due to the fact that the bacteria possess the peculiar ability of thriving on an exclusively inorganic nutrient medium, as was shown by Hueppe [3] and Heræus.[4]

They satisfy their nitrogen and carbon requirements from ammonium

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[1] Elements of Agricultural Chemistry, **1814.**

[2] S. Winogradsky: Compt. rend. **110,** 1013 (1890).

[3] Tageblatt Naturforscher-Versammlung Wiesbaden, **1887.**

[4] Zentr. Bakt. **3,** Nr. 13 (1887).

carbonate.[1] Nitrification is not a simple process. It requires the simultaneous activity of several varieties of bacteria. One oxidizes the ammonia to nitrite, and another converts the nitrite into nitrate. The nitrifying bacteria are found everywhere. They play an extremely important part in the economy of nature. They effect the nitrogen cycle.

Even the nitrogen which the animal organism utilizes for its nutrition, is finally returned to the ground again as ammonia. We shall see later that the largest part of albuminous nitrogen reappears in the form of urea in the urine of mammals. Under the action of specific bacteria this is broken down into ammonia, which is then converted into nitrates. The plants utilize this anew for the synthesis of albumins, and the nitrogen completes its cycle of usefulness, first, in the form of inorganic, and then as organic compounds. This process is not as simple in practice as the statement indicates. A large amount of free nitrogen is produced simultaneously with the combined nitrogen. When nitrogenous organic material undergoes combustion, free nitrogen is obtained as well as ammonia. The amount of the former may be very considerable under favorable conditions. This is the case when the combustion is carried out at a high temperature with a liberal supply of air. Nitrogen is also liberated in large quantity by the explosion of gunpowder. It is liberated not only by artificial processes, but also through the intermediacy of organisms occurring in nature. To be sure the assumption that free nitrogen is liberated in the metabolism of higher plants has been disproved by exact investigations, just as the oft-repeated question as to whether nitrogen is eliminated as such, from albuminous material in the animal organism, has been answered in the negative. We are acquainted on the other hand with a large number of organisms of common occurrence which are incapable of liberating nitrogen from organic compounds, but can do so from nitrates. This process, also called *denitrification*, has been known for a long time.

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[1] It may be interesting to note that there are also other organisms capable of utilizing inorganic instead of organic materials. The group of sulphur bacteria is best known. Kernels of sulphur are found in their cell-bodies. They thrive in sulphur springs and produce therein a characteristic flora. They constitute the group of *Beggiatoa*, and are aërobic. The *Beggiatoa* are capable of oxidizing sulphuretted hydrogen, when in the presence of oxygen, to sulphur. The stored-up sulphur is then further utilized in the cells, sulphuric acid being formed, which seems to be their characteristic product. They need only small amounts of organic material. During the oxidation of a gram-molecule sulphuretted hydrogen to sulphuric acid, 62.4 cal. of heat energy are obtained by the bacteria.

Many thread-bacteria, especially *Leptothrix ochracea*, form other examples. They oxidize ferrous carbonate into a ferric salt, which is decomposed with the formation of ferric hydroxide. Winogradsky, to whom we owe our knowledge of these sulphur and iron bacteria, suggests the possibility of the latter participating in the formation of bog-ore deposits.

Davy [1] called attention to the fact that gaseous nitrogen was set free from decomposing organic material in the soil. Gayon and Dupetit [2] were, however, the first to announce that the nitrogen originated from the nitrates. Since that time a large number of bacteria have been isolated which produced nitrogen from nitrates. Gayon and Dupetit cultivated two varieties of anaërobic bacteria from the soil, which they called *bacterium denitrificans*, α and β. Denitrifying bacteria can live without oxygen. They utilize the nitrates as a source of energy. They work, in a sense, in constant opposition to the nitrifying bacteria. With a liberal supply of oxygen the latter will predominate, while if the oxygen supply be diminished, the reverse will be true. There is no doubt but that appreciable amounts of nitrogen are being continually set free. Nitrogen would be constantly withdrawn in this way from the organized world were it not for the fact that other processes are at work to recombine it. We also know that atmospheric nitrogen and oxygen are combined under the influence of electrical discharges, producing nitric acid. The amount of nitrogen combined in this manner must necessarily be small. It is certainly insignificant in comparison with the production of nitrogen from other sources. During recent years various bacteria have been isolated which possess the faculty of assimilating, or " fixing," the atmospheric nitrogen. Berthellot [3] first called attention to this process. He found an enrichment of soils which were free from higher plants, and whose only source of nitrogen was the atmosphere. Winogradsky [4] was the first to succeed in isolating a bacterium which was capable of fixing nitrogen directly from the air. This was the anaërob *Clostridium Pasteurianum*. Winogradsky showed that a culture of this bacillus, shut off from every other source of nitrogen except the air, was capable of assimilating 24.7–28.9 grams nitrogen in 15–20 days. It is interesting to note that this *Clostridium* is not found alone, but is accompanied by two aërobic bacteria. Evidently this is a case of symbiosis. The aërobic bacteria remove the oxygen which is deleterious to the development of the *Clostridium*. They undoubtedly receive nitrogenous material from the latter in return. Since this discovery other bacteria have been isolated, which possessed the ability of assimilating free nitrogen. Krüger and Schneidewind [5] describe a bacterium, a culture of which in 62 days converted 4.6–8.5 grams atmospheric nitrogen into albuminous nitrogen. It is noteworthy that the *Clostridium* has been found in the slime of ocean bottoms, and in the plankton of salt

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[1] Elements of Agricultural Chemistry.

[2] Compt. rend. **95**, 644 (1882).

[3] *Ibid.* **101**, 775 (1885); **104**, 205 and 625 (1887); **106**, 569, 1049, 1214 (1888); **107**, 372 (1888); **108**, 700 (1889); **109**, 277 and 417 (1889); **115**, 569 (1892); **116**, 842 (1893).

[4] Compt. rend. **116**, 1385 (1893); **118**, 353 (1894).

[5] Landwirtsch. Jahrb. **29**, 801 (1900).

and fresh water. The following observations of Kühn [1] are mentioned to give an idea of the significance of the activity of these nitrifying bacteria. A field, which for twenty years had not had any nitrogenous fertilizer added to it, gave an average return of 1976 kilograms of grain per hectare. Not only was there no decrease in the annual yield due to the gradual removal of the nitrogen of the soil, but it actually showed an increase of 11.6 per cent in grain produced. There was annually withdrawn from a hectare of land in crops of rye, from 25–30 kilograms of nitrogen. This amount of nitrogen must have been taken from the air and transferred to the soil. Even the fallen leaves in forests assimilate nitrogen by the activity of the bacteria contained within themselves. It is not at all impossible that these bacteria are the pioneers in converting decomposed rock into arable land.

It is a well-known fact that some plants, for instance the legumes, enrich the soil with nitrogen, while others only deplete it. The practical farmer utilizes this fact by not planting cereals year after year on the same soil, but rotates the legumes and the grains. Hellriegel [2] and Willfarth [3] have satisfactorily explained the whole subject as a result of their experiments. They proved that the legumes assimilated the nitrogen, and showed that this formation was intimately connected with the enlargements of the so-called " root-nodules or tubercles " of these plants. It was also shown that the legumes could be made to grow nodules on sterilized soil if infusion of ordinary soil be sprinkled over it. There must evidently be micro-organisms present in the soil which cause the formation of these nodules. The infusion obtained from the soil loses its activity on being heated. The *graminæ* act entirely different. They are not influenced in their consumption of nitrogen by any infusion from the soil. Their nitrogen assimilation is dependent on the nitrates already present in the soil. Free nitrogen is of no service to them. The legumes, on the other hand, are entirely independent of any increase of nitrates. If the legumes are grown in sterilized soil, they behave like the graminæ. They lose the faculty of fixing the free nitrogen, and rely entirely upon the nitrates in the soil. The following experiments are referred to as illustrating the assimilation of nitrogen through the nodules of the legumes. Schloesing and Laurent [4] cultivated legumes in sterilized soil and in sterilized glass cylinders. The amounts of carbon dioxide, oxygen and nitrogen in the air

[1] Frühlings landw. Ztg. p. 2, 1901; quoted by F. Czapek: Biochemie der Pflanzen: G. Fischer, p. 131, 1905.

[2] Tageblatt Naturforscher-Vers. Berlin, **1886**, p. 290.

[3] Tageblatt Naturforscher-Vers. Berlin, Wiesbaden, **1887**, p. 362; Zeit. Ver. Rübenzuckerind. Beilageheft, **1888**, p. 234, and Ber. botan. Ges. **7**, 138 (1889). For further literature see J. Vogel: Zent. Bakt. u. Parasitenkunde 15, 11, 33, 1905.

[4] Compt. rend. **111**, 750 (1890); **113**, 776 (1891); **115**, 881, 1017 (1892); Ann. Inst. Pasteur. **6**, 65 and 824 (1892).

present were exactly known. Sterilized water was added in one experiment, while the others were watered with infusions of powdered nodules. Three months later the air was removed from the cylinders and the amounts of nitrogen present estimated. It was found that it was diminished only in those cases where nodule extractions had been added. Two of these experiments in which root nodule infusions were used gave the following values:

	I	II
Nitrogen present in air at beginning of experiment	2681.2 cm.³	2483.3 cm.³
Nitrogen present in air at end of experiment	2652.1 cm.	2457.4 cm.
Nitrogen absorbed	= 29.1 cm. = 36.5 mg.	= 25.9 cm. = 32.5 mg.

The nitrogen absorption can be shown even better by the following table. In experiment III there were no root nodules present, whereas I and II contained these:

	I	II	III
Nitrogen in the soil and in the leguminous seeds (peas) at the start	32.6 mg.	32.5 mg.	32.5 mg.
Nitrogen in the soil at the end	73.2 mg.	66.6 mg.	33.1 mg.
Nitrogen taken up by the plants	40.6 mg.	34.1 mg.	0.6 mg.

These root nodules contain bacteria, as has been proved by Beijerinck.[1] They live in symbiosis with the cells of the nodules. Beijerinck names the bacillus *B. radicicola*. It is widely distributed in land and water. Recent investigations indicate that this nitrifying organism is not a separate individual. It seems as if various bacilli are assigned to the different varieties of *Papillionacæ*. Successful inoculations of the nodules have only been possible with closely related members of this family. For instance, we have not succeeded in forming nodules on the robinia roots by means of the bacteria from peas. It is also very interesting to note that *Soja Hispida* very often fails to produce nodules in European gardens, but will do so when impregnated with Japanese earth. To indicate the importance of these discoveries we may add that the nodule bacteria have become an article of commerce.

It is problematical whether these nodule bacteria are restricted to the *Papillionacæ*. There are indications that they are also found in other plant species. They are believed to be present in the *Rhinantacæ*, *Elæag-*

[1] Bot. Zeit. (1888) p. 725.

nacæ, Cycadeæ, Coniferæ, etc. The assumption has also been made that the hypomycetes, which often exist symbiotically with the roots of higher plants, have a similar function to that of the nodules. These experiments have not yet been completed. Many observations on the wild plants seem to indicate a wider distribution of such symbioses. We know of many plants which, year by year, always grow in the same spot with the usual profusion, while many others suddenly spring up and after a short " period of blossoming " gradually fade away. In this way the dominant species in a meadow, and especially in a dump-heap, may follow one another in rapid succession, probably because these short-lived plants rely exclusively on the nitrates of the soil.

It is of great importance to us in considering this subject to realize that nature possesses ways and means of assimilating the free nitrogen of the air. On the one hand, nitrogen is set free; and on the other, it is recombined. We are not prepared to state the relations existing between these two processes, — whether they maintain an equilibrium, or whether the liberation of nitrogen far exceeds that of recombination. It would be very interesting to know the compounds into which these organisms convert the nitrogen.[1] At present we have no knowledge concerning this. We assume that the final substance produced is albumin, which is then, in part, assimilated by the plants with the help of fermentation.

The discovery that ordinary nitrogen can be directly assimilated closes the chain of the nitrogen cycle, which had apparently been broken open by the discovery of the denitrifying organisms. We have forgotten to mention one point. We shall see later, when considering the inorganic nutrient materials, that the earth possesses the power of " fixing " certain constituents. This applies, for instance, to the important elements, phosphorus, potassium, ammonia, etc., which are so necessary for the development of the plants. As soon as their solutions come in contact with certain constituents of the earth they are changed into insoluble compounds, and are thus protected from being washed away by rain-water. The salts of nitric acid, the nitrates, behave quite differently. They are not absorbed by the earth. They are readily soluble in water, and are continually being washed away, carried to brooks and streams, and finally appear in the ocean. The amount of nitrogen abstracted yearly from the soil in this manner is really enormous. K. Brandt[2] estimates it at 40,000,000 kilograms per year. We are confronted with the question of how this large amount of nitrogen is returned to the general nitrogen cycle. That this must take

[1] Recently it has been found possible to convert technically large amounts of atmospheric nitrogen into its compounds.

[2] Wissenschaftlichen Untersuchungen. Report of the Kommission zur Untersuchung der deutschen Meere, **1899** and **1901.** Cf. also, E. Schulze: Schweizer. landwirtschaftl. Zentralblatt. **1902.**

place is evident from the fact that the vegetation which has grown for thousands of years still continues doing so in the customary manner, in spite of the leaching of the mainland which has taken place. There is practically no difference in the behavior of the plants and animals of the ocean and those of the mainland. Marine plants assimilate carbon dioxide; this process also requires the assistance of the sun's energy, which accounts for the absence of plant life at great depths to which the sun's rays cannot penetrate. Marine plants also require nitrates for the formation of their albuminous constituents. The fishes in the ocean also obtain their albumin ultimately from the vegetable world. Marine vegetation is incapable of utilizing all of the immense amounts of nitrogen presented to it. Nitrogenous compounds are set free from dead plants and animals through putrefactive processes. These are changed into ammonia, which then goes over into nitrites and nitrates in the same manner as on the mainland. The ocean occasionally casts large masses of sea-weed on the shores. The amount of nitrogen from this source is infinitesimal in comparison with that leached from the soil. It is, therefore, of great interest to learn that the ocean also possesses denitrifying bacteria, which are continually setting nitrogen free. They complete the cycle of the nitrogen washed into the ocean as nitrogen compounds.

The significance of the great value of the denitrification process now becomes apparent, in contradistinction to its undesired appearance in the soil. The great part which the nitrogen-assimilating bacteria play in nature, is now explained. The living requirements of the whole world of organisms guarantee an interchange of material! These smallest living beings furnish us with the fundamental requirements of our existence. The discovery of the denitrifying bacteria has also solved an apparent contradiction. It is well known that the concentration of plant and animal life on the mainland diminishes from the equator towards the poles. This is not the case in the ocean. This circumstance is very striking, as one would be led to expect much better development of conditions in the tropical seas, owing to the great preponderance of light, in comparison with the dark arctic regions. It is probable that this state of affairs may be traced to the denitrifying bacteria in some manner. A tropical environment is ideal for them and their activities. They develop to best advantage at a temperature of 25°–30°C. They would, therefore, abstract more nitrogen from marine plants in tropical seas, than in the seas of the arctic zone. We will at once state that this is merely offered as an explanation. We know that the growth of all organisms is governed by the Law of the Minimum, i.e., of all the substances which are accessible to the organism, the amount utilized is governed by the amount of that substance present to the smallest extent. While the marine plants may have a large quantity of available nitrogen in the form of nitrates, it may

be that the supply of, say, phosphorus, for example, is unusually small. The plants would then only be capable of utilizing the nitrates in proportion to the amount of phosphorus present. It is perhaps possible that the conditions of nourishment are different in different zones.

In any case, the free nitrogen in organic nature plays an exceedingly important part in the nitrogen cycle. The amounts of nitrogen produced artificially, whether by the combustion of organic substances or by the explosion of gunpowder, although large, to be sure, have little effect upon the equilibrium of the nitrogen cycle. Such amounts are in time recombined and again take part in the natural cycle.

Albumin contains, besides nitrogen, also carbon, hydrogen, and sulphur. We have already pointed out the central position that the carbohydrates in the plant organism play in the assimilation of carbon dioxide, and called attention to the fact that it evidently forms the starting point of the synthesis of albumin. In another place we shall go into this matter more in detail. Here we shall merely suggest that certain relations are known to exist between the simple carbohydrates and individual amino acids, so that we can easily understand the formation of the latter from the former. Thus carbon and hydrogen for the synthesis of albumin originate in the air and water. In this form the animal organism gives back these elements to the vegetable kingdom again.

The plants obtain their sulphur from the soil, in which this element is present as sulphates of the alkalies and alkaline earths. Plants utilize sulphur almost exclusively for the formation of albumin, and it also reaches the animal organism in this form. In the animal, this sulphur is largely converted into sulphuric acid, and given back to the general cycle in the form of its alkali salts.

It is difficult to estimate the value of albuminous substances to the vegetable kingdom, from the experiments at hand. Our knowledge of the metabolism of albumin in plants is remarkably slight. We are certain that it by no means plays the same part in the plant that it does in the animal organism. We should like above all to know whether the plants consume albumin at all, i.e., oxidize it. Oxidation processes, as we have seen in considering the assimilation of carbonic acid, play a subordinate *rôle* in plants to processes of reduction. They undoubtedly take place to some extent. We do know that the albumin in the animal organism is almost entirely decomposed, partly into urea, or partly into uric acid. Such substances have been looked for in vain in the vegetable kingdom.[1] It is interesting, however, to learn that many plants produce substances closely allied to the uric acid group, namely, methylated-xanthine derivatives, such as theo-bromine and caffeine, both of which are important con-

[1] A discovery of urea has been reported among the varieties of *Lycoperdaceæ*. Cf. Max Bamberger and Anton Landsiedl: Monatsh. **24**, 218 (1903).

stituents of table accessories. Concerning the relations which the purine bases, and their accompanying intermediate products, bear to the metabolism of albumin, nothing can be affirmed. It is, of course, possible that they are derived from the nucleins. The alkaloids have often been assigned a relationship to albumin. We know little about their origin. It is possible that the dyestuff, indigo, is related to albumin metabolism. In discussing the decomposition products of proteins we met with tryptophane, skatole-amino-acetic acid, and have seen that putrefaction decomposes this into indole, skatole, skatole-acetic acid, and skatole-carboxylic acid. Skatole is rarely found in plants. The Japanese wood, *Ulmacaæ, Celtis reticulosa Miq.*, contains approximately 1 per cent of skatole. Recent investigations also indicate the occasional appearance of indoxyl among plants. We know nothing definite about the relations of the indoxyl derivatives to the albumin decompositions in the plant organism.

We are not much better informed concerning the processes participating in albumin syntheses. The nitrates which the plant takes up must be reduced. It is now usually assumed that the leaves are mainly instrumental in effecting the albumin syntheses; the idea being that amino acids are first formed, which by recombining among themselves produce higher complexes, and finally albumin itself. Although the leaves themselves are incapable of utilizing the nitrogen of the air, as such, observations have been made indicating that they can absorb small amounts of ammonia. It seems that light has also an effect upon the production of albumin. Albumin is, to be sure, formed in the dark, but the synthesis proceeds much more rapidly in the sunlight. We are still entirely in doubt as to how the amino acids are formed. We can assume, as previously indicated, that they are derived from simple carbohydrates, — for instance, from glycerose; or we can just as easily imagine that the formation of the amino acids is a more direct assimilation process. The manner in which the nitrates are utilized presents a difficult problem. It is certain that they must be reduced. The nitrite formation has also been followed directly. We have assumed that HNO_3 goes over into HNO_2, and this into HN:O. The addition of water would produce hydroxylamine, $NH_2 . OH$, which, in conjunction with formaldehyde, obtained by the reduction of carbonic acid, produces formamide, $HCO . NH_2$.[1] The formation of hydrocyanic acid and the reduction of a nitrate to ammonia have also been suggested. It is almost impossible at present to draw any positive conclusions. The synthesis of albumin in the organism of plants, or, perhaps better stated, the formation of the amino acids, as yet remains entirely unexplained.

We have a better conception of albumin metabolism in germinating seeds. Ripe seeds contain large stores of albumin. They, therefore, act

[1] A. Bach: Compt. rend. **122,** 1499 (1896).

as sources for the production of plant proteins. We note great changes as soon as germination begins; in fact, the entire cell contents are drawn upon. We have already considered the conversion of the carbohydrates into fats, and vice versa. Besides such processes hydrolysis undoubtedly causes other metabolic changes to take place. The proteins are disintegrated into their components by the activity of proteolytic ferments. Complicated products, "peptones,"[1] are first formed, and finally amino acids, which, at least in part, are then decomposed further. We may compare the beginning of germination with the intestinal processes. The purpose is in many respects the same. The germinating cell disintegrates, in order to utilize the various elementary components for the construction of a new cell body. We are still undecided whether the protein molecule is completely, or only partially, disintegrated by hydrolysis. Asparagine has been detected in germinating legumes, while glutamine has been observed in other cases. The amount of asparagine may be increased by germinating in the dark. We are still unaware of the significance of the formation of asparagine. It is possible that it does not participate further in the construction of albumin, but that it acts as an intermediate step to other nitrogenous substances; or, even, that it has nothing further to do with such substances, but now enters into relations with the carbohydrates and fats.

That asparagine does not directly participate in the synthesis of albumin, which immediately follows its breaking down, is evident from the fact that it does not diminish to the same extent that the albumin formation progresses.

We will add here that the seedlings at the beginning of their existence also disintegrate their other constituents, the nucleins, fats, etc., into the components. It reconstructs everything anew.[2] We may compare the metabolism of this germinating seedling with that of the animal.

When we take everything that we know about the formation of the proteins in the vegetable kingdom and their albumin metabolism into consideration, we find it very difficult to formulate any distinct conception of what actually occurs, based on experimental results. We have felt that we ought to consider briefly this subject here, because, as we have repeatedly said, we can expect to have a complete understanding of biological processes only when we have as broad a foundation as possible. There is no sharp dividing line between the plant and animal kingdoms. It would be a gross error to try to separate the biological investigations in these two fields. The absolute dependence of the animal organism on the products of the vegetable kingdom forces us to consider in detail the biological chemistry of plants.

[1] W. R. Mark: Z. physiol. Chem. **42**, 259 (1904).

[2] Cf. among others, J. R. Green and H. Jackson: Pr. Roy. Soc. **77** (B), 69 (1905).

Let us now return to the relations of the animal organism to the albumins it obtains in its food. They are not at all attacked by saliva, with which they first come into contact. This secretion does not contain any ferment which can act upon the proteins.

The albumins are next subjected to the action of pepsin in the stomach. Spallanzani [1] was the first to give a clear demonstration of the digestive action of the gastric juice. Normal gastric juice reacts acid. It contains free hydrochloric acid. This was definitely established by Bidder and Karl Schmidt.[2] They estimated quantitatively the total chloride present in the stomach, and also all the bases, — potash, soda, lime, magnesia, iron oxide and ammonia, — and found after computing the amount of hydrochloric acid required to combine with these, that some remained uncombined. We shall discuss the composition of gastric juice and its secretions more in detail later, confining ourselves at present to the statement that the proteolytic ferment mentioned, i.e. pepsin, is only active when in acid solution. It was first believed that the pepsin was united with the hydrochloric acid and exercised its functions as pepsin-hydrochloric acid. It was, however, soon shown that the hydrochloric acid could, on the one hand, be substituted by other acids, for instance, lactic acid, while, on the other hand, other acids did not replace the hydrochloric acid in equivalent amounts. The amount of hydrochloric acid in the gastric juice is very appreciable. The gastric juice of dogs contains 0.5–0.6 per cent hydrochloric acid; that of cats 0.5 per cent; while for human beings from 0.2–0.3 per cent is reported. The attempt has been made to assign to the hydrochloric acid content of the stomach an antiseptic action as its greatest function. Although there is undoubtedly such an action, the fact also remains that hydrochloric acid participates in the digestion of albuminous substances. The mechanism of its activity has, however, not been thoroughly explained. It may be summed up as follows: If we add hydrochloric acid, or even gastric juice, to albumin, a peculiar change takes place. The albumin swells up and fills the entire vessel as a gelatinous mass. Large amounts of hydrochloric acid are simultaneously combined. The quantity of free hydrochloric acid diminishes. We can imagine that the hydrochloric acid enters into combination with the albumin, producing soluble albumins, the so-called "acid-albumins." It is possible that the hydrochloric acid loosens up the albumin molecule, i.e., changes it in some way, preparing it for the action of pepsin. There are,

[1] Versuche über das Verdauungsgeschäft. German by Michaëlis. Leipsic, 1785. Eberle: Physiolog. Verdauung auf natürlichem und künstlichem Wege Würzburg, 1834. Cf. also Gamgee: Physiologische Chemie der Verdauung: Leipsic and Vienna, 1897. W. Beaumont: Neue Versuche und Beobachtungen über den Magensaft und die Physiologie der Verdauung. German by B. Luden. Leipsic, 1834.

[2] Bidder and Schmidt: Die Verdauungssäfte u. d. Stoffwechsel, Mitau u. Leipsic, 1852.

for instance, many albuminoids nearly immune to the action of the gastric juice, which, under the influence of mineral acids in the cold, are so changed that they are then appreciably disintegrated by pepsin. It seems, however, that the hydrochloric acid, besides exercising this effect on the albumin, also, in some manner, directly influences the pepsin. This is evident from the fact, that, when all the hydrochloric acid has combined with the albumin and its cleavage-products in an artificial digesting mixture, the pepsin digestion then ceases, and can only be brought to renewed activity by the addition of fresh hydrochloric acid. We are justified in believing that the albumin combines with more hydrochloric acid, in proportion to the greater number of cleavage-products formed. Direct observation has also shown that the hydrochloric acid disappears in proportion to the time of digestion.

The breaking down of the proteins by the action of the gastric juice is not at all extensive. Complicated peptones are mainly formed, accompanied, of course, by some of the lower cleavage-products, evidently of the group of simple polypeptids. Amino acids, under normal conditions, are not to be detected.[1] The digestion of albuminous substances in the stomach evidently serves to prepare them for the action of trypsin, with which they next come in contact. Test-tube experiments have shown that tryptic digestion is quicker and more intense when preceded by a pepsin-hydrochloric acid digestion.[2] Certain difficultly digestible albuminous substances, as for instance serum-globulin, show this property very plainly. It is evident that the preliminary digestion in the stomach of the different albuminous substances is of varying importance. It is of little significance for those easily digested. The advantage of such a preliminary decomposition becomes especially clear when we consider how rapidly the absorption of the albumin cleavage-products follows in the duodenum and the remaining small intestine. In spite of an extremely liberal diet of meat, we find only small amounts of digesting material in the duodenum. Accordingly as the stomach is emptied through the pylorus, trypsin digestion and the absorption of cleavage-products take place in rapid succession. A much larger field of action is presented to the trypsin ferment at one time. Instead of acting upon an albumin molecule, it can immediately attack a large number of cleavage-products, and quickly disintegrate them into more simple components.

The proteids, when in the stomach, are disintegrated first of all into their constituents. Hematin is split off from hemoglobin, and the globin is digested by itself. The nucleoproteids give off nuclein, which, being

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[1] Emil Abderhalden: Z. physiol. Chem. **44**, 17 (1905).

[2] Emil Fischer and Emil Abderhalden: *ibid.* **40**, 215 (1903).

only slightly attacked by the pepsin, remains undissolved for the most part, and for this reason was first discovered.

The pepsin of the gastric juice also possesses another action besides that of direct disintegration. It causes milk to curdle. This striking property, not yet entirely explained, is due to a specific ferment, known as rennin,[1] or chymosin. We will state at once that the assumption of a separate ferment has been questioned. Pawlow and Parastschuk[2] conclude, from their experiments, that the two actions attributed usually to pepsin and rennin are due to the same ferment. They arrive at this conclusion from the fact that the proteolytic- and milk-coagulating effects of the gastric juice take place parallel to one another. Both actions are accelerated and retarded by the same influences, not only qualitatively, but also quantitatively. We know that neither the pepsin nor the rennin is secreted as such in the walls of the stomach. Both are found in the inactive form as *zymogen*. It is only by the action of acid that this zymogen is converted into active ferment. Activating these ferments of the gastric juice is another important function of its acid contents. The ferments are only present in their inactive state, and are incapable of doing their work when the free acid of the gastric juice is missing. Pepsin and rennin are both rendered active by the same agent; in fact, to the same extent. This parallelism has caused the Russian authors above mentioned to stop speaking of two ferments, but of two different actions of one and the same ferment. We cannot, at this time, accept Pawlow's decision. The more we study the action of ferments, the clearer it becomes that they are delicately adjusted for definite compounds, and are influenced by very slight differences of configuration. In fact, such differences in the configuration of molecules are oftentimes first indicated by the fact that a ferment does or does not act upon a certain substance, whereas its ordinary chemical behavior has not led us to suspect such a difference. This of itself is sufficient to make it seem improbable that a ferment could have two such different actions. An important objection immediately arises. We do not exactly know how to interpret the activity of rennin. It is, of course, possible that this activity may produce the same result as does pepsin, namely, an hydrolysis. We know that the essential function of rennin consists, not, as formerly believed, in the curdling of the casein, but in the conversion of casein into another albuminous body, possessing entirely different characteristics. If the assumption be correct that this is merely a hydrolysis, then the analogy to that of pepsin would be complete. We would only have to assume that the nature of the

[1] Cf. O. Hammarsten: Sitzber. kgl. Gesellsch. Wissensch. Upsala, **1877.**

[2] Z. physiol. Chem. **42,** 415 (1904), and Die Identität des Pepsins und Chymosins. Verhandl. Sektion Anat., Physiol. mediz. Chem. Vers. nordischer Naturforscher u. Aerzte in Helsingfors. **7,** 12, July, 1902, p. 28.

cleavage-products was due to the peculiar nature of casein, which we will soon consider. In this case it would be more correct not to speak of a milk-coagulating function, but of that of the proteolytic ferment, pepsin. We are inclined to look upon the pepsin and rennin ferment as probably identical, not from the fact that these have never been isolated in a satisfactorily pure condition, but more especially because of the interesting observations of Pawlow and Parastschuk. They found that the secretions of the pancreatic gland act towards casein in exactly the same manner as does the gastric juice, with this modification, that the proteolytic ferment of the pancreatic juice, trypsin, acts only in alkaline, neutral, or weakly acid solutions. The establishment of this fact has settled one thing. We must either assume that different ferments exist which will coagulate milk, i.e., one which acts in distinctly acid reaction, and another which is efficient in neutral or alkaline solution (the rennin of the stomach and the trypsin from the pancreas are activated by entirely different agents); or, as is far simpler, we must assume that only one process takes place, namely, a hydrolysis. Coagulation occurs as a secondary effect in the general decomposition of casein. It is caused by the precipitation of the early cleavage-products. It is possible that this stage of decomposition, which probably takes place before the formation of peptones, is common to all proteins. On the other hand, it is also possible that casein occupies a unique position, and that perhaps, corresponding to its functions, it represents a particularly complicated protein. We should like to place stress upon the above observations of Pawlow rather than upon the established similar behavior of the two ferments, and will state once more that we do not wish to speak of two different actions of *one* ferment. Until we have an accurate knowledge of these relations we will retain the conception of two separate ferments, pepsin and rennin, in our presentation of the subject.

Rennin is very widely distributed in the whole animal kingdom, and ferments acting in an analogous manner are undoubtedly found in the vegetable world. It has been found in animals which chew their cud, and especially in the fourth, so-called rennet-stomach of the calf. Its main function has generally been considered to be the curdling of milk. It has been found, however, that this precipitation of casein is not the primary process. The nature of casein is changed first of all by the action of the rennin. Another protein with different properties is produced. Curdling depends on the formation of insoluble calcium salts, arising from the casein, which has been changed by the rennin. That this conception is correct is evident from the fact that casein will not be curdled by rennin if the solution is free from calcium salts, but it will, nevertheless, undergo a change. If the casein, treated in the above manner, is then boiled, — thus destroying the rennin, — it will curdle on the addition of calcium

salts. The latter property is therefore dependent on the presence of calcium salts, and has no direct relation with the rennin as such. The albumin formed, *para-casein*, differs essentially from casein, inasmuch as it is precipitated by calcium salts. The precipitate contains large amounts of calcium phosphate. We do not know what relation this salt has to the curdling.

According to the general conception, the pepsin action follows the precipitation of the para-casein, disintegrating it in the same manner as it does the remaining proteins. The phosphoric acid portion, the so-called *pseudonuclein* (a compound as yet insufficiently investigated), is thrown out at this stage. It does not possess any of the ordinary components of the nucleins. The purpose of the rennin action is not at all clear. It is certainly significant that we always find evidence of the activity of rennin wherever there are proteolytic ferments. Rennin is found in the intestine and in the organs. It cannot be denied that the conception that the activity of rennin is to be regarded as being an hydrolytic attack, and the precipitation by the calcium salts as only an intermediate effect, depending on the specific characteristics of the first cleavage-products of casein, these being then subjected to the further disintegrating action of the pepsin, is certainly a very attractive one. The behavior of casein during digestion is thus removed from its special position, while the assumption of Pawlow and Parastschuk that rennin and pepsin are identical is given a further support, to be sure in the sense that it is a case of one and the same kind of fermentation, and not that of two different actions. These processes are still very obscure.

The opinion has often been expressed that pepsin and rennin are not simple, individual ferments. The various species of animals are supposed to possess different kinds of ferments. The rennins from human beings and from swine are supposed to be different from that obtained from the calf. Bang[1] has isolated a rennin, parachymosin, from the stomach of the calf, whose properties are essentially different from those obtained from other sources. Not even the pepsins from different animals are alike. Differences undoubtedly exist, which may possibly be due to the nature of the different nutrient albumins supplied to the animals. As long as we are not acquainted with the ferments as such, and are unable to study their activity in uniform materials, it will be difficult to pass judgment on the results obtained with different ferments. It is to be hoped that the transference of such investigations to the complicated polypeptides of known structure and configuration will throw light upon this subject.

We must refer to another peculiar phenomenon as yet entirely unex-

[1] Pflüger's Arch. **79**, 425 (1900).

plained. If some rennin is added to a clear solution of the so-called albumoses and peptones, a flocculent precipitate is formed. This is called *plastein*, and is supposed to occur only in the presence of albumoses. Its amount has been variously estimated, from 3 to 27 per cent. It is suggested that the closer the cleavage-products stand in relation to albumin, the more readily will they be precipitated by rennin. The formation of plastein is still unexplained. It has been looked upon as a synthetic process, although this assumption has not been substantiated by any experimental proof.[1]

The albumin, already partly disintegrated, passes from the stomach into the duodenum, being there subjected to the influence of the pancreatic juice, and especially to the trypsin contained therein. This also is not delivered to the intestines, as such. It is secreted in the zymogen form, and only becomes active in the intestine. There is a substance, called "enterokinase," in the intestinal juice, which converts the trypsin-zymogen into the active ferment. We shall return to this particular process later. We were a long time uncertain regarding the extent of the decomposition of the albumins reaching the duodenum in the form of a mixture of peptones of different degrees of complexity. We assumed, until recently, that, as a rule, only the so-called albumoses and peptones were formed, and that these were absorbed directly. Such a conception is particularly plausible, if, as has been generally done, digestion is only regarded as a means of preparing the nutrient material for absorption. This assumption was still believed, even when free amino acids, especially leucine and tyrosine, were repeatedly found in the alimentary canal. This view obtained added support from the experiment of Hofmeister.[2] He put a piece of the stomach or intestinal wall of a recently killed animal in a moist chamber for a time at 40° C., and showed that its peptone content had diminished when compared with a piece of the same size, whose peptone content had been immediately estimated. In fact, after two to three hours, all the peptones had disappeared. Salvioli [3] also showed that peptones quickly disappeared when introduced into a ligated intestine. There seems to be no doubt that peptones are absorbed; in fact, it has even been asserted that albumin itself is directly taken up. It was shown by the researches of Kutscher and Seeman,[4] and of O.

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[1] Cf. A. Danilewsky and Okunew: Inaug. Diss. St. Petersburg, **1895**. M. Lawrow: Inaug. Diss. St. Petersburg, **1897**. Sawjalow: Diss. Jurjew. **1899**, and Pflüger's Arch. **85**, 171 (1901). H. Bayer: Hofmeister's Beiträge, **4**, 554 (1903). M. Lawrow and S. Salaskin: Z. physiol. Chem. **36**, 277 (1902). Kurajeff: Hofmeister's Beiträge, **1**, 121 (1901); **2**, 141 (1902).

[2] Pflüger's Arch. **19**, 8 (1885).

[3] Arch. Anat. Physiol. Sup. **1880**, p. 95.

[4] Z. physiol. Chem. **34**, 528 (1901 and 1902); **35**, 432 (1902).

Cohnheim,[1] that the breaking down of the proteins in the intestine was more extensive than was originally thought. The former succeeded in isolating crystalline cleavage-products from the intestinal contents of dogs which had been fed on a diet rich in albumin, the animals being killed at varying times, — for instance, at intervals of six hours.

The discovery of O. Cohnheim, that the mucous membrane of the intestine contains a ferment, erepsin, which further disintegrates the peptones, casts doubt upon the conclusions drawn from the above-mentioned experiments of F. Hofmeister and Salvioli.

Recent investigations, from various standpoints, indicate a considerable disintegration of the albumin molecule. It has been shown for one thing that intestinal digestion is very similar to that artificially produced by trypsin. Amino acids, e.g., tyrosine, leucine, alanine, glutamic acid, aspartic acid, lysine, arginine, and histidine, are formed in the intestinal canal and even the polypeptides which are observed in artificial digestion with trypsin, and are attacked with difficulty, if at all, by ferments. It is at present uncertain as to how far the disintegration goes in individual cases, as to whether polypeptides with a small number of amino acids result, or that the digestion stops while the chains are more complicated. We have already shown that we can draw no conclusion as to the extent of the decomposition simply on account of the appearance of free amino acids. More complex substances may be present at the same time.

We have reached a like conclusion concerning the decomposition of the proteins in the intestinal tract from another standpoint.[2] The significance of the function of digestion is not merely to prepare the food for absorption. It goes far beyond this point. The separate components of the food are not in a condition suitable for the economy of individual beings. Every species of animal — in fact, every individual — has its own specifically constituted tissues and cells. If the diet were always the same, the formation of the tissues might bear some close relation to the components of the food. The diet varies, however, and, especially in the case of human beings and the omnivora, is exceedingly diverse in nature. In order to maintain the individuality of the animal, and to make its organism independent of the outer world in the matter of food taken, it disintegrates the nutrient it receives, and utilizes those components which may be of service to it in building up new complexes. This conception of the process of digestion, as a whole, will become especially clear when we consider the most important food of growing mammals, i.e., milk.

[1] *Ibid.* **33**, 451 (1901).

[2] Emil Abderhalden: Z. physiol. Chem. **44**, 17 (1905); Zent. Stoffwechs.-Verdauungs-Krank. **5**, 647 (1904); Med. Klinik. **1**, Nr. 1 and 2 (1905); **1**, Nr. 46 and 47 (1905).

All of the tissues are formed from this. The only proteins contained in milk are lactoalbumin, lactoglobulin, and casein. From these, and also in the case of animals in which the albumin content of the milk is less prominent than is true of human milk, all sorts of different proteins with their varying functions must be formed. We need only refer to the albuminous substances of the blood, to serum-globulin, serum-albumin, hemoglobin, then to the numerous albuminous constituents of the tissues, and all of the other proteins. A glance at the following table will give a good conception of the great changes which one protein must undergo to produce all the others.

	Casein.	Serum-albumin.	Serum-globulin.	Globin from hemoglobin.
Glycocoll	—	—	3.5	
Alanine	0.9	2.7	2.2	4.2
Aminovaleric acid	1.0	present	—	present
Leucine	10.5	20.0	18.7	29.0
Proline	3.1	1.0	2.8	2.3
Phenylalanine	3.2	3.1	3.8	4.2
Glutamic acid	11.9	7.7	8.5	1.7
Aspartic acid	1.2	3.1	2.5	4.4
Cystine	0.065	2.3	0.7	0.3
Serine	0.23	0.6	—	0.6
Tyrosine	4.5	2.1	2.5	1.5
Lysine	5.8	—	—	4.3
Arginine	4.8	—	—	5.4
Histidine	2.6	—	—	11.0

	Fibrin.	Histon from thymus gland.	Elastin.	Keratin.
Glycocoll	3.0	0.5	25.75	4.7
Alanine	3.6	3.5	6.6	1.5
Aminovaleric acid	1.0	—	1.0	0.9
Leucine	15.0	11.8	21.4	7.1
Proline	2.5	1.5	1.7	3.4
Phenylalanine	2.0	2.2	3.9	—
Glutamic acid	8.0	0.5	0.8	3.7
Aspartic acid	2.0	—	—	10.0
Cystine	—	—	—	0.6
Serine	present	—	—	—
Tyrosine	3.5	5.2	0.34	3.2
Lysine	—	6.9	—	—
Arginine	—	15.5	0.3	—
Histidine	—	1.5	—	—

Even if all the proteins so far investigated are not all derived from the same animal, and the methods of analysis are not so employed as to give exact results, it is, nevertheless, clear that casein must undergo great changes in order to make possible the transformation into these very different products. We have disregarded, to be sure, the other albuminous components of milk, albumin and globulin. It is possible that certain of the proteins in the body are more closely related to these than to casein, — at least, as far as their composition is concerned. Such a discovery would not alter our conception, as there can be absolutely no doubt but that the casein plays a large part in the economy of the nursling. This is evident from the large amount present in milk. It might, of course, be objected that casein is mainly utilized as a combustible material, and does not participate to any great extent in the building up of the body. While such an assumption is not yet supported by any proof, still on the other hand, we can reply that our knowledge of the composition of the lactoalbumin and lactoglobulin is such as to warrant the belief that they can only participate to a limited extent in the formation of the albuminous components of the body. They would also have to undergo great changes in order to make them available for the requirements of the cells of the body.

It is not difficult to imagine the formation of all of the varied albuminous substances from one primitive body, if we take into consideration the fact that a very extensive decomposition occurs even in the alimentary tract. From the complicated albumin, the intestine receives the individual constituents either as such or in long or short chains. The intestine is able to unite these in varying proportions, forming definite products to meet its requirements. The same process can take place in every cell.

We might expect to obtain an insight into the digestion of the albumins by studying the blood. It were conceivable that the cleavage-products are only recombined in the various organs. Such a conception has much to commend it. We must not, however, forget that the organism strives to maintain a constant composition for its blood-stream. The blood has important functions to fulfill, and any disturbance is accompanied by far-reaching results. It were, in fact, not a matter of indifference to have the various decomposition products introduced into the blood. The cells would require that all the elementary components be brought to them and in definite proportions, in order that they might build up their own albumins, a condition of affairs hardly probable with a large part of these components.

We have not yet succeeded in definitely isolating any peptones or other protein cleavage-products from the blood.[1] The serum of the blood

[1] E. Abderhalden and C. Oppenheimer: Z. physiol. Chem. **42**, 155 (1904). Cf. also P. Morawitz and R. Dietschy: Arch. exp. Path. Pharm. **54**, 88 (1905).

undoubtedly carries mainly albumin, which occupies the same relation to the albumin metabolism that grape-sugar does to the transport of the carbohydrates. As the sugar content of the blood is very constant, so, also, the sum total of the albumins in the blood-serum is subject to but little variation. The serum-albuminous bodies are mainly composed of albumin and globulin. Their relative amounts vary. During starvation the former gradually diminishes, while the latter increases. We could imagine that the composition of the serum-albuminous bodies were dependent on that of the albumin in the food. This ought to be subject to proof by direct experiment.[1] Six liters of blood were taken by venesection from a horse, which had been fed mainly on hay and oats, and the amounts of tyrosine and glutamic acid present in the serum were estimated. The animal was then made to fast a whole week in order to guarantee that the intestines were entirely emptied of their contents. Another sample of blood (six liters) was taken, and the amounts of tyrosine and glutamic acid present in the serum again determined. The animal was now fed an albuminous substance which possessed 36.5 per cent glutamic acid and 2.37 per cent tyrosine. The serum-globulin of the horse contains, under normal conditions, about the same amount of tyrosine, but only 8.5 per cent glutamic acid. Serum-albumin contains 7.7 per cent glutamic acid. The animal under investigation, therefore, was fed an albumin, *gliadin*, which possessed five times as much glutamic acid. The following table gives a summary of the results:

EXPERIMENT I.

	Normal.	After 8 days' fasting.	After feeding 1500 g. gliadin.	After feeding 1500 g. gliadin.
	Per cent.	Per cent.	Per cent.	Per cent.
Tyrosine	2.43	2.60	2.24	2.52
Glutamic acid	8.85	8.20	7.88	8.25

EXPERIMENT II.

	Normal.	After 7 days' fasting.	After feeding 2500 g. gliadin.
	Per cent.	Per cent.	Per cent.
Tyrosine	2.50	2.55	2.48
Glutamic acid	9.52	8.52	8.00

[1] E. Abderhalden and F. Samuely: Z. physiol. Chem. **46,** 193 (1905).

These experiments show clearly that the composition of the serum-albuminous bodies remains unchanged and is independent of the nature of the albumin administered. The amounts of glutamic acid remained very constant, in spite of the fact that the horse had to renew a supply of serum-albumin, due to a large loss of blood. The albumin in the food must certainly have undergone a complete change before it entered into the general circulation. The blood was taken from the *Vena jugularis* in these experiments. As the albuminous substances do not seem to enter the general circulation through the lymph-stream, but only through the blood, it was conceivable that the transformation of the nutrient albumin, that is, the synthesis of the cleavage-products, was carried out in the liver. We have, as yet, no exact proof of this. Our present knowledge indicates that a synthesis of the albuminous cleavage-products takes place in the walls of the intestine. From the various different albumins in the food, the serum-albumins are formed first. From the latter, each cell constructs the protein that it requires. The cell, therefore, obtains the same nourishment entirely independent of external conditions. The functions of the intestine and of the digestive ferments are, according to this conception, to be regarded in a quite particular light. First of all, they guarantee collectively the correct course of the general metabolism. The digestive ferments act before the intestine does. They furnish the intestine with the building materials from which it forms homogeneous products for the cell-metabolism. It now becomes apparent why any derangement of the alimentary tract should have such a far-reaching effect upon all processes of metabolism. It is not the deranged absorption which is so important. It is the deranged assimilation. The intestine itself is one of the most important organs. Many important syntheses and changes are carried on within its walls.

That syntheses play necessarily in albumin-metabolism a part as important as in the case of fats and carbohydrates, is evident from the fact that animals which are supplied with greatly disintegrated albuminous material, can be easily maintained in nitrogen equilibrium, as the experiment on the next page shows.[1]

The casein decomposed by tryptic digestion consisted of 80–85 per cent of simple cleavage-products, the amino acids constituting, by far, the larger portion, while the smaller part was composed of substances akin to polypeptides. At most, 15–20 per cent of the total material administered consisted of complicated polypeptides, which, however, no longer gave the biuret reaction. Whether the organism is capable of producing albumins from the amino acids alone remains undecided, and at present is not sus-

[1] E. Abderhalden and P. Rona: Z. physiol. Chem. **42**, 528 (1904); **44**, 108 (1905). Cf. O. Loewi: Arc. exp. Path. Pharm. **48**, 303 (1902).

ceptible to direct proof; because, in the first place, we are not acquainted with all the albumin cleavage-products, and, on the other hand, many of the amino acids are evidently destroyed during the complete hydrolysis by acids and alkalies.

Date. 1905.	N. in Food.	Urine.		Dried Fæces.		Total N.	N. Balance.	Weight	Observations.
		Amt.	N.	Amt.	N.	Outgo.			
Jan. 12	—	—	—	—	—	—	—	—	fasting.
13	—	—	—	—	—	—	—	—	"
14	2 g.	120	2.30	14.7	0.27	2.57	−0.57	2.740	fed per day:
15	"	115	2.00		0.27	2.27	−0.27	2.750	33.3 g. sliced meat.
16	"	130	1.98		0.27	2.25	−0.25	2.785	25.0 g. fat.
17	"	118	1.46	7.3	0.38	1.84	+0.06	2.790	50.0 g. starch.
18	"	105	1.85	10.0	0.14	1.99	+0.01	2.800	10.0 g. cane-sugar.
19	"	110	1.50		0.14	1.64	+0.36	2.820	5.0 g. grape-sugar.
20	2 g.	100	1.72	16.6	0.23	1.95	+0.05	2.825	
21	"	95	1.55		0.23	1.78	+0.22	2.830	
22	"	110	1.38	20.1	0.47	1.85	+0.15	2.840	fed per day:
23	"	115	1.35	20.4	0.36	1.71	+0.29	2.870	23.5 g. digested
24	"	110	1.39		0.36	1.75	+0.25	2.880	casein.
25	"	120	1.50	23.6	0.37	1.87	+0.13	2.900	25.0 g. fat.
26	"	105	1.31		0.37	1.68	+0.32	2.945	50.0 g. starch.
27	"	100	1.29	15.3	0.34	1.63	+0.37	2.960	10.0 g. cane-sugar.
28	"	120	1.34		0.34	1.68	+0.32	2.970	5.0 g. grape-sugar.
29	"	105	1.39	15.9	0.35	1.74	+0.26	2.985	
30	"	100	1.64	12.6	0.19	1.83	+0.17	3.010	
31	"	90	1.36	28.8	0.52	1.88	+0.12	3.030	
Feb. 1	"	100	1.35		0.52	1.87	+0.13	3.045	
2	"	105	1.51	20.4	0.38	1.89	+0.11	3.030	
3	"	95	1.53		0.38	1.91	+0.09	3.040	
4	"	110	1.58	16.1	0.45	2.03	−0.03	3.010	
Total	32 g.	—	23.19	—	5.86	29.05	+3.01	—	
Average	2 g.	—	1.45	—	0.36	1.85	+0.19	—	

Closely related to this question, is the problem whether the organism is capable of getting along with albuminous substances which are deficient in specific groups. Under normal conditions we constantly consume a mixture of proteins. Based on the above conception, regarding the degradation and reconstruction of the albumins, we can imagine that it is immaterial whether one or the other protein, or this or that elementary constituent, is absent. The fact that they are all finally present in the digesting mixture is the important consideration. We can also imagine that the animal organism possesses the ability of producing certain amino acids from others; for instance, glycocoll. That an organism can get along with an albuminous substance in which glycocoll is entirely absent, is evident from the feeding experiment mentioned, in which the cleavage-products of casein were used.

The reason that many albuminous substances, like the keratins, are not looked upon as food material, is due to the fact that their amino acid constituents are in such combinations that they are attacked with difficulty, or not at all, by trypsin. Among the albuminous substances so far considered, we mentioned one which was characterized by the absence of almost all of the members of the aromatic series. This is gelatin. Tyrosine and tryptophane are entirely absent, while phenylalanine is present in only very small amount. We are justified in concluding, from the idea previously suggested, that gelatin is not a satisfactory substitute for albumin in the ordinary sense. The animal organism seems unable to synthesize tyrosine and tryptophane. It would have to produce aromatic compounds from substances of the fatty acid series. It is, in fact, impossible to maintain the nitrogen balance by feeding gelatin exclusively. It is, of course, possible to substitute gelatin for a part of the nutrient albumin, in the same manner as when we do this with a large supply of carbohydrates or fats. Taking the above-described metabolic processes into consideration, we may conclude that gelatin is a much better "albumin-sparer" than the nitrogen-free foodstuffs mentioned, for the reason that it delivers to the organism a whole series of albumin constituents which it is capable of uniting with the other albumin degradation products to form its serum-albumins. We must expect that it can act as a substitute for more nutrient albumin, in proportion to the richness of the latter in aromatic groups. Experiments to confirm this have not yet been undertaken, although efforts have been made to increase the "nutritive index" of gelatin by the addition of the missing ingredients, tyrosine and tryptophane. It has in fact been found possible to increase greatly the amount of gelatin used as substitute by adding simultaneously these amino acids.[1] Cystine was used in these experiments. The reason that we have so far been unable to replace completely albumin with gelatin, and the addition of the missing amino acids, may be due to the fact that we are not yet acquainted with all of the albumin components. It seems far more probable, however, that gelatin contains numerous combinations, which are very resistant to the action of the proteolytic ferments, and, possibly, it also restricts the rapid decomposition and reconstruction in cell-metabolism.

Effort has also been made to obtain values, as albumin-sparers of substances closely related to albumin, especially of asparagine, which occurs so abundantly in germinating seeds.[2] The interesting discovery was

[1] M. Kauffmann: Pflüger's Arch. **109,** 1 (1905). Cf. also the earlier investigations of Eschle: Vierteljahresschrift naturforsch. Ges. Zürich, **1876,** 36. K. H. Lehmann: Sitzber. Münchener morphol.-physiol. Ges. March 10, 1885.

[2] O. Kellner: Z. Biol. **39,** 313 (1900). Politis: *ibid.* **28,** 492 (1891). S. Gabriel: *ibid.* **29,** 115 (1892). C. Voit: *ibid.* **29,** 125 (1892). Mauthner: *ibid.* **28,** 507 (1891). I. Munk: Virchow's Arch. **94,** 441 (1883). Weiske: Z. Biol. **17,** 415 (1881); *ibid.* **30,** 254 (1894). W. Völtz: Pflüger's Arch. **107,** 360 (1905); **107,** 415 (1905).

made that this substance acted differently in the organisms of the carnivora than in that of the herbivora. With the former and with the omnivora, asparagine cannot be utilized as a substitute for albumin; but this substance does act as an albumin-sparer with the herbivora. It is not easy to interpret this result. We cannot exactly realize how asparagine can act as a substitute for albumin. It is, of course, possible, in fact very probable, that the animal organism is capable of forming one amino acid at the expense of another; we cannot, however, believe it possible to produce albumin from asparagine alone. Such an assumption is entirely out of the question. It seems more probable that it is active in another direction. It has been thought that the asparagine in the intestines protects the albuminous material of the food, before disintegration, against the attacks of micro-organisms; in fact, it has even been suggested that the bacteria in the intestines synthesize albumin from asparagine, which is then absorbed by the organism. It is interesting to note that ammonium acetate [1] and succinamide [2] are credited with having the same effect as that of asparagine. We cannot consider this question as solved, from the investigations at hand; only this much is certain, that asparagine cannot be looked upon as a substitute for albumin in the sense that gelatin is. Its action is an indirect one.

In this connection we may state that the lower forms of life, like the molds, can utilize an individual amino acid as a starting-point in the synthesis of albumin. This is not so remarkable, for, if the nitrate-nitrogen is available, then the amino acid-nitrogen ought also to be of value. Experiments with *Aspergillus niger* [3] indicate that, within certain limits, the production of albumin is entirely independent of the source of the nitrogen. This mold synthesized its albumin just as efficiently in a potassium nitrate medium as when it was supplied with glycocoll or glutaminic acid as its sole source of nitrogen. Furthermore, the examination of the albumin in the mold showed it to be composed of apparently the same relative amounts of individual amino acids in all three experiments. Glycocoll, alanine, leucine, glutamic acid, and aspartic acid were all obtained from the mold. This phenomenon might be explained by assuming that this mold evidently decomposes the amino acids presented as nutriment, i.e., possibly splits off the amino group, and starting from ammonia begins the synthesis anew. In the same manner it probably produces the same substances from the nitrates, and eventually forms most complicated compounds. Czapek [4] and O. Emmerling [5] have already

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[1] O. Kellner: Z. Biol. **39**, 339 (1900).
[2] Weiske: Z. Biol. **20**, 279 (1884).
[3] E. Abderhalden and P. Rona: Z. physiol. Chem. **46**, 179 (1905).
[4] F. Czapek: Hofmeister's Beit. **1**, 542 (1902).
[5] O. Emmerling: Ber. **35**, 2289 (1902).

shown that the amino acids act very efficiently as sources of nitrogen. The latter also called attention to the interesting fact that the molds will only attack the α-amino acids, while other acids, with the amino groups differently situated, are unacted upon. We may add that *Aspergillus niger* will also act on the polypeptides produced from the α-amino acids, as well as on the polypeptides which are not decomposed by trypsin; for instance, glycyl-glycin and dileucyl-glycyl-glycin. This is not very unusual, for we know that the animal organism possesses ferments in the cells which are capable of breaking down compounds unattacked by trypsin. That this assumption is correct, is evident from the previous description of the behavior of individual polypeptides in the animal organism.[1]

Ammonium oxalate[2] has been observed as a metabolic end product in mold activity, but only during growth with a supply of certain amino acids; for instance, glycocoll, alanine, serine, aspartic acid, glutamic acid, and proline. On the other hand, no oxalic acid is produced from leucine, phenylalanine, lysine, arginine, and histidine. Ammonia is very often one of the end-products of mold and bacterial metabolism. We would refer, for example, to the cleavage of urea by bacterial action with the formation of ammonium carbonate. The different varieties of mold and bacteria, moreover, produce unequal amounts of ammonia. *Bacillus mycoides*, for instance, converts as much as forty-six per cent of the nitrogen in albumin into ammonia.[3]

We might expect that carnivorous plants, unlike the *Aspergillus niger* which we have just considered, would be able to assimilate directly the amino acids and higher albuminous cleavage-products, synthesizing them into albumin in the same manner as does the animal organism, i.e. without preliminary decomposition. The metabolism of such plants has, unfortunately, been studied but little, and we do not even know how they digest albumin. That there are organisms in the vegetable world which are only capable of forming albumin from its cleavage-products, is evident from the researches of Beijerinck,[4] from which it appears that the conidial-alga, *Cystococcus humicola*, the alga of the lichen, *Physica parietina*, with which it lives in symbiosis, prepares peptones for the nourishment of the latter. It would be interesting to study the true parasites and the saprophytes from this point of view.

Let us return to the behavior of the albuminous substances in the intestine. Not all the cleavage-products of the proteins are absorbed. A part is decomposed in another manner, and is lost for the further synthesis of albumin in the animal body. Bacteria are present in the intes-

[1] Cf. p. 203.

[2] O. Emmerling: Zentr. Bakt. u. Parasitienkunde, II, **10**, 273 (1903).

[3] E. Marchal: Zentr. Bakt. II, **1**, 1753 (1895).

[4] Beijerinck: Bot. Zeit. 1890, No. 45, Zentr. Bakt. **13**, 368 (1893).

tines, living at the expense of our food materials and especially the albuminous substances. They are found in the small as well as in the large intestine, and occur there as aërobes and anaërobes.[1] The intestinal "flora," that is, the bacterial content of the intestines, is very dependent upon the nature of the nourishment, and varies with it. The anaërobic bacteria, and especially the *Bacillus putrificus*, are largely responsible for the putrefaction of albumin. The activity of this bacillus is accelerated by the presence of aërobic bacteria, especially *Bacterium coli* and *lactis aërogenes*. They predominate when a large amount of oxygen is present; the activity of the anaërobes then becomes restricted, as a result of which the albumin putrefaction is also diminished. When, on the other hand, there is a small amount of oxygen present, the anaërobic bacteria become active. That the anaërobic and aërobic species of bacteria act together is due to the fact that the latter consume the oxygen which restricts the living processes of the former; while the anaërobes, on the other hand, form products by their activity which serve as sources of nutriment for the aërobes. Naturally, the action of anaërobic bacteria alone on albumin will not give the same products as when the two kinds of bacteria act together. The bacteria themselves are introduced into the intestines with the food. The intestines of the new-born are sterile.[2] No bacteria can be found in their meconium, the first excretory product from the intestine. When a definite bacterial flora has settled itself in the intestines, the amount present is but slightly dependent upon any further addition from the food-supply. The fate of the bacteria is, then, determined by the food presented to them, their nutrient medium. There is, furthermore, a vigorous struggle for existence in the intestines. Those bacteria whose nutrient requirements are satisfied most favorably triumph over the others. On the other hand, a form of symbiosis develops by which one variety of bacteria will consume the products of another, thus giving rise to quite a variety of bacteria. Their development is kept within certain limits in a number of ways, so that the putrefactive processes do not play any great part in the intestines, and only a small portion of our food is sacrificed to them.

Great importance has been attached to the hydrochloric acid in the stomach for restraining bacterial activity; in fact, for a long time it was believed that this was its most important function. The amount of hydrochloric acid in the gastric juice varies in different animals, as already mentioned. It is sufficient, however, to restrict the activity of the putre-

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[1] Cf. also Escherich: Die Darmbakterien des Säuglings, Stuttgart, 1886. A. Macfayden, M. Nenki, and N. Sieber: Arch. exp. Path. Pharmak. **28**, 311 (1891). D. Gerhardt: Ueber Darmfäulniss. Ergebnisse der Physiologie (Asher and Spiro), **3**, 1, 107 (1904).

[2] Bienstock: Arch. Hyg. **36**, 335 (1899); *ibid.* **39**, 301 (1901).

factive bacteria, as has been shown by N. Sieber.[1] Even Spallanzani[2] was acquainted with this property. He showed that a lizard which had been swallowed by a snake did not show any indication of putrefactive changes during sixteen days in which the digestion of the animal in question was carried out. He also found that when he inserted decaying meat into an animal's stomach that the putrefactive changes were diminished and that the putrefactive odor disappeared. It is, therefore, certain that the free hydrochloric acid of the stomach is of service in this direction. It is, however, questionable whether, as some observers maintain, the putrefactive changes in the intestines are prevented to any extent by the hydrochloric acid from the stomach. At any rate, no increase in putrefaction has been observed, even after the stomach was completely removed. Hydrochloric acid is often absent in human beings under certain pathological conditions, while, in other cases, it is often secreted in excess. No definite influence on putrefactive changes is discernible under such conditions. On the other hand, we should like to call attention to an indirect significance of the hydrochloric acid of the stomach in this connection. We have already mentioned that the hydrochloric acid undoubtedly plays an important part in the preliminary preparation of the albuminous bodies for their disintegration. This splitting up of the proteins into numerous larger and smaller cleavage-products in the stomach has for its main object, according to our present conception, the presentation of the largest possible surface of attack to the trypsin, which facilitates the further rapid degradation and absorption. The more quickly these processes are carried out, the less opportunity do the bacteria have to attack the cleavage-products. That this is the correct assumption, is evident from the researches of Ortweiler.[3] He found that two patients afflicted with carcinoma of the stomach — a tumorous formation in which the free hydrochloric acid is generally absent — showed a smaller elimination of indican after the administration of hydrochloric acid. Indican is one of the putrefactive products of albumin, as we shall shortly see. That this retardation is not due to any direct action of hydrochloric acid on the bacteria is evident from the fact that the administration of pepsin will have the same effect. The preliminary decomposition of the albuminous bodies was, therefore, the cause of the diminished putrefaction. The putrefactive bacteria could not develop their activity in the stomach under normal conditions. We also introduce air into the stomach with the food. This is the reason why the aërobic bacteria develop there. They themselves cannot cause putrefaction. Their action is confined mainly to

[1] Nadina Sieber: J. pract. Chem. **19**, 433 (1879).

[2] Spallanzani: Expériences sur la digestion. Trad. par Senebier. Nouvelle édition, Genève, 1784; in German: Versuche über das Verdauungsgeschäft, Leipzig, 1875.

[3] Ortweiler: Mitteil. a. d. med. Klinik zu Würzburg, **2**, 1886.

the carbohydrates, whose fermentation is an especially vigorous one when the free hydrochloric acid is absent, as is indicated by the appearance of butyric and lactic acids in such cases. The antiseptic action of hydrochloric acid is, therefore, more to be sought for in this direction.

To the bile, and especially the acid present in it, has also been ascribed an influence on the putrefactive changes in the intestines. There are, however, many observations which do not support this view. Friedrich Müller [1] observed no increase in the amount of indican excreted in a case of icterus, i.e. an obstruction in the biliary passages, thus preventing the flowing of bile into the intestine; nor was there any appreciable increase in putrefactive changes observed in the case of a dog in which a biliary fistula was made.

The putrefactive processes increase in the intestines only when some stagnation of the intestinal contents exists. Jaffé, who first called attention to this phenomenon, also showed that only when a stoppage occurred in the *small* intestine did any marked increase of indican appear in the urine. If we observe any increase in the elimination of indican, and there is an obstruction in the large intestine, we are, therefore, justified in concluding that the stoppage reacts back upon the contents of the smaller intestine. That a stoppage of the large intestine of itself has but little effect upon the elimination of indican, is evident from the fact that the greater part of the albumin is absorbed in the small intestine, while only small amounts, depending on the nature of the food, succeed in reaching the large intestine. Ellinger and Prutz [2] have determined the effect of stoppages in various parts of the alimentary tract in a very ingenious manner. They cut out pieces of the intestine of a dog, and replaced them so that the oral end of each excised piece was joined to the distal end of the whole intestine; and, conversely, the distal end was joined to the portion remaining attached to the stomach. Such a piece of intestine retains its original peristalsis and prevents the further progress of the chyme and fæces, in that it continually opposes the activity of the remainder of the intestine. We shall subsequently take up in detail the compounds resulting from the putrefactive processes upon the cleavage-products of albumin.

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[1] Z. klin. Med. **12**.

[2] Z. physiol. Chem. **38**, 399 (1903).

LECTURE XI.

ALBUMINS OR PROTEINS.

V.

Decomposition of Proteins in the Tissues. The End-products of Albumin Metabolism.

According to our present conception of the digestion and assimilation of albuminous substances, we must assume that the different proteins of our food are converted into the albuminous bodies of the plasma. In this form the individual cells obtain the nitrogenous material which is absolutely necessary for its maintenance, and, from our knowledge of metabolism during fasting,[1] we must conclude that the cells themselves give up their albumin only in this form to the blood for further transportation. We know as little about the manner in which the cells produce their albumin from the proteins of the blood as we do of the reason why the animal organism, under all conditions, requires such relatively large amounts of albumin for the proper maintenance of its corporal existence. In this connection it is especially noteworthy that the growing nursling consumes as much albumin, proportionately, as does the fully developed organism. E. Feer[2] gives 951 grams as the daily quantity of milk consumed by a boy 29 to 30 weeks old, and weighing 8.23 kilograms. This amount would contain 15.2 grams albumin, 32.3 grams fat, 58.0 grams sugar, and 1.9 grams ash. At this rate a grown-up person weighing 70 kilograms would consume daily:

Albumin	129 grams
Fat	275 "
Sugar	494 "
Ash	16 "

The following values have been found actually to be the average food requirement of an adult:

Albumin	118 grams
Fat	56 "
Sugar	500 "

[1] See Lecture on Metabolism.

[2] Jahrbuch f. Kinderheilkunde, N. F. **42**, 195, 196.

The fully developed organism has, of course, losses. We need only refer to its secretions, to the constant changes of the epidermal structures, the cells of the mucous membrane, and to all of the observations regarding the organs themselves, which participate in a continuous decomposition and reconstruction in the tissues. It is, of course, possible that these latter processes are much more extensive than we have any idea, and that new groups are constantly entering the cell contents, and others leaving. This is in accord with the fact that the animal organism disintegrates the nutrients to such an extent and adapts them to all of the bodily requirements. On the other hand, we can form no clear conception of the manner in which protoplasm and its components are used up, nor understand at present why the cells should tear down its own components in order to make way for the constituents of the nourishment. As the different cells, according to the tissues of which they are a part, and the function which they serve, have a specific structure, and above all possess individualized protein materials, we must consequently assume that the cells at every moment take their own characteristic building material from the homogeneous mixture of the blood proteins, and to some extent transform them in a quite complicated manner. It is possible that the whole phenomenon is an hereditary one. The single-celled organisms are constantly engaged in multiplying themselves. They develop rapidly, producing new individuals in quick succession. We meet the same phenomenon in some of the more highly organized invertebrates. From a single individual, thousands and tens of thousands of new, rapidly growing creatures are formed. The whole cell material is engaged in constant growth and change. We also find analogous phenomena among the vertebrates. In these cases they are confined in the grown individual to the sexual organs. Here, also, we observe a constant increase in the number of cells, and a continual delivery of cell material, on the one hand eggs, and on the other spermatozoa. The coöperative efficiency of the other body-cells, in promptly preparing thousands and tens of thousands of new cells, is evident from the tremendous production of leucocytes at the beginning of any infection. The invading army is surrounded in the shortest space of time by thousands of white blood-corpuscles. They form a thick wall, and protect the remainder of the organism. The leucocytes also play some other, as yet undiscovered, important *rôle* in the animal organism. For instance, they are present in the intestines in large numbers during digestion. Every one of these newly formed cells must have a completely-constructed protoplasm. They must in every case contain all the elementary components; moreover, they also contain proteins, which alone are capable of giving to their complicated structure its true individuality.

The great facility with which the animal organism produces new cells

from its tissues may possibly throw some light on the fact that the animal cell is continuously using up proteins in its economy. Every individual body-cell is capable of forming new cells, either in renewing its own structure, or in giving off cells. It obtains the other necessary elementary constituents from the storehouses of the tissues. Fats and carbohydrates are held in reserve. The animal organism has no storeroom for its albumin. It is only capable of accumulating albumin under very specific conditions. Even these stores are removed when the nutrition returns to its normal state. Every cell is evidently restricted to a definite amount of albumin, to which it closely adheres. If the animal organism possesses more fat or carbohydrate than it requires, it will store them up. There is no increase in the metabolism. If, on the other hand, the amount of albumin administered be increased, then the metabolic changes are increased. The albumin governs the whole animal organism. We will add, that from the chemical point of view it is perfectly possible for the cell, under certain conditions, to produce all its other organic requirements from albumin. It is possible that it may produce fats and carbohydrates from albumin. Albumin, from this point of view alone, is therefore a valuable food-material. Whether such changes actually occur under normal feeding, that is, with a sufficiency of fats and carbohydrates, is very problematical, in fact hardly to be assumed.

We will furthermore add that the albumin in the nourishment, which certainly serves for manifold purposes, may not all participate in the true cell metabolism. We arrive at this conclusion, from the fact that the animal organism evidently produces its body albumin from such differently constituted proteins in our foods. Waste products may very easily arise. Let us recall the experiment of feeding a horse with gliadine, which is so rich in glutamic acid. As this animal was only capable of utilizing one-fifth of this amino acid for the synthesis of serum-albumins, the remainder must have been consumed in some other manner. It is possible that the animal cells possess the ability of producing other amino acids from a specific one, but we can also imagine that when the nutrient albumin is transformed into body albumin, many of the elementary components are cast aside and directly consumed. We must not forget that we are now entering into a realm which has so far been but little investigated. We might expect from this assumption that the animal organism would be satisfied with the smallest amounts of those albuminous substances whose compositions were closely related to its own proteins. In this case most of the elementary components would be available. At any rate, we are not justified in drawing the conclusion that the proteins in the food take part in the metabolism of the cells from the fact that the nitrogen — in the case of mammals — reappears in a short time as urea. We find that amino acids and polypeptides are eventually broken

down in the same way as the proteins themselves, although they hardly ever come into intimate contact with the cells. It has, moreover, never been found possible to spare albumin by means of a mixture of amino acids. The animal organism is evidently unable to build up its own albumin from such material because other essential components are wanting.[1] Why should not groups result under normal conditions which are unsuitable for further synthesis and which are, therefore, immediately deprived of the amino group and consumed?

The large albumin-requirement of the animal organism would be partly explained by its constantly striving to obtain as much as possible of its individual building-material. Here also the Law of the Minimum holds, i.e. the extent of the syntheses taking place depends upon the amount of that substance which is present relatively to the least extent. Again, in the formation of the cell material from the serum-albumin, all of the components of the latter are not utilized to the same extent. Under some circumstances entire large groups may not be used at all. According to this conception, it might be that a cell decomposition and replacement, which of itself was not very extensive, might require a large amount of proteins. But even with this assumption we are still unable to explain the fact that a definite amount of albumin is necessary to maintain an equilibrium in the metabolism. We would expect that the amount of albumin used would be now more, now less. But the grown organism maintains its metabolism in a very definite manner, and keeps it, with very little variation, at a constant level. We ought to get a good idea of all of these relations by exactly following the course of nitrogen metabolism of the salmon during its stay in fresh water. Remarkable transformations occur in its organism. Its muscle-albumins finally produce the protamines of entirely different composition. Are all of the elementary components of the former utilized? If this be so, then the animal organism must be capable of converting one component into another. These suggestions, needless to state, are insufficient to remove the haze which still thickly envelops the whole metabolism of albumin.

If we wish to consider this entire problem which is so important to biology, we cannot neglect any single phase of the whole subject of the transformation of material; and from this point of view it will be worth our while to follow the utilization of the nutrient proteins and their value in the animal organism.

Although, in considering the questions concerning the assimilation of the albumin from food, we met with great difficulties, these become apparently unsurmountable when we attempt to follow the proteins till they are taken up by the cells of the body. The enigmas begin with the

[1] E. Abderhalden and P. Rona: Z. physiol. Chem. **47** (1906)

cell itself. Here are problems wholly unsolved. We cannot be led away from this fact by the discovery of more and more nerve fermentation processes and new cell-ferments. They only suggest the way in which the cell disintegrates material, and leave us to imagine the process by which the cells obtain their energy from the nourishment. We know very little about their real metabolism.

We must, therefore, give up trying to trace the further behavior of absorbed albumin in the animal organism, and confine ourselves to the discussion of the metabolic end-products. We may be able to obtain some information of cell-metabolism in this way. The albuminous bodies contain characteristic elements, namely nitrogen and sulphur, which aid us in recognizing their decomposition products. We know, to be sure, of other nitrogenous substances besides proteins, which participate in metabolism, and need only refer to the lecithins and nucleins, etc., as examples. Their amounts, however, are extremely small in comparison with the proteins, and we are able, from the chemical constitution of the end-products of metabolism, to indicate definitely their origin. The extent of the decomposition of proteins in the animal organism varies, depending on the kind of animal; this at least applies to the final end-products. In mammals we find the larger part of the nitrogen introduced into the organism reappears in the urine in the form of urea, $CO(NH_2)_2$. The amounts are especially large in the case of the carnivora, and less so with the herbivora. Only a very small amount of urea occurs in the urine of birds and reptiles, while it plays a very important part in the economy of the amphibia and many varieties of fishes. Urea has also been found in the tissues and the blood of mammalia. It is very striking that the blood and tissues of many fishes, especially the selachia, contain very large amounts of urea.[1] The part they play in the economy of these animals has not yet been determined.

That urea is one of the end-products in the albumin metabolism, is evident from the fact that its amount is dependent on the extent of albumin decomposition taking place in the tissues. The human urine for twenty-four hours contains, under normal conditions of nutrition, about thirty grams urea. The quantity of urea is greater on increasing the amounts of albumin administered, or by reason of an increased decomposition of the albumin; and, conversely, any diminution of changes in the albumin will produce less. This applies only within certain limits. In many cases an increased disintegration of albumin is accompanied by

[1] W. v. Schröder: Z. physiol. Chem. **14**, 576 (1890). Cf. also O. Hammarsten: Z. physiol. Chem. **24**, 322 (1898). S. Baglioni: Zentr. Physiol. **19**, 385 (1905). V. Diamare: *ibid.* **19**, 545 (1805).

a small, or even no, increase in the amount of urea in the urine, for the reason that other nitrogenous decomposition products are also formed, which, in part, are direct indications of incomplete combustion of the albumin. We observe an increased albumin disintegration during many pathological processes, such as fevers, phosphorus, arsenic and antimony poisoning, and also with an insufficient supply of oxygen. We shall see later that an increased elimination of nitrogen follows the administration of the thyroid gland, and of the hypophysis, or of extracts obtained from these organs. In *Morbus Basedowii* we shall become acquainted with a disease, which is evidently related to the changes in metabolism of the thyroid gland, and is characterized by an increased disintegration of albumin.

We are here chiefly interested in this question: How is urea produced from albumin? We cannot give a direct answer. We may in fact add that the formation of urea from albumin has never been satisfactorily explained. We are accustomed to assume that a hydrolytic cleavage precedes the oxidation of the absorbed and assimilated nourishment. The cell evidently does not consume glycogen, but *d*-glucose, and perhaps not even this directly, but only after the cell has altered it in a manner as yet undetermined so that oxygen can attack it. We assume likewise that the fats are decomposed into their components and are then completely oxidized. Many observations indicate that the proteins in the cell-metabolism are first hydrolyzed and then the cleavage-products are consumed.

An observation by Drechsel [1] seemed to place the proteins in an exceptional position. Drechsel obtained urea from albumin simply by hydrolysis, although the yield was very small. We know to-day that the amount of urea thus formed is dependent on the quantity of arginine present in the protein.

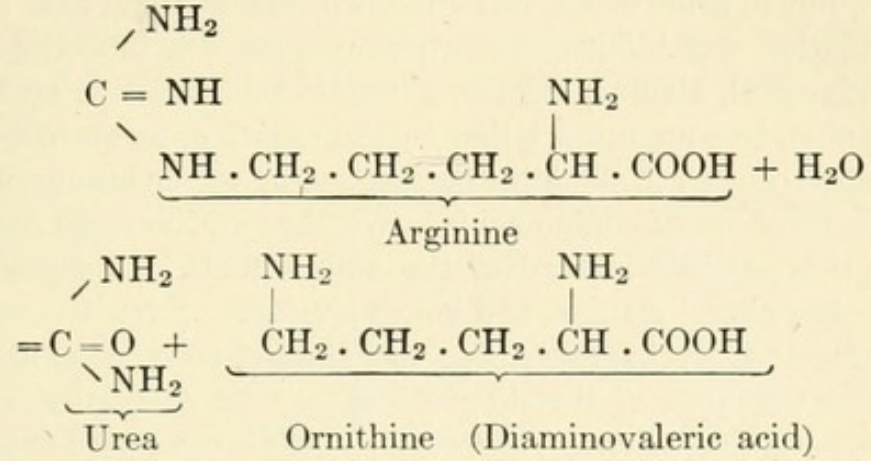
A. Kossel and H. D. Dakin [2] have also recently shown that urea may be obtained from arginine in the tissues. They permitted erepsin to act on clupein sulphate, and found that this protamine was attacked by the ferment. After a time all of the arginine present in the molecule was found in the digesting fluid. A repetition of the experiment, using another erepsin preparation, failed to remove the biuret reaction, even when the process was allowed to continue through a long period of time. Higher complexes remained unattacked.

On analyzing the digesting mixture Kossel and Dakin found the following products: (1) protone, the peptone of the protamine; (2) arginine; (3) ornithine; (4) urea; and (5) amino-valeric acid. This discovery indicates that the erepsin further hydrolyzes a portion of the arginine into

[1] Ber. **23**, 3096 (1890).

[2] Z. physiol. Chem. **41**, 321 (1904); **42**, 181 (1904). Münchener med. Wochenschrift **No. 13**, 1904.

its components. These investigators also succeeded in showing that the splitting off of urea was due to the action of a specific ferment which they called *arginase*. This ferment has been isolated from the liver, kidneys, thymus and lymphatic glands, and the chopped-up intestine of the dog. Small amounts are also present in the blood and muscles, but it is absent in the suprarenal glands, in the spleen, and in the pancreatic juice. The formation of urea from arginine proceeds according to the following scheme:



Now arginine is formed in the alimentary tract by the action of trypsin, and we can easily imagine that this diamino acid is also formed in the tissues by the breaking up of the proteins. One of the sources of urea is, therefore, explained by this discovery. But the proteins used as food-material contain far less arginine than they do of protamines. Only the smallest part of urea present in the urine can originate from this source, as the following table shows:

	100 gms. Albumin contains gms. Arginine.
Salmine	87.4
Histon from the thymus glands	15.5
Gliadin	3.4
Gluten-casein	4.4
Casein	4.8
Edestin	11.7
Gelatin	7.6

Another interesting discovery accompanied the conclusion that urea was split off from arginine in the tissues; namely, the formation of *ornithine*. Jaffé had already proved that as a matter of fact this diaminovaleric acid is formed in the organism, by showing that ornithuric acid was excreted when benzoic acid was fed to birds. Ornithuric acid is the dibenzoyl derivative of ornithine, and has the formula $C_5H_{10}(C_6H_5CO)_2 . N_2O_2$. The intermediate product of metabolism, ornithine, is undoubtedly

protected against further oxidation by its combination with benzoic acid, and is excreted as such.

The question now arises regarding the manner in which urea is formed from ornithine, and the other amino acids. We can say that it has been shown that urea is actually formed from amino acids in the animal organism.[1] This discovery is of great significance. It had always been thought possible that the proteins were broken down, not through the amino acids, but in some other, still unknown, manner, and that the secret of urea formation was hidden somewhere in such decomposition. If we administer glycocoll, alanine, leucine, glutamic acid, aspartic acid, asparagine, or arginine, to a mammal, either by the mouth or subcutaneously, we find an increase of urea in the urine corresponding to the amounts of nitrogen added in the form of amino acids. We observe the same result when we use the polypeptides instead of the amino acids, e.g. glycyl-glycine, diglycyl-glycine, alanyl-alanine, and leucyl-leucine.[2] Even the anhydrides so far investigated, glycine anhydride and alanine anhydride, are disintegrated by the organism of the dog into urea. An especially interesting case is that of arginine, already referred to, which is composed of two parts, guanidine and diaminovaleric acid (ornithine). We have already called attention to the ease with which urea is formed from the first substance. W. H. Thompson has shown [3] that when arginine is administered to a dog, the nitrogen of the first component, guanidine, quickly reappears in the urine as urea. Ornithine itself is more slowly converted into urea. Even 100 per cent of the nitrogen of arginine can be found in the urine of dogs as urea. Very appreciable differences sometimes occur in different individuals. S. Salaskin [4] has shown that the liver probably plays an important part in the production of urea from the amino acids. He passed glycocoll, leucine and aspartic acid through a dog's liver and found that the blood used as a circulating medium showed an increase in its content of urea. His method of proof is not very satisfactory, owing to the fact that his method of estimating urea is not free from criticism.

Before discussing the statements which give us an idea of the chemical processes participating in the formation of urea, we wish to mention those hypotheses, which, being based upon experimental results, will give us the best conception of the formation of urea from albumin and its cleavage-products. We find it necessary to add that it is extremely difficult, from

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[1] O. Schultzen and M. Nencki: Ber. **2,** 566 (1869), and Z. Biol. **8,** 124 (1872). M. Nencki: Ber. **5,** 890 (1872). W. Knieriem: Z. Biol. **10,** 264 (1874). E. Salkowski: *ibid.* **4,** 54 and 100 (1880).

[2] E. Abderhalden and Y. Teruuchi: *loc. cit.* E. Abderhalden and Franz Samuely: *loc. cit.* E. Abderhalden and B. Babkin: Z. physiol. Chem. **47** (1906).

[3] J. Physiol. **32,** 137 (1905), and **33,** 106 (1905).

[4] Z. physiol. Chem. **25,** 128 (1898).

the literature at hand, to draw a correct conclusion regarding the subject. In very many cases we have contented ourselves with a more or less exact method of determining the urea or its antecedents. This proof is generally an indirect one. We shall, therefore, confine ourselves to the most important investigations, abstracting only the essentials from each of them.

M. Nencki [1] characterizes the urea formation as analogous to a type of synthesis, which takes place to a considerable extent in the animal organism; namely, the combination of two substances with elimination of water. He uses the formation of hippuric acid from glycocoll and benzoic acid, as an example:

$$\underbrace{C_6H_5 . CO . OH}_{\text{Benzoic acid}} + \underbrace{H . NH . CH_2 . COOH}_{\text{Glycocoll}} = \underbrace{C_6H_5CO . NH . CH_2 . COOH}_{\text{Hippuric acid}} + H_2O.$$

Again,

$$\underbrace{C_{23}H_{39}O_3 . COOH}_{\text{Cholic acid}} + \underbrace{H . NH . CH_2 . COOH}_{\text{Glycocoll}}$$

$$= \underbrace{C_{23}H_{39}O_3 . CO . NH . CH_2 . COOH}_{\text{Glycocholic acid.}} + H_2O.$$

We can add other innumerable examples to these. We need only refer to the formation of glycogen, fats, and albumin from their components, and to the numerous examples of the conjugation of glycocoll, glucuronic acid, and sulphuric acid, with other foreign bodies. Thus, we have as an example of each:

$$\underset{\text{Phenol}}{C_6H_5OH} + HO . SO_3K = \underset{\text{Pot. phenolsulphate}}{C_6H_5 . O . SO_3K} + H_2O.$$

$$\underbrace{C_{10}H_7COOH}_{\text{Naphthoic acid}} + \underbrace{NH_2 . CH_2 . COOH}_{\text{Glycocoll}} = \underbrace{C_{10}H_7CO . NH . CH_2 . COOH}_{\text{Naphthuric acid}} + H_2O$$

$$\underbrace{(CH_3)_3 . COH}_{\text{Trimethyl-carbinol}} + \underbrace{C_6H_{10}O_7}_{\text{Glucuronic acid}} = \underset{\text{Conjugated Glucuronic acid}}{(CH_3)_3 . CO . C_6H_9O_6} + H_2O.$$

M. Nencki considers the formation of urea from ammonium carbamate to take place similarly by the elimination of water:

$$\underbrace{NH_2 . CO . O . NH_4}_{\text{Ammonium-bamate}} = \underbrace{NH_2 . CO . NH_2}_{\text{Urea}} + H_2O.$$

[1] Ber. **5**, 890 (1872).

Schmiedeberg[1] is of the same mind, only he believes that the urea is produced from ammonium carbonate. We can imagine that the ammonium carbamate is an intermediate product, as indicated by the following formulæ:

$$\begin{array}{c} \diagup O\,(NH_4) \\ C = O \\ \diagdown O\,(NH_4) \\ \text{Ammonium} \\ \text{carbonate} \end{array} \rightarrow \begin{array}{c} \diagup O\,(NH_4) \\ C = O \\ \diagdown NH_2 \\ \text{Ammonium} \\ \text{carbamate} \end{array} \rightarrow \begin{array}{c} \diagup NH_2 \\ C = O \\ \diagdown NH_2 \\ \text{Urea} \end{array} + H_2O$$

Consequently no important difference seems to exist between the last two views. Hoppe-Seyler[2] and Salkowski[3] look upon the formation of urea in another manner. They assume that cyanic acid and ammonia are first produced from albumin:

$$\underbrace{NCOH}_{\text{Cyanic acid}} + NH_3 \rightarrow \underbrace{CN\,.\,O\,.\,NH_4}_{\text{Ammonium cyanate}} \rightarrow \underbrace{\begin{array}{c} \diagup NH_2 \\ CO \\ \diagdown NH_2 \end{array}}_{\text{Urea}}$$

F. Hofmeister[4] has finally proposed a third hypothesis. He assumes that urea is produced by an oxidation synthesis. From this point of view, a $CONH_2$ group is formed by the oxidation of albumin or its amino acid, which, at the moment of its formation, unites with the NH_2 residue of ammonia, when the latter is oxidized, thus producing urea.

Of the three theories mentioned, that of Hoppe-Seyler and Salkowski seems to us the least probable. It has the least experimental support. Again, cyanic acid has not been discovered in the organism. The Nencki-Schultze-Schmiedeberg and the Hofmeister hypotheses, on the other hand, have many observations substantiating their claims. In the first place, numerous experiments have established the fact that ammonium carbonate, and all those other ammonium salts which are capable of being converted into it in the tissues, are changed into urea by the animal organism.[5] This applies to the carnivora as well as to the herbivora. It had seemed, it is true, as if the former were an exception. After the administration of sal-ammoniac, NH_4Cl, to rabbits, the increased elimination of urea corre-

[1] Arch. exp. Path. Pharm. **8,** 1 (1879).

[2] Physiologische Chemie, Berlin, **1881,** pp. 809 and 810.

[3] Z. physiol. Chem. **1,** 1 and 374 (1877).

[4] Arch. exp. Path. Pharm. **33,** 198 (1894); **37,** 426 (1896).

[5] W. v. Knieriem: Z. Biol. **10,** 263 (1874). E. Salkowski: Z. physiol. Chem. **1,** 1 (1877). Cf. also L. Feder: Z. Biol. **13,** 256 (1877). I. Munk: Z. physiol. Chem. **2,** 29 (1878–79). E. Hallervorden: Arch. exp. Path. Pharm. **10,** 124 (1879). F. Walter: *ibid.* **7,** 148 (1877). Corander: *ibid.* **12,** 76 (1880). J. Pohl and E. Münzer: *ibid.* **43,** 28 (1900).

sponded exactly with the amount of nitrogen added in the form of sal-ammoniac. The results were not so definite with human beings and dogs. A part of the ammonia appeared in the urine, and, from the uncertain increase of urea, it remained undecided whether this was due to the ammonia diet or an increased disintegration of albumin. The cause of the different behavior between the carnivora and the herbivora was soon discovered. It depends on the following: The food of the herbivora yields an alkaline ash, and during its combustion in the organism it forms potassium carbonate which can react with ammonium chloride. Ammonia is liberated, and can then be utilized for the production of urea. The conditions are entirely different with the carnivora. Its food furnishes an acid ash. The hydrochloric acid is not separated from the ammonia in the tissues, and consequently the latter is not available for the production of urea. If, on the other hand, we feed some ammonium carbonate to a dog, we likewise observe an increase of urea. These experiments, therefore, indicate that the organism of mammals is capable of utilizing ammonia for the production of urea.

The observations of N. Nencki, J. Pawlow, and J. Zaleski [1] have indicated the probability that ammonia normally — i.e. without being artificially administered — participates in the formation of urea. They showed that portal blood contained much more ammonia than did the venous blood of the liver. The intestine evidently sends ammonia to the liver, where it is transformed. If the liver be extirpated, we no longer observe any difference between portal blood and that obtained from any other part of the body.

W. v. Schröder [2] has shown that the liver can produce urea from ammonium carbonate and ammonium formate. He passed blood, to which he had added these ammonium compounds, through the liver of a dog, and could soon detect an increase in the amount of urea therein. There can, therefore, no longer be any doubt that the liver plays a very important part in the production of urea. Analogous experiments were carried out with the kidneys and muscles, without, however, showing any increase in the formation of urea in these organs.

That the liver is not to be looked upon as the only place where urea is formed, is evident from the fact that its production is continued, even if less in amount, after the liver has been entirely extirpated. These discoveries only became possible after it was known how to make the so-called "Eck's fistula." [3] Mammals do not tolerate the complete extirpation of the liver. They die shortly after the operation. They can,

[1] Arch. exp. Path. Pharm. **37,** 26 (1895).

[2] Arch. exp. Path. Pharm. **15,** 364 (1882); **19,** 373 (1885).

[3] M. Hahn, O. Massen, M. Nencki, and J. Pawlow. Arch. exp. Path. Pharm. **32,** 161 (1892).

however, be kept alive for a considerable time, if the liver is cut off from the general circulation while the portal vein is sewed to the *Vena cava inferior* near the hilus of the liver, and communication then established between the two veins. The blood then passes directly from the intestine into the general circulation. The experimental results obtained after this operation are not always uniform, owing to the fact that some of the blood will often find its way through the liver on account of a collateral circulation which may develop.

If, thus, the utilization of ammonia in the formation of urea has been established, we must now determine whether it is to be assumed that all, or at least the greater part, of the amino groups present in the tissues are split off as ammonia, and thus take part in the production of urea. Such an assumption has much in its favor. In recent years a number of processes have been discovered in the animal organism which indicate the presence of ferments which cause the removal of the amino group, and, in fact, such processes are known in the vegetable as well as in the animal kingdom. We can imagine that entirely analogous to the breaking down of carbohydrates and fats in the tissues, the proteins are first hydrolysed with the formation of the separate amino acids, from which ammonia is split off. Nitrogen-free carbon chains would then remain, by the combustion of which the cells could then obtain their energy. Unfortunately, we know nothing further about these carbon chains. It is possible that they enter into relations with the carbohydrates and with the fats. The CO group for the production of urea does not necessarily have to originate from the albumin itself.

We have already called attention to the assumption that carbamic acid may occur as an intermediate product between the amino acids and urea.

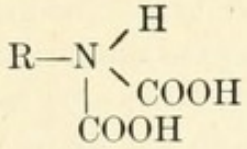
It is not known as the free acid: $\begin{array}{l} \diagup NH_2 \\ CO \\ \diagdown OH \end{array}$, but as its ammonium salt.

The close relations between carbamic acid, $\begin{array}{l} \diagup NH_2 \\ CO \\ \diagdown OH \end{array}$, and urea, $\begin{array}{l} \diagup NH_2 \\ CO \\ \diagdown NH_2 \end{array}$, are evident without further comment. We may consider urea as the amide of carbamic acid. It is difficult to decide, from the investigations at hand, whether this acid is a normal metabolic product. There are many observations at hand which indicate that carbamic acid is present in urine, — in fact, normally so. Abel [1] states that he has succeeded in obtaining this

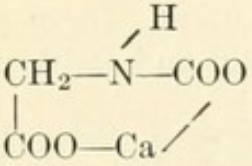
[1] E. Drechsel and J. J. Abel: Arch. Anat. Physiol. **1891**, 236. J. J. Abel and A. Muirhead: Arch. exp. Path. Pharm. **31**, 15 (1893). Cf. also E. Drechsel: J. pract. Chem. **12**, 417 (1875); **22**, 476 (1880).

acid in large amounts from the urine of human beings and dogs after the administration of milk of lime. Furthermore, it has been observed that dogs with an Eck's fistula showed severe indications of poisoning, and that the same symptoms could be obtained by the introduction of carbamates into the blood-stream. This method of proof is not convincing. Macleod and Haskins [1] have recently indicated the normal presence of carbamates in the urine and of an increased appearance after extirpation of the liver. We must admit that the estimation of the carbamic acid was only an indirect one. On the other hand, the formation of carbamates corresponds very well with the assumption of the production of urea from ammonium carbonate, as previously mentioned. We wish to emphasize, however, that its normal occurrence and its relations to the production of urea have not yet been absolutely proved.

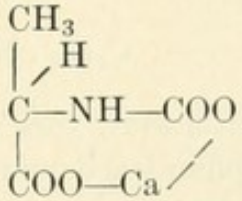
It will be appropriate at this point to call attention to the observation of M. Siegfried,[2] which may have some bearing on the formation of urea as indicated above. M. Siegfried found that when carbon dioxide was led into a mixture of equal parts of normal glycocoll and baryta solutions no immediate precipitation of barium carbonate appeared, as was to be expected, but the mixture remained clear for some time. It gradually became turbid on standing. In studying this subject further, Siegfried found that salts of the following type were formed:



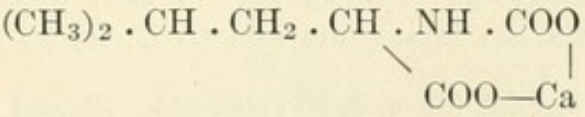
Siegfried analyzed several of these salts, for instance, calcium glycocoll-carbonate (calcium carbamoacetate):



Also the calcium alanine-carbonate (calcium carbamopropionate):



The composition of calcium leucine-carbonate is:



[1] Am. J. Physiol. **12**, 444 (1905).

[2] Z. physiol. Chem. **44**, 85 (1905); **46**, 401 (1905).

We will only refer here to these interesting investigations, and await further developments.

We must also consider Hofmeister's hypothesis and its claims. Hofmeister succeeded first of all in producing urea by the oxidation of albumin, and then also of amino acids, in the presence of ammonia. From 10 grams glycocoll he obtained 3 grams urea. The assumption of an oxidizing synthesis in the formation of urea has much in its favor. On the one hand, the conditions in the animal organism are very favorable for such a production of urea from amino acids; and then again, the whole process harmonizes very well with our conception of the degradation of the proteins. The hypothesis, however, has not yet been proved.

If we take everything that we know about the formation of urea in the animal organism, into consideration, we can conclude that a part of urea is directly produced by the hydrolytic cleavage of albumin, the amount depending on the nature of the latter. Arginine is the only source so far known for this process. This does not, however, exclude the possibility that other analogous complexes to that of this amino acid may be present among the as yet unknown elementary constituents of albumin. We also know that amino acids and polypeptides, when incorporated in the animal organism, go over into urea. We do not, however, know the manner in which this further decomposition is accomplished. It is not impossible that the anhydride formation, or the oxidizing synthesis, plays an important part. Both assumptions are supported by experimental evidence. As far as the place of formation of the urea is concerned, we are certain that the liver produces it. We do not know whether other organs also participate in its formation.

It has been desired to draw definite conclusions from the presence of the following compounds in the urine. If we administer aminobenzoic acid to the animal organism we will find carbaminobenzoic acid in the urine:

$$NH_2 . C_6H_4 . COOH \longrightarrow NH_2 . CO . NH . C_6H_4 . COOH.$$[1]

After the administration of ethylamine (carbonate) we find ethylurea:

$$C_2H_5NH_2 \longrightarrow C_2H_5 . NH . CO . NH_2.$$[2]

Taurine, under analogous conditions, goes over into carbaminoisethionic acid, sulphanilic acid into sulphanilcarbamic acid, and *o*- and *p*-amino-salicylic acid into the corresponding carbamino acids.

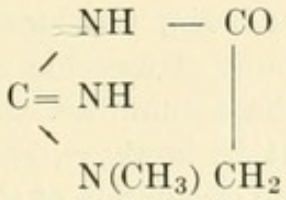
We can easily imagine a conjugation of urea with the compounds in question, and ascribe an analogous *rôle* to it, as to glycocoll, sulphuric

[1] E. Salkowski: Z. physiol. Chem. **7**, 93 (1883-83). Cf. also R. Cohn: *ibid.* **17**, 274, 292 (1893).

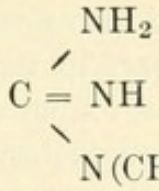
[2] O. Schmiedeberg: Arch. exp. Path. Pharm. **8**, 1 (1877).

acid, and glucuronic acid. The three last compounds unite with a large number of other substances, as is well known, thus protecting the tissue-cells from being attacked. Effort has been made to utilize the above observations as proof of the occurrence of cyanic acid, while, on the other hand, Hofmeister has looked upon it in the light of his assumption of an oxidizing synthesis. We are, therefore, still unable to decide the exact manner in which urea is produced.

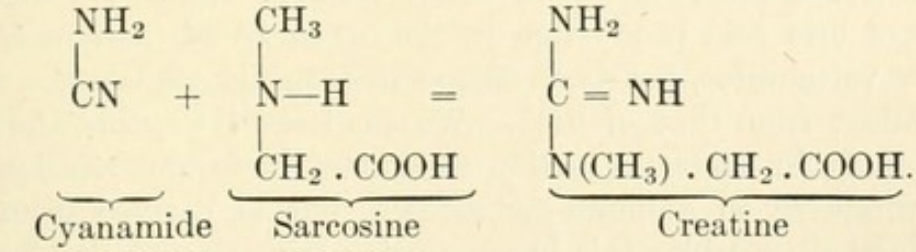
We also desire to call attention to a compound present in urine, although only in small amount, which has often been mentioned in relation to urea. This is *creatinine:*



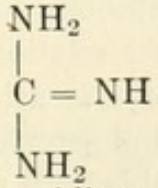
The amount excreted daily by human beings varies from 0.6 to 1.3 gram. It is the anhydride of creatine, which is present in the muscles, and is a methyl-guanidine-acetic acid:



On boiling with baryta water creatine decomposes, adding water, and forms urea, sarcosin, and other products. It may also be obtained synthetically by heating sarcosine (methyl-glycocoll) with cyanamide in a sealed tube to 100 degrees,[1] or by adding a few drops of ammonia in the cold to a saturated solution of sarcosine containing the equivalent amount of cyanamide, and allowing the mixture to stand:[2]



Creatine may also be considered a substituted guanidin, as the following formula shows:


Guanidine

[1] J. Volhard: Zeit. f. Chem. **1869**, 318.

[2] A Strecker: Jsb. Chem. **1868**, 686.

We have already indicated, while discussing arginine and arginase, that urea can be formed from guanidine. Creatine, therefore, may also be looked upon as an antecedent of urea, although we do not possess any confirmatory proof of this. The position of creatine in the general metabolism is, in fact, extremely uncertain. We do not even know its source. It is generally considered as being directly related to the proteins. Its formation from albumin has, as yet, never been proven. It appears far more probable that it is more directly related to the nourishment; at any rate, the elimination of creatine depends upon the introduction of food. It is principally found in the muscles, although it has also been isolated from the blood, brain, transudates, and in the amniotic fluid. It is stated that the amount of creatine is increased by muscular work. This has given rise to the assumption that its formation is related to muscular contraction. It is, however, also possible that its elimination may be due to an increased circulation of the blood and the changes which occur in a working muscle.

Uric acid must also be looked upon as a source of urea. It has been shown that a part, at least, of this substance when administered to the organism of mammals, is changed into urea.[1] This form of urea production plays only an insignificant part in mammals. It was long thought that uric acid was first formed from albumin, and that this was then converted into urea. It was believed to have been observed that a diminished oxidation in the tissues was responsible for an increase in the amount of uric acid at the expense of urea. Such an assumption was supported by the observation that those animals, namely the reptiles, whose metabolism is very slow, showed only uric acid in their excreta. Opposed to this assumption was the fact that birds, whose metabolism we generally consider to be a very rapid one, likewise excreted the larger part of the nitrogen from their food in the form of uric acid. To-day, the question of uric acid production in the organism of mammals, and also the other vertebrates, has been more satisfactorily explained. It is quite independent from that of urea. We shall see later, that the uric acid of mammals, in contradistinction to that of birds and reptiles, has no relation whatever to albumin-metabolism, but is derived from the nucleins. We shall consider this in connection with the constitution of uric acid and related compounds, and shall deal with the subject here only in so far as is related to the metabolism of albumin.

There are, undoubtedly, direct relations between the decomposition of albumin and the elimination of uric acid in those animals in which the larger part of the nitrogen administered reappears in the form of uric acid. This is especially noticeable with reptiles. Here the elimination of uric acid stands in the same relation to the albumin taken into the

[1] Wöhler and Frerichs: Ann. **65**, 335 (1848); Neubauer: Ann. **99**, 206 (1856).

system, as does urea in the case of mammals. The analogy between uric acid and urea has received further support from the discovery that the same materials which produce an increase in urea in mammals, will cause an increased elimination of uric acid in birds. Thus, von Knieriem [1] showed such an influence by the administration of amino acids, while von Schröder [2] observed the same thing by feeding ammonium salts. Urea,[3] also, will cause an increased elimination of uric acid.

Minkowski [4] obtained a further insight into the production of uric acid in birds, by extirpating the liver of geese. These animals will survive the operation for 20 hours, because, in them, the portal vein is not the only outlet of the splanchnic vessels, but is supplemented by another, the *Vena communicans*. The elimination of nitrogen during this operation was somewhat diminished. The greatest change occurred in the uric acid of the urine. Normal geese eliminate from 60–70 per cent of the total nitrogen in the urine as uric acid, while those whose livers had been removed showed only 3.6 per cent. Ammonia, in large amounts, took the place of uric acid. Minkowski also noticed at the same time a large amount of sarcolactic acid in the urine, a substance which is entirely absent in the urine of normal geese. The question now arises, What relation do the eliminated ammonia and lactic acid bear to the formation of uric acid?

We can imagine that the increased formation of ammonia arises from a secondary process due to the appearance of the lactic acid. We are aware of the fact that in ammonia the animal organism has a valuable weapon to protect itself against acids. An increased appearance of ammonia in the urine is very often a direct indication of an increased production of acid in the organism. That the presence of the lactic acid was an important factor in the increased production of ammonia is evident from the fact that an addition of alkali caused an appreciable diminution of the amount of ammonia in the urine.[5] At any rate, a part of the ammonia must be looked upon as exerting a neutralizing effect.

Hoppe-Seyler [6] has shown that the presence of the lactic acid was due to a diminished oxidation in the tissues, as a result of the operation. It is well known that a lactic acid formation often accompanies a diminished supply of oxygen. Minkowski [7] has replied to this objection by showing that the total extirpation of the liver is not the only way an

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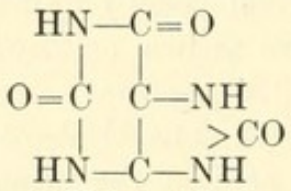
[1] Z. Biol. **13,** 36 (1877).
[2] Z. physiol. Chem. **2,** 228 (1878).
[3] H. Meyer: Inaug. Diss. Königsberg, 1877.
[4] Arch., exp. Path. Pharm. **21,** 89 (1886); **31,** 214 (1893).
[5] S. Lang: Z. physiol. Chem. **32,** 320 (1901).
[6] Festschrift zu Vichow's 70 Geburtstag, **1891.** Cf. also Araki: *loc. cit.*
[7] Arch. exp. Path. Pharm. **31,** 214 (1893).

excretion of lactic acid can be caused, but that even ligating the vessels of the liver is sufficient to bring this about. Lactic acid will only appear in the urine at the moment when the last branch of the hepatic artery has been tied. There is no lactic acid formed, if a single branch is left free.

Kowalewski and Salaskin [1] have shown that the appearance of lactic acid after the extirpation of the liver is actually to be traced back to a disturbance in the formation of uric acid. They could detect the formation of lactic acid by merely leading ammonium-lactate through the liver of birds. H. Wiener [2] also showed relations between the lactic acid and the formation of uric acid. He fed lactic acid and urea to birds, and noticed an increase in the uric acid.

In order to give an idea of the relationship of lactic acid to uric acid it will be necessary to give the constitution of uric acid. It is a 2, 6, 8 trioxypurine:



Uric acid

Horbaczewski has obtained it synthetically by heating urea and glycocoll together, and also by heating trichlorlactamide with an excess of urea. Uric acid is decomposed on strongly heating into urea, hydrocyanic acid, cyanuric acid, and ammonia. If uric acid is heated with concentrated sulphuric acid in a sealed tube at 170° C. it breaks up into glycocoll, carbon dioxide, and ammonia. Strecker,[3] to whom we owe this discovery, compares the uric acid production from the components glycocoll and cyanic acid with the production of hippuric acid from glycocoll and benzoic acid.

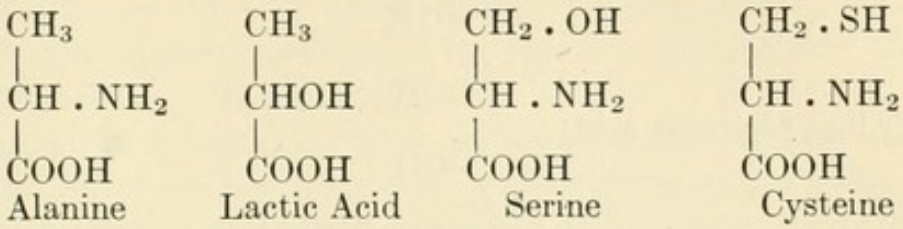
We observe from this that the synthesis of uric acid from lactic acid and ammonia, or urea, is a very plausible one. The standpoint that uric acid is a direct degradation product of albumin has long been discarded. Uric acid, which obtains its nitrogen from albumin, can only be produced synthetically. We wish to call attention at this point to the very close analogy between the production of uric acid and that of urea. It can be shown in both cases that ammonia, directly or indirectly, plays a part, as does also an acid (carbonic acid or lactic acid). The ammonia in both cases may have the same origin, being derived from albumin or its cleavage-products. The organisms of the birds and reptiles evidently are also capable of causing the removal of the NH_2 group. This is appar-

[1] Z. physiol. Chem. **33,** 210 (1901).

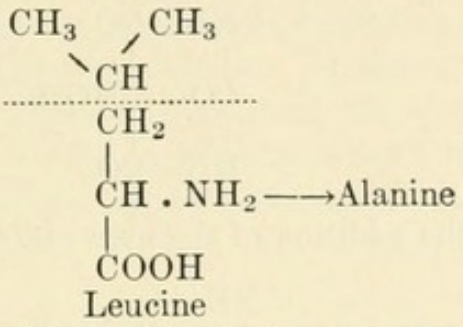
[2] Verh. XVII, Kong. Med. **1889,** p. 622, and Arch. exp. Path. Pharm. **42,** 375 (1899); Verh. XIX, Kong. Med. **1901,** 383. Hofmeister's Beit. **2,** 42 (1902). Cf. also H. Wiener: Die Harnsäure, Ergebnisse der Physiologie (Asher and Spiro) **1,** I, 555 (1902).

[3] Ann. **146,** 142 (1868).

ent from the comparatively large production of lactic acid. The latter may be derived from various sources. Here, the carbohydrates, as well as the amino acids, come into consideration. We know some of these which are closely related to lactic acid. We would refer especially to alanine:



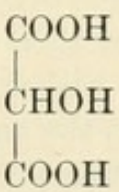
We can also imagine that serine and cysteine may bear some relation to the formation of lactic acid. Leucine might also produce lactic acid, if we assume that its carbon chain is broken in the middle:



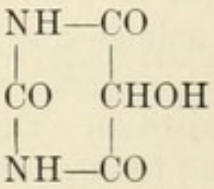
We can, therefore, easily derive all of the components of uric acid direct from albumin. It is not to be implied, however, that lactic acid may not also arise from other sources.

It might be thought that some idea of the formation of urea could be obtained from the way uric acid is produced. We, however, know that there are several ways in which the uric acid formation may be explained. We can take all of our theories for the formation of urea and apply them directly to that of uric acid. It is possible that the synthesis in this case is primarily carried out with the elimination of water; although it is also conceivable that an oxidation synthesis may be a factor. It is not at all impossible that the formation of urea may play a part in the synthesis of uric acid.

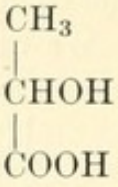
Wiener's investigations will give us an idea of these relations. He found that tartronic acid:



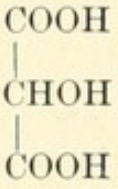
and its ureide (dialuric acid):



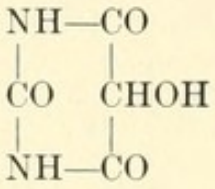
easily go over into uric acid; in fact, when isolated organs are used, this result has been accomplished. Wiener assumes from this that lactic acid:



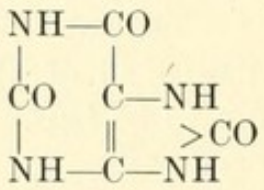
goes over into tartronic acid:



which then forms dialuric acid:



and finally, by the addition of the urea radical, produces uric acid:



We should, therefore, have to assume that the main cause of the disturbance in the synthesis of uric acid, after the liver had been removed, was the non-oxidation of lactic acid into tartronic acid, and, evidently, also, the non-formation of urea.

It is clear that we are not yet prepared to state that the formation of uric acid proceeds normally in this manner. Wiener's investigations at all events indicate the manner in which the synthesis *may* proceed. Wiener also advances the opinion that the organisms of human beings and mammals in general synthesize at least a part of their uric acid, and he sees no real difference in principle between their metabolism of albumin and especially in the formation of their end-products from that of birds and reptiles. The distinction is rather a quantitative one. Birds and reptiles likewise produce urea, but the amount formed is less than that of uric acid. On the other hand, urea predominates in human beings and mammalia. It cannot be denied that such an assumption has much to commend it. Nowhere in the animal kingdom do we observe any sharp demarcations, especially in those processes which are of such great importance as is the case with metabolism. The synthetic formation of uric acid among mammals, according to our present knowledge, must, never-

theless, be relegated to the background. Even if such a process does take place, the amount produced thereby is so small in comparison with that obtained from other sources, that it possesses little significance. Wiener has also tried to show that uric acid is synthetically produced by mammals. His conclusions have, however, been contradicted.[1] The synthetic production of uric acid by the mammalian organism cannot, at present, be accepted with certainty.

The question now arises, In what organs does the uric acid formation take place? The liver seems to play an important part in this process among birds. W. v. Schröder[2] extirpated the kidneys from hens, and succeeded in keeping these birds alive for 5–10 hours. Schröder examined after their death the blood and organs for uric acid, and found that a very appreciable accumulation of this acid had resulted. The uric acid production had evidently continued after the extirpation of the kidneys. The same behavior was noticed with snakes. It seems, therefore, that the kidneys are of little, or even no, service in this process. This decision was the more striking, as the kidneys were for a long time looked upon as the most important place for the production of uric acid. This opinion had originated from the way uric acid is distributed after the ureter has been ligated.

Both of the albumin metabolic substances so far considered are connected with the whole albumin molecule and all its cleavage-products. Ammonia ought also to be included in this group, as it occurs in varying quantities in urine. It was formerly believed that an increased elimination of ammonia indicated an insufficient production of urea. Little by little the cause was more accurately investigated, and it was discovered that the increase in the amount of ammonia was not a primary effect, but that it was due to an increased production of acid. Thus, in a diabetic, the appearance of acetoacetic acid and of β-hydroxy-butyric acid is associated with an increased elimination of ammonia. F. Walter[3] even showed that the administration of hydrochloric acid to human beings and dogs caused an increased elimination of ammonia. A. Schittenhelm and A. Katzenstein[4] have recently shown that the amount of ammonia present in the urine is directly related to the total nitrogen of the urine. It rises and falls with the consumption of albumin, so that the ratio of ammonia to the total nitrogen remains constant within narrow limits. The amount of ammonia excreted is not affected by the administration of urea or ammonium carbonate. It is also interesting to note that, not only does an increased elimination of ammonia follow an increased

[1] Cf. R. Burian: Z. physiol. Chem. **43**, 497 (1905).
[2] Arch. Anat. Physiol. **1880**, p. 113, Supplement.
[3] *Loc. cit.*
[4] Arch. exp. Path. Therapie, **2**, 541 (1905).

diet of albuminous material, but that this also occurs after the addition of the free amino acids, glycocoll and alanine. It is very significant that the administration of ammonium carbonate is followed by a sharp decline in the formation of ammonia, so that the ratio of ammonia-nitrogen to the total nitrogen in the urine becomes less than under normal conditions. We cannot conceive that the amino acids mentioned are capable of producing *acidosis* on their own account, although we can imagine that Siegfried's discovery, that the amino acids take on carbon dioxide forming carbamic acids, is here expressed. On the other hand, this intermediate *acidosis* gives us an indication of the manner in which the decomposition of the albumin cleavage-products is carried out in the tissues. It does not seem improbable that acids are temporarily formed after the ammonia has been split off, which cause an increase in the production of ammonia. Our knowledge of the intermediate metabolism of albumin is so slight at present that we have hardly any conception of these relations.

We will now consider the end-products of albumin metabolism, which occupy a different position from those just mentioned in that they are not derived from the albumin cleavage-products as a whole, but can be traced to definite amino acids. Among these is hippuric acid. It was discovered in horse urine by Liebig. Its method of formation was known to Keller and Wöhler.[1] These authors noticed that when benzoic acid was administered *per os*, it did not reappear as such in the urine, nor could cleavage-products be found which were related to it. Keller and Wöhler, however, noticed an appreciable increase in the amount of hippuric acid. Its manner of formation is evident from its constitution. It is broken down on boiling with strong mineral acids or alkalies, with the addition of water, into benzoic acid and glycocoll:

$$\underbrace{C_6H_5.CO.NH.CH_2.COOH}_{\text{Hippuric acid}} + H_2O = \underbrace{C_6H_5.COOH}_{\text{Benzoic acid}} + \underbrace{NH_2.CH_2.COOH}_{\text{Glycocoll}}$$

It may be synthetically produced from benzamide and monochloracetic acid:

$$\underbrace{C_6H_5.CONH_2}_{\text{Benzamide}} + \underbrace{Cl.CH_2.COOH}_{\text{Monochloracetic acid}} = \underbrace{C_6H_5.CO.NH.CH_2.COOH}_{\text{Hippuric acid}} + HCl$$

It may also be obtained by heating glycocoll with benzoic acid in a sealed tube for 1 or 2 hours at 160 degrees.

The observation of Keller and Wöhler, that benzoic acid appears in the urine united to glycocoll, has been confirmed by numerous investigations

[1] Ann. **43**, 108 (1842); Wöhler and Frerichs; **65**, 335 (1848); Wilhelm Wiechowski; Hofmeister's Beit. **7**, 204 (1905).

through administrations by the mouth, as well as by subcutaneous injections. We know to-day that this synthesis, which at that time created much excitement on account of the fact that it was the first instance in which a synthetic process had been shown to take place in the animal organism, is not unique. Thus, R. Cohn [1] found that naphthoic acid administered to rabbits and to dogs reappeared in the urine as naphturic acid. Its formation is exactly analogous to that of hippuric acid:

$$\underbrace{C_{10}H_7.COOH}_{\text{Naphthoic acid}} + \underbrace{NH_2.CH_2.COOH}_{\text{Glycocoll}} = \underbrace{C_{10}H_7.CO.NH.CH_2.COOH}_{\text{Naphturic acid}} + H_2O$$

Salicylic acid unites with glycocoll in the same manner.[2] Hydroxyhippuric acid is formed:

$$\underbrace{OH.C_6H_4.COOH}_{\text{Salicylic acid}} + \underbrace{H_2N.CH_2.COOH}_{\text{Glycocoll}} = \underbrace{OH.C_6H_4.CO.NH.CH_2.COOH}_{\text{Hydroxyhippuric acid}} + H_2O$$

It is also interesting to note that alkylated benzoic acids, for instance, toluic acid,[3] likewise unite with glycocoll, reappearing as alkylated hippuric acids:

$$\underbrace{CH_3.C_6H_4.COOH}_{\text{Toluic acid}} + \underbrace{NH_2.CH_2.COOH}_{\text{Glycocoll}} = \underbrace{CH_3.C_6H_4.CO.NH.CH_2.COOH}_{\text{Toluric acid}} + H_2O$$

Phenylacetic acid similarly appears in the urine as phenaceturic acid:[4]

$$\underbrace{C_6H_5.CH_2.COOH}_{\text{Phenylacetic acid}} + \underbrace{NH_2.CH_2.COOH}_{\text{Glycocoll}} = \underbrace{C_6H_5.CH_2.CO.NH.CH_2.COOH}_{\text{Phenaceturic acid}} + H_2O$$

We have already seen, when discussing glucuronic acid, which plays a *rôle* in the animal organism very analogous to that of glycocoll, that the cells are able to adapt compounds, which of themselves would not unite together, partly by oxidation, partly by reduction, and sometimes by both methods. Thus, toluene is first converted into benzoic acid and then united to glycocoll. Ethyl- and propylbenzenes are changed in the same manner.[5] Xylene is likewise oxidized to toluic acid. It is interesting to note that aldehydes are oxidized to acids; as an example we will cite

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[1] Z. physiol. Chem. **18**, 112 and 119 (1894).

[2] Bertagnini: L. Ann. **97**, 248 (1856); Z. physiol. Chem. **1**, 244 and 253 (1877–78).

[3] Schultzen and Naunyn: Du Bois' Arch. **1867**, 352.

[4] E. and H. Salkowski: Z. physiol. Chem. **7**, 161 (1882–83); **9**, 229 (1885).

[5] M. Nencki and P. Giacosa: *ibid.* **4**, 325 (1880).

the conversion of nitrobenzaldehyde into nitrobenzoic acid, and the subsequent formation of nitrohippuric acid:[1]

$$\underbrace{NO_2 \cdot C_6H_4 \cdot CHO}_{\text{Nitrobenzaldehyde}} + O = \underbrace{NO_2 \cdot C_6H_4 \cdot COOH}_{\text{Nitrobenzoic acid}}$$

$$\underbrace{NO_2.C_6H_4.COOH}_{\text{Nitrobenzoic acid}} + \underbrace{NH_2.CH_2.COOH}_{\text{Glycocoll}} = \underbrace{NO_2.C_6H_4.CO.NH.CH_2.COOH}_{\text{Nitrohippuric acid}} + H_2O$$

The conversion of benzamide[2] into hippuric acid is especially noteworthy on account of the fact that water must be added to form benzoic acid:

$$\underbrace{C_6H_4 \cdot CONH_2}_{\text{Benzamide}} + H_2O = \underbrace{C_6H_4 \cdot COOH}_{\text{Benzoic acid}} + NH_3$$

The ability of the organism to unite other substances with glycocoll is not confined to benzoic acid or its derivatives, but the same is true of the carboxylic acids of furan-, thiophen- and pyridine-nuclei. Thus, from thiophenaldehyde[3] thiophenic acid is formed, which in the presence of glycocoll goes over into thiophenuric acid:

$$\underbrace{C_4H_3S \cdot CHO}_{\text{Thiophenaldehyde}} + O = \underbrace{C_4H_3S \cdot COOH}_{\text{Thiophenic acid}}$$

$$C_4H_3S.COOH + NH_2.CH_2.COOH = \underbrace{C_4H_3S.CO.NH.CH_2.COOH}_{\text{Thiophenuric acid}} + H_2O$$

Such reactions in the animal organism are of interest in more than one way. Such investigations give an idea of the activity of the animal cell. We notice that oxidation and reduction processes are carried out with the greatest ease, while water is split off, or added, as the case demands.

We ask ourselves, Where does the animal organism obtain the glycocoll which enters into these combinations? We have seen that this amino acid is found among the many cleavage-products of the albumins. There can be no doubt that the animal cells obtain the necessary glycocoll by the decomposition of proteins. The fact that glycocoll will unite with benzoic acid and analogous compounds under technical conditions, permits us to draw valuable conclusions regarding the decomposition of albumins in the tissues. There is little doubt that this also applies to the amino acids. The glycocoll formed is usually further disintegrated into urea; if, however, benzoic acid happens to be present in the tissues, the glycocoll, produced as an intermediate substance, is removed from further metabolism. We have already seen that other albumin cleavage-

[1] *Ibid.* **17**, 274 and 292 (1893).

[2] L. v. Nencki: Arch. exp. Path. Pharm. **1**, 420 (1873).

[3] R. Cohn: Z. physiol. Chem. **17**, 281 (1893). Cf. E. Fromm: Die chemischen Schutzmittel d. Tierkörpers bei Vergiftungen, K. J. Trübner, Strassburg, 1903, p. 14; also M. Nencki: Opera omnia (Vieweg and Sohn, Braunschweig, 1905).

products can be combined in like manner. Thus, on administering benzoic acid to birds we obtain a definite amount of ornithine. In this case hippuric acid does not appear in the urine, being replaced by ornithuric acid, the dibenzoyl compound of ornithine. As previously stated, we can unite cystine with brombenzene, in a dog, thus protecting it from further oxidation. All of these discoveries indicate that the disintegration of albumin in the tissues proceeds in an analogous manner to that of the fats or carbohydrates. Glycogen is first split up into its components and then consumed. The same kind of action takes place with the fats.

We might ask ourselves whether the glycocoll withdrawn from the body by the benzoic acid administered can be directly derived from the amount of decomposed albumin. An answer to this question must be obtained by studying the increased elimination of hippuric acid caused by the administration of benzoic acid, and tracing at the same time the disintegration of the albumin. Such experiments have been made,[1] and it appears that more glycocoll is excreted than can be derived from the disintegrated albuminous substance. Such results are to be very cautiously accepted, as we know comparatively little about the decomposed albumins in the intermediate metabolism. We must always consider the possibility that the animal cell may be capable of producing glycocoll from other amino acids. We have already called attention to a striking example of this by showing that it was impossible to change the composition of the individual amino acids in the serum-albumins by feeding a protein which was especially rich in a certain amino acid.[2] In the case indicated, the " normally " constituted serum-albuminous bodies were evidently produced from gliadin. A glance at the following table will show the changes which must have taken place. We find it necessary to state, in order to prevent any misunderstanding, that our conclusions were naturally only superficial and incomplete. The configuration and stereochemistry will at some time undoubtedly be taken into consideration in studying such changes.

	100 parts of Albumin contain	
	Serum-albumin.	Gliadin.
Glycocoll	3.5	0.7
Alanine	2.2	2.7
Aminovaleric acid	present	0.33
Leucine	18.7	6.0
Proline	2.8	2.4
Phenylalanine	3.8	2.6
Glutamic acid	**8.5**	**31.5**
Aspartic acid	2.5	1.3
Tyrosine	2.5	2.4
Tryptophane	present	present

[1] Cf. W. Wiechowski: Arch. exp. Path. Pharm. **53,** 435 (1905).

[2] E. Abderhalden and F. Samuely: *loc. cit.*

The question arises, What has become of the large amount of glutamic acid? It is possible that it was completely disintegrated in the intestine. This phenomenon may perhaps indicate the reason for the marked increase in the amount of ammonia in the blood of the portal vein during digestion. We might, however, assume that the glutamic acid was converted into other amino acids and utilized in the synthesis of albumin. We are unable to form a definite opinion. It is important, however, to point out the possibility of such transformations, because the conclusion has been drawn from the considerable amount of glycocoll which can be withdrawn from the organism by means of benzoic acid, that the breaking down of all the amino acids to urea passes through the glycocoll stage.[1] We are not justified in making any such assumption. There is no foundation for it. It is far more probable that the organism in any given case utilizes its albuminous substance richest in glycocoll, or, in case of necessity, forms glycocoll from other amino acids. It must not be forgotten that benzoic acid is a poison to the cells, causing an increase in the disintegration of albumin, thus leading to a direct increase in the amount of glycocoll. We must also notice another possible source of glycocoll. We shall soon see that the animal organism is able, to a marked degree, to decompose uric acid. It is assumed, to be sure without an entirely satisfactory proof, that glycocoll is formed as a decomposition product. The amount of this amino acid thus formed is necessarily small among mammals. Finally, we must consider the possibility of glycocoll being produced synthetically, for instance, from ammonia and acetic acid. We have, to be sure, not yet succeeded in detecting such a synthesis.[2]

All of the benzoic acid administered is not changed into hippuric acid. A part is excreted as such, and another portion cannot be traced, evidently being transformed in some unknown manner.

The next question is, In which organ is the hippuric acid produced? G. v. Bunge and O. Schmiedeberg [3] studied first of all the livers of frogs. These survive the extirpation of the liver very well indeed, and live for 3 or 4 days after the operation. They formed hippuric acid when benzoic acid was introduced into the dorsal lymph sac, and particularly large amounts when glycocoll was added at the same time. Hippuric acid has never been obtained from the organisms of frogs or their excretions without the previous administration of benzoic acid. The liver of the frog is, therefore, not the only organ in which the benzoic acid unites with glycocoll, if, indeed, the liver participates at all in this synthesis.

Bunge and Schmiedeberg next tested the kidneys to see if they were able to produce hippuric acid from benzoic acid and glycocoll. They

[1] W. Wiechowski: *loc. cit.*

[2] R. Cohn: Arch. exp. Path. Pharm. **53,** 435 (1905).

[3] Arch. exp. Path. Pharm. **6,** 233 (1877).

ligated the vessels of the kidneys of dogs, and then introduced benzoic acid and glycocoll into the remaining circulation. The animals experimented upon were killed after 3 or 4 hours, and the blood and liver tested for hippuric acid. Benzoic acid, but never hippuric acid, was found. The exact proof that the kidneys of the dog is as a matter of fact the place where the synthesis of hippuric acid is effected, Bunge and Schmiedeberg determined by direct experiment. They cut out the kidneys from a dog that had been just killed, and passed defibrinated blood, to which benzoic acid and glycocoll had been added, through the renal arteries. It flowed away through the veins of the kidneys and returned through the arteries, this process being continued for several hours. Hippuric acid was then found in this blood as well as in the fluid which flowed from the ureter. The other kidney and a part of the original blood were used as a control, but no hippuric acid was found in them. The surviving kidney had, therefore, produced hippuric acid from glycocoll and benzoic acid. When the experimenters added only benzoic acid, but no glycocoll, they found that the amount of hippuric acid formed was very small. This, however, quickly increased when glycocoll was added and passed through the kidney. The synthesis was just as satisfactory at the room-temperature as it was at 37° C.

The red blood-corpuscles and the cells of the kidneys are of great importance in the synthesis of hippuric acid. When the kidney tissues are destroyed by chopping, or, better yet, by rubbing them up with pulverized glass, we find that the conjugation of glycocoll with benzoic acid no longer takes place. When the kidneys are cooled to − 20 degrees and then raised to 40 degrees, it is also found that hippuric acid is no longer produced from its components. Again, the synthesis could not be effected if the serum of the blood, instead of the blood itself, was utilized. It has been shown, by the investigations of A. Hoffmann, that oxygen plays an important part in this synthesis.[1] He led blood through the kidneys, in which the oxygen had been displaced by carbon dioxide, and found that no synthesis of hippuric acid resulted. Quinine also prevented the kidney cells from producing hippuric acid.

It seems very probable that the synthesis of hippuric acid from glycocoll and benzoic acid is due to a ferment, water being split off. The attempt has been made to isolate such a ferment. Recent investigations in which, contrary to earlier experiments, it was found possible to detect the synthesis in the chopped up kidneys, lead to the hope[2] that the

[1] A. Hoffmann: Arch. exp. Path. Pharm. **7**, 233 (1877).

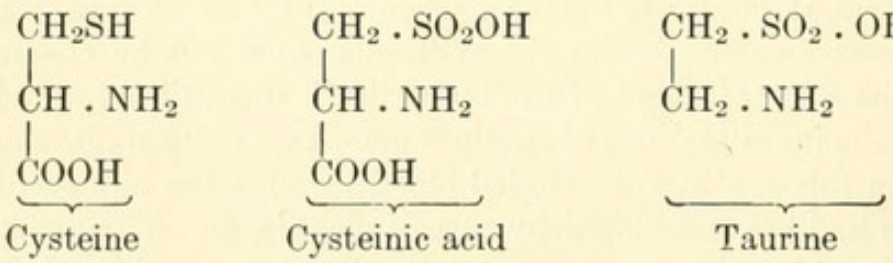
[2] W. Kochs: Pflüger's Arch. **20**, 64 (1879). M. R. Berminzone: Bol. accad. med. di Genua **16**, No. 1 (1901). J. E. Abelous and H. Ribaut: Compt. rend. Soc. Biol. June 9, 1900.

conjugation of glycocoll with benzoic acid can be accomplished without the direct use of organs or cells.

As regards the place where the hippuric acid is formed, it is to be noted that what has been said applies only to dogs. Frogs produce hippuric acid even after the extirpation of the kidneys. Salomon [1] also observed hippuric acid in large amount after the administration of benzoic acid to a rabbit whose kidneys had been removed. It is possible that the synthesis of hippuric acid is more localized in the carnivora than it is with the herbivora, because the formation of hippuric acid by the former under normal conditions is only very small in amount. The quantity of hippuric acid daily excreted by human beings under an ordinary diet is about 0.7 gram. It may be increased to more than 2 grams by a liberal diet of vegetables or fruit.

Glycocoll not only participates in the production of hippuric acid and of the other artificially introduced products just mentioned, but is also a component of *glycocholic acid* and *glycocholeic acid*. Both are decomposed, in the same manner as is hippuric acid, by boiling with acids or alkalies into glycocoll, cholic acid, or choleic acid, respectively. These last two acids are both found as constituents of the bile.

Besides these, there is another acid containing sulphur called taurocholic acid which is found in the bile of most animals, and is likewise related to one of the albumin cleavage-products; i.e. to cystine. When taurocholic acid is heated with acids or alkalies, it is decomposed into taurine and cholic acid. The relations of taurine, which is an amino-ethyl-sulphonic acid, to cystine and cysteine, are evident from the following formulæ:



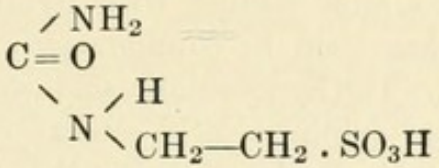
Friedmann has succeeded, as previously mentioned, in converting cysteine into cysteinic acid, and this into taurine. Shortly after the chemical relations between these compounds had been settled, experiments with animals also indicated the probable derivation of taurine from cysteine. W. v. Bergmann [2] fed dogs, in which he had made a complete biliary fistula, with cysteine, and estimated the amount of taurocholic acid separating out with the bile. He could not detect any increase in the sulphur content of the bile in these experiments, but did notice such

[1] Z. physiol. Chem. **3**, 365 (1879).

[2] Hofmeister's Beitr. **4**, 132 (1903).

when sodium cholate, that is, the other component of taurocholic acid, was added at the same time with the cysteine. Wohlgemuth [1] confirmed these experiments, and showed with rabbits that the amount of sulphur in the bile and the sulphur content of the liver increased with the administration of cysteine alone.

We know nothing definite concerning the further history of taurocholic acid or of the taurine contained in its molecule. Salkowski [2] found that, after the administration of taurine to human beings and dogs, a part of this taurine and a substituted urea,



appeared in the urine.

Almost all of the sulphur of the taurine reappears in the urine as sulphuric and sulphurous acids, when fed to a rabbit.

Salkowski could not detect any increased elimination of sulphuric acid nor of sulphurous acid, after taurine had been fed to human beings and dogs. Cysteine increases the elimination of sulphuric acid in the urine of human beings and dogs.[3] The same also is observed in the case of rabbits, while here salts of hyposulphurous acid are found in addition. We will add that thiosulphuric acid has been found in the urine of cats and dogs.

It has been shown recently [4] that cystine, in the form of polypeptides, is apparently decomposed during metabolism in the same manner as when cystine itself is administered. It is interesting to note that in these experiments there was a distinct increase in the oxidized sulphur of the urine corresponding to the duration of the experiment. The urine always contains a part of the sulphur in an unoxidized form. This portion is also called " neutral " sulphur. Its amount varies and to some extent is directly related to the oxidized sulphur.

It is at present impossible to give a clear outline of the relations of the components containing sulphur of the urine to albumin or its cleavage-products, because we are not yet able to recognize all the constituents of proteins which contain sulphur. We can only consider the fact as settled that the sulphur in the cystine administered to an animal organism largely reappears in an oxidized form in the urine; in fact, as sulphuric acid. There is no doubt but that cystine is also formed during a normal disinte-

[1] Z. physiol. Chem. **40**, 81 (1903).

[2] Virchow's Arch. **58**, 460 (1873).

[3] Cf. E. Goldmann: Z. physiol. Chem. **9**, 260 (1885). C. H. Rothera: J. Physiology, **32**, 175 (1905). L. Blum: Hofmeister's Beit. **5**, 1 (1903).

[4] E. Abderhalden and F. Samuely: Z. physiol. Chem. **46**, 187 (1905).

gration of the albumins, and that this is decomposed in the same manner as the cystine which is artificially introduced.

The sulphuric acid of the urine has been subjected to thorough study. It was found that it occurred in various combinations. If we add barium chloride to urine which has been previously acidified, barium sulphate will precipitate at once. A further turbidity will appear after filtering this off and boiling with hydrochloric acid. E. Baumann [1] satisfactorily explained this behavior of sulphuric acid in urine. The sulphuric acid at first precipitated is derived from sulphates — salts of sulphuric acid. That which is obtained after boiling with hydrochloric acid is due to sulphuric acid which has been in combination with different aromatic substances in the urine. The hydrochloric acid decomposes these aromatic compounds — also called sulphuric acid esters — into the aromatic component and sulphuric acid, the latter being then precipitated by barium chloride. The sulphuric acid esters themselves form soluble barium salts. We shall see that sulphuric acid forms the same kinds of compounds with these that we found it does with glycocoll and glucuronic acid. We wish to add at this point that some sulphur compounds still remain in solution even after the sulphur in the sulphuric acid esters have been precipitated. This is the "neutral sulphur" mentioned above. In order to detect this sulphur it is necessary to oxidize it, thus converting it into sulphuric acid, when it will be precipitated by barium chloride. By these methods we are able to isolate all three varieties of combined sulphur in the presence of one another. The determination of the total sulphur together with that of the total nitrogen in the urine gives us a very good conception of the course of the disintegration of albumin.

We must not forget to mention that sulphocyanic acid, or thiocyanic acid, HCNS, also occurs in urine. Gscheidlen [2] found it invariably present in the urine of human beings, horses, calves, dogs, cats, and rabbits. In human urine 0.2 to 0.8 gram is eliminated daily. The sulphocyanic acid comes from the saliva, being formed in the salivary glands. This acid reaches the blood-stream by absorption. If all the ducts of the salivary glands are cut and the saliva discharged externally, sulphocyanic acid no longer appears in the urine. Its origin and significance have never been explained.

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[1] Ber. 9, 54 (1876).

[2] Tageblatt 47, Versammlung deutscher Naturf. u. Aerzte in Breslau, 1874.

LECTURE XII.

ALBUMINS OR PROTEINS.

VI.

Metabolic End-Products.

We have already mentioned the fact that putrefactive processes always take place in the intestines to a greater or less extent. A part of the products thus formed is absorbed and eliminated in the urine. Only a small proportion of these compounds is excreted unchanged, or combined with alkalies. By far the largest percentage of the albuminous cleavage-products produced by putrefaction appear in the urine in the form of complex combinations. Baumann [1] and Brieger [2] have shown that here the acid esters of sulphuric acid are most important. The organism produces glucuronic acid only when there is a deficiency of sulphuric acid. There always seems to be in the urine a small amount of conjugated glucuronic acid compounds. The decomposition products are largely the aromatic components of albumin; in fact, chiefly tyrosine and probably phenylalanine as well. Tryptophane is likewise an important factor. We have seen that tyrosine, which is *p*-hydroxyphenyl-α-aminopropionic acid, is changed by loss of ammonia into *p*-hydroxyphenylpropionic acid; the latter on further oxidation and loss of carbon dioxide finally decomposes into *p*-cresol and phenol. From tryptophane skatole and indole are the end-products obtained.

The most important of the conjugated sulphuric acid compounds are phenyl-, tolyl-, indoxyl-, and skatoxyl-sulphuric acids. Catechoyl-sulphuric acid is also, although not invariably, found in human urine in small quantity. We may state that some of the sulphuric acid combinations have not yet been identified. The amounts of such substances in horse urine are especially large. In human urine, on the other hand, there is much less present than of the other sulphur compounds. From 0.1–0.6 gram is excreted on an average every 24 hours. Experience has shown that no definite values can be given. They vary considerably, and are naturally dependent on the putrefactive changes in the intestines. The excretion of the acid esters of sulphuric acid can be increased artifi-

[1] E. Baumann: Ber. **9,** 54 (1876); **9,** 1389 (1876); **9,** 1715 (1876); **10,** 685 (1877); **11,** 1907 (1878); **12,** 2166 (1879); Pflüger's Arch. **12,** 63 (1876); **12,** 69 (1876); **13,** 285 (1876); Z. physiol. Chem. **1,** 60 (1877–78); **2,** 335 (1878–79); **3,** 250 (1879); **4,** 304 (1880); **10,** 123 (1886); **17,** 511 (1893).

[2] E. Baumann and L. Brieger: *ibid.* **3,** 254 (1879); **3,** 156 (1879).

cially, for instance, by the administration of phenol. If this be introduced into the animal organism, it appears in the urine as potassium phenylsulphate.[1] We assume it to be formed as follows:

$$\underbrace{C_6H_5OH}_{\text{Phenol}} + HO \cdot SO_3K = \underbrace{C_6H_5 \cdot O \cdot SO_3K}_{\text{Potassium phenylsulphate}} + H_2O$$

We will add that not only substances like phenol are eliminated in this way, but in place of phenol we may find: the cresols, $CH_3 \cdot C_6H_4OH$; thymol, $C_3H_7(CH_3)C_6H_3 \cdot OH$, also the dihydroxy-benzenes, $C_6H_4(OH_4)_2$; methylquinol, $CH_3 \cdot O \cdot C_6H_4OH$; orcinol, $CH_3 \cdot C_6H_3(OH)_2$; pyrogallol, $C_6H_3(OH)_3$; tribromphenol, $Br_3C_6H_2OH$; *o*-nitrophenol, $NO_3.C_6H_4.OH$; *p*-amidophenol, $NH_2 \cdot C_6H_4 \cdot OH$; protocatechuic acid, $COOH.C_6H_3(OH)_2$; tannin, salicylamide, *m*- and *p*-hydroxybenzoic acids.

Before discussing the sulphuric acid esters which normally occur in urine, we will mention the fact that in addition to the above compounds produced by the artificial introduction of aromatic compounds, substitution products of the phenols and hydroxyl derivatives of other cyclic compounds may also appear in urine conjugated with sulphuric acid. Such an example is hydroxyquinolin sulphate, which, to some extent at least, appears in urine as an acid ester of sulphuric acid. Here also it is interesting to note that in the organism substances are prepared for combination which of themselves are incapable of reacting together. For example, benzene is first oxidized to phenol, and then united with sulphuric acid. We shall soon see that indole and skatole are also first oxidized to indoxyl and skatoxyl, and then further made to combine:

$$\underbrace{NH : C_8H_6}_{\text{Indol}} + O = \underbrace{HN : C_8H_5OH}_{\text{Indoxyl}}$$

$$\underbrace{HN \cdot C_8H_5 \cdot OH}_{\text{Indoxyl}} + OH \cdot SO_3K = \underbrace{HN : C_8H_5 \cdot O \cdot SO_3K}_{\text{Pot. indoxylsulphate}} + H_2O$$

It is also interesting that substances, themselves capable of combination, are likewise oxidized. This part of the phenol is oxidized to quinol and appears then as quinol sulphuric acid:

$$\underbrace{C_6H_5 \cdot OH}_{\text{Phenol}} + O = \underbrace{HO \cdot C_6H_4 \cdot OH}_{\text{Quinol}}$$

$$HO \cdot C_6H_4 \cdot OH + HO \cdot SO_3K = \underbrace{HO \cdot C_6H_4 \cdot O \cdot SO_3K}_{\text{Potassium quinol sulphate}} + H_2O$$

These reactions indicate that the formation of sulphuric acid esters is dependent on the presence of aromatic compounds, whether these are

[1] E. Baumann and E. Herter: Ber. **9**, 1747 (1876); **1**, 244 (1877–78).

[2] C. Brahm: Z. physiol. Chem. **28**, 439 (1899).

artificially administered or normally produced from the food. It is very probable that, under normal conditions, all the conjugated sulphuric acid compounds are the outcome of intestinal putrefaction. The amount of sulphuric acid esters has even been suggested as indicating the extent of putrefaction taking place in the intestines. We may, however, state that a true conception of the putrefactive changes in the intestines cannot be obtained by the determination of sulphuric acid esters alone. Their amount is naturally dependent, first of all, on that of the absorbed products of putrefaction, and the absorption depends on the time the material remains in the intestines. During diarrhea large amounts of putrefactive products are withdrawn from the organism. The quantity in the fæces would also have to be determined. Moreover, only a part of the putrefactive products leave the organism in an unaltered condition. If indole or phenol is administered to the animal organism, it is partially destroyed, or, more correctly expressed, it cannot be detected in the urine, having been evidently transformed in some manner.

We must also bear in mind that not all the aromatic putrefactive substances appear in the urine in the form of conjugated sulphuric acids, but they are often present as salts, or even in an unaltered condition. We must also remember that the sulphuric acid present is derived from the albumins themselves, and is necessarily limited in amount. It is very probable that larger quantities of glucuronic acid than usual would be obtained in place of the sulphuric acid esters, if albumins, low in sulphur content, were fed to the organism. On the other hand, we must admit the possibility of the organism covering up any such deficit in sulphur by breaking down proteins rich in sulphur from its own tissues, just as well as our present knowledge indicates that, within certain limits, the formation of hippuric acid is independent of the glycocoll in the albumin of the food. As the amount of stored-up sulphur compounds is necessarily small, it follows that the animal organism will soon have to rely upon glucuronic acid when the amount of aromatic putrefactive products exceeds a certain limit. The compounds conjugated with glucuronic acid are naturally not detected in determining the amounts of sulphuric acid esters.

Baumann assumed that the acid esters of sulphuric acid were produced by the combination of aromatic substances with the sulphuric acid residues which circulated in the body in the form of sulphates. This theory has recently been questioned. Tauber[1] only succeeded in obtaining an increased amount of phenylsulphuric acid in administering large amounts of phenol only when he introduced sulphites at the same time; while this was not the case with sulphates. It seems, therefore, that the reaction between the aromatic compound and the radical containing sulphur

[1] Tauber: Arch. exp. Path. Pharm. **36,** 197 (1895); Z. physiol. Chem. **2,** 366 (1878/89); Habilitationsschrift, 1878.

occurs before the latter has been oxidized to sulphuric acid. It is very probable that the final oxidation to sulphuric acid only takes place after the substances have united. We must not neglect to call attention to the analogy existing between the formation of the sulphuric acid compounds and the conjugated glucuronic acids. The latter are also of secondary nature, only appearing after the dextrose has combined with some of the conjugating substance.[1]

We shall now consider those compounds which are produced by the aromatic cleavage substances of proteins being conjugated with sulphuric acid. Let us consider first of all phenylsulphuric acid:

$$C_6H_5O \cdot SO_2 \cdot OH$$

and the *p*-tolyl sulphuric acid:

$$C_6H_4\begin{cases} CH_3 \\ O \cdot SO_2 \cdot OH \end{cases}$$

Both have the same origin and are always classed together. They are found in urine as alkali salts. The quantity of each varies, and depends, as can easily be imagined, on the intensity of the intestinal putrefaction and the amount of resulting products that is absorbed. Tyrosine is the mother-substance of the phenols. As we have seen, all the phenol administered to the body is not excreted as such combined with sulphuric acid in the urine. A part is changed in another way, probably consumed, while another portion is in some cases found in the urine, oxidized to quinoylsulphuric acid. This cannot be detected under normal conditions, although catechoylsulphuric acid is often present in urine, if only in small amount. Catechol is *o*-dihydroxybenzene. It has never been definitely decided whether its sulphuric acid ester is produced by the oxidation of phenol, or arises from some constituent in the food which is not directly related to the proteins.

There is some evidence indicating that catechoylsulphuric acid results from a vegetarian diet, but is not formed in a diet exclusively of meat. It has been suggested that protocatechuic acid is the mother-substance of the sulphuric acid ester mentioned.

We will add to the phenyl- and tolylsulphuric acids the other decomposition products of tyrosine which occur in urine. They may be considered as intermediate products between tyrosine and phenol. Thus, there has been found in urine: *p*-hydroxyphenylpropionic acid (*p*-hydrocumaric acid): $C_6H_4 \cdot OH \cdot CH_2 \cdot CH_2 \cdot COOH$,

and the *p*-hydroxyphenylacetic acid: [2]

$$C_6H_4 \cdot OH \cdot CH_2 \cdot COOH.$$

[1] Cf. Lecture II, p. 33.

[2] E. Baumann: Z. physiol. Chem. **4**, 304 (1880); **6**, 183 and 234 (1882).

They have both been obtained from urine, partly as conjugated sulphuric acids, but mainly in the form of salts.

It is questionable whether these two compounds are always and invariably produced by intestinal putrefaction. It is possible, in fact probable, that these two hydroxy-acids are produced by the decomposition of tyrosine in the tissues. Confirming this is the fact noted by H. Thierfelder and Nuttal,[1] that these two acids were observed in the urine of guinea pigs which had been kept sterile,[2] i.e. they had grown up with their intestinal tracts free from bacteria, consequently there could not be any putrefaction. It is important that the urine of these animals did not contain any typical putrefactive products of proteins; i.e. the phenols.

Para-hydroxymandelic acid,

$$C_6H_4 . OH . CH . (OH) . COOH,$$

has also been found in the urine, although only under specific conditions. Schultzen and Ries [3] found it in acute cases of atrophy of the liver.

Blendermann, [4] after feeding tyrosine to a dog, found a dihydroxyphenylpropionic acid:

$$HO . C_6H_4 . C_2H_3 (OH) . COOH,$$

in the urine.

If we review the investigations just mentioned, we shall see that the same groups of decomposition products are obtained by intestinal putrefaction of tyrosine as are obtained by the same process outside the animal organism.[5] From tyrosine,

p-hydroxyphenylaminopropionic acid: $C_6H_4\begin{cases}OH\\CH_2 . CH (NH_2) COOH\end{cases}$

we obtain the following decomposition products:

p-hydroxyphenylpropionic acid: $C_6H_4\begin{cases}OH\\CH_2 . CH_2 . COOH\end{cases}$

p-hydroxyphenylacetic acid: $C_6H_4\begin{cases}OH\\CH_2 . COOH\end{cases}$

p-cresol: $C_6H_4\begin{cases}OH\\CH_3\end{cases}$

phenol: $C_6H_5 . OH$

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[1] H. Thierfelder and Nuttal: *ibid.* **21,** 109 (1896); **22,** 62 (1897).

[2] Cf. Lecture IV, p. 64.

[3] Schultzen and Ries: Ueber akute Phosphorvergiftung u. Leberatrophie, Berlin, 1869.

[4] H. Blendermann: *loc. cit.*

[5] Cf. Lecture VIII, p. 171.

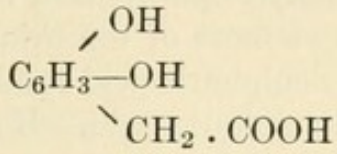
We will state once more that according to the experiments at hand, para-cresol and phenol are to be regarded solely as products of putrefaction, whereas it is still a question with regard to the other products as to how much is formed in the intestine and how much is caused by the intermediate breaking down of albumin, or of tyrosine, in the cells. It is unquestionably certain that *p*-hydroxyphenylpropionic acid and *p*-hydroxyphenyl-acetic acid may be formed beyond the intestine. This discovery serves to give us an interesting insight into the decomposition of tyrosine in the tissues. We first observe that the amino group is split off and that oxidation then sets in. It is still questionable as to how far these observations may be applied to the decomposition in the metabolism of the cells. Some observations seem to indicate that the elimination of the amino group is the first stage of the decomposition of the amino acids.

We must refer to another specific property of tyrosine. In discussing the digestion of albuminous substances by trypsin, we called attention to the fact that this amino acid, together with tryptophane, is very quickly split off from the albumin. It is very possible that this fact may cause tyrosine and tryptophane, — which we shall soon learn to recognize as the mother-substance of skatole and indole,— to fall easy prey to the putrefactive bacteria.

Another question of considerable interest confronts us: What becomes of the other aromatic amino acid, phenylalanine? Investigations on tryptic digestion show that this amino acid shows an entirely different behavior from that of tyrosine. It is not set free by trypsin. In the putrefaction of albumin outside the body, phenylaminopropionic acid breaks down into phenylpropionic acid and phenylacetic acid. The former is not found as such, in urine, but is combined with glycocoll as phenaceturic acid. In this form it has been isolated from normal horse urine. Whether it occurs in human urine, or not, has never been decided. If it were present to a considerable extent, one might, aside from the possibility of its formation from other sources, e.g. from the decomposition products of tyrosine, and its formation during the decomposition processes in the tissues, draw the conclusion that albumin, or a higher complex of amino acids such as a polypeptide, is attacked by the bacteria of putrefaction. The increased appearance of the other two acids would be an indication of intense putrefactive changes taking place in the intestines.

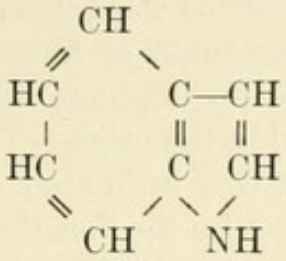
Tyrosine and phenylalanine have also been supposed to participate in the production of hippuric acid. If so, they would furnish the benzoic acid radical. According to the above, it is evident that phenylalanine can hardly play a part here, — at least, as far as it is a question of putrefactive processes in the intestines. Tyrosine alone is to be considered in this connection. It is, of course, possible that phenylalanine, and tyrosine as well, are used for the production of hippuric acid in the metabolism of the cell.

In this connection we would add, that two other acids, closely related to tyrosine and phenylalanine, namely, homogentisic acid and uroleucic acid, have also been found in rare cases in urine. The former is a dihydroxyphenylacetic acid:

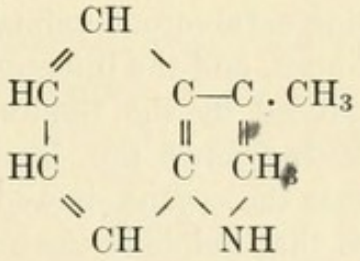


the latter, a dihydroxyphenyllactic acid. They are both found during an abnormal metabolism in the so-called " alcaptonuria."

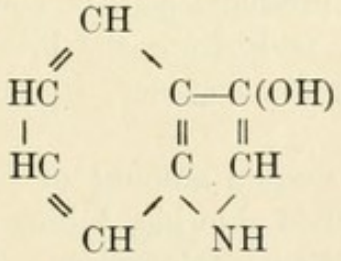
Let us turn from our discussion of the tyrosine and phenylalanine decomposition products to the sulphuric acid esters. We have already considered the occurrence of indoxyl- and skatoxyl-sulphuric acids in urine. Intestinal putrefaction of albumin produces indole:



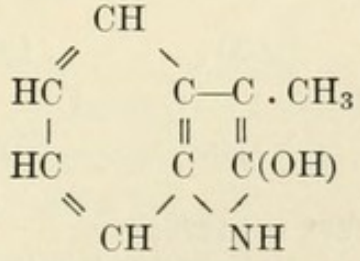
and skatole (methyl-indole):



They are oxidized in the tissues to indoxyl:



and skatoxyl:

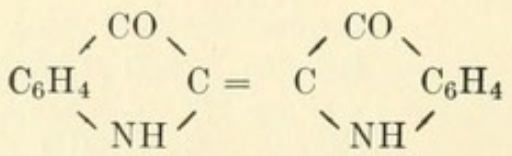


and then combined with sulphuric or glucuronic acids.

We will at once state that skatoxylsulphuric acid has not been detected

with sufficient certainty in urine. It is even problematical whether skatoxyl occurs in urine. The suggestion has been made that it is present in combination with glucuronic acid. At any rate, its presence is only indirectly established. Indoxylsulphuric acid is found as an alkaline salt in urine. It is the source of most of the urine indigo. Jaffé [1] was the first to discover that indoxylsulphuric acid resulted from the combination of indole and indoxyl with sulphuric acid. He injected indole subcutaneously into dogs, and then found large amounts of indoxylsulphuric acid in the urine. The suggestion has also been advanced that the indoxylsulphuric acid in the urine is not only produced by intestinal putrefaction, but that indole and indoxyl are also formed in the tissues. The researches of A. Ellinger and M. Gentzen [2] have made this improbable. These authors showed first of all that tryptophane, which is skatoleaminoacetic acid, is the antecedent of indole, and the latter is formed from it during putrefaction. When tryptophane was introduced directly into the small intestine of rabbits, large amounts of indoxyl quickly appeared in the urine. When, however, the tryptophane was injected subcutaneously, no indoxyl could be detected in the urine. That the excretion of indoxylsulphuric acid in the urine is very intimately connected with the intestinal putrefactive changes, is shown by the fact that when there is an intestinal stoppage, especially in the small intestine, the quantity quickly increases. Observations with fasting carnivorous animals have shown that the elimination of indoxyl continues, and the inference was drawn that indole and indoxyl were also produced by the tissues. F. Müller [3] has, however, shown that, during fasting, only the intestinal contents gave an intense indole test, whereas the organs showed this only to a slight extent. He, therefore, concludes that indole in the urine is exclusively of intestinal origin. A limitation is certainly necessary, and this applies also to the phenols. Putrefactive products can, of course, also be formed in other parts of the organism, aside from the intestines, wherever putrefactive processes are at work; for instance, in putrid empyema, decomposing tumors, etc.

If we add to urine an equal amount of hydrochloric acid containing a little free chlorine or ferric chloride, a blue coloring matter is produced, which can be shaken out with chloroform. This is indigo-blue:



[1] Jaffé: Z. med. Wiss. **1872** and **1875**.

[2] A. Ellinger and M. Gentzen: Hofmeister's Beit. **4**, 171 (1903).

[3] F. Müller: Mit. Würzburger med. Klinik, **2**, 1886; A. Ellinger: Z. physiol. Chem. **39**, 44 (1903).

It is produced by the decomposition of indoxylsulphuric and indoxylglucuronic acids into their components, and the simultaneous oxidation of the indoxyl to indigo-blue. This coloring matter occurs in the indigo plant in the form of a glucoside, called indican.[1] Indigo-blue can occasionally be observed on the surface of putrid urine as a copper-red scum, with a metallic luster. There is also another coloring material present, an isomer of indigo-blue. This is indirubin, indigo-red. This coloring matter has also been assumed to be related to skatoxyl, although this substance has not yet been isolated, as such from normal urine. This question must be left open for the present.

In the putrefaction of tryptophane, skatoleacetic acid and skatolecarboxylic acid are also produced. Up to the present time, only the latter has been shown to be probably present in urine.[2]

Furthermore, cadaverine and putrescine are also to be mentioned among the putrefactive products of the intestines. Cadaverine is formed with loss of carbon dioxide from lysine (diaminocaproic acid), and is a pentamethylenediamine.

$$\underbrace{CH_2 . CH_2 . CH_2 . CH_2 . \underset{|}{CH}(NH_2) . COOH}_{\text{Lysine}} = \underbrace{CH_2 . CH_2 . CH_2 . CH_2 . \underset{|}{CH_2}(NH_2)}_{\text{Cadaverine}} + CO_2$$

Putrescine, tetramethylenediamine, is produced from ornithine, diaminovaleric acid, a constituent of arginine:

$$\underbrace{CH_2 . CH_2 . CH_2 . \underset{|}{CH}(NH_2) . COOH}_{\text{Ornithine}} = \underbrace{CH_2 . CH_2 . CH_2 . \underset{|}{CH_2}(NH_2)}_{\text{Putrescine}} + CO_2$$

Phenylethylamine is produced from phenylalanine:

$$C_6H_5 . CH_2 . CH(NH_2) . COOH = C_6H_5 . CH_2 . CH_2 . NH_2 + CO_2$$

and hydroxyphenylethylamine from tyrosine:

$$\begin{matrix} \diagup OH \\ C_6H_4 \\ \diagdown CH_2 . CH(NH_2)COOH \end{matrix} = \begin{matrix} \diagup OH \\ C_6H_4 \\ \diagdown CH_2 . CH_2 . NH_2 \end{matrix} + CO_2$$

Recently [3] the attempt has been made to trace the production of phenylethylamine and of hydroxyphenylethylamine to the action of trypsin or of pepsin. A great many experiments have been carried out, with pure pancreatic and gastric juices, taking great care to prevent any bacterial

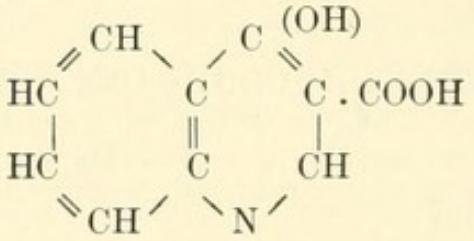
[1] The indoxyl of urine is also wrongly called indican.

[2] E. Salkowski: Z. physiol. Chem. **9**, 1, 23 (1885).

[3] R. C. Emmerson: Hofmeister's Beit. **1**, 501 (1902).

contamination, without finding any ground for such an assumption. That, on the contrary, carbon dioxide is split off by the action of putrefactive bacteria, has been convincingly shown by Ellinger.[1] *Cadaverine* and *putrescine* are only found in the fæces and urine under exceptional conditions, as, for example, in dysentery and acute enteritis. It is particularly well known that these diamines appear in *cystinuria*, a disturbance in the metabolism of albumin which we shall soon take up more in detail. It is still questionable what the relation is between the appearance of these two diamines and this metabolic irregularity. At all events, these compounds are not always observed when cystine is eliminated in the urine.

We have now mentioned all those products of urine which are to be traced to putrefaction in the intestines, and will now turn our attention to an acid which has been found only in the urine of certain animals, especially dogs, namely *kynurenic acid*, whose mother-substance has been quite recently recognized to be tryptophane. It is γ-hydroxyquinolin-β-carboxylic acid:[2]



The formation of kynurenic acid from tryptophane was proved by Ellinger, who fed some of the tryptophane, inclosed in a gelatine capsule, to a dog, and estimated the amounts of kynurenic acid before and after the feeding. The increase caused by tryptophane, was very appreciable. Rabbits, which ordinarily do not excrete any kynurenic acid, did so after the administration of tryptophane.[3] Human beings, on the other hand, were not found to produce any kynurenic acid.

These decomposition products give us a very good idea of the protein decomposition in the tissues. We will not go very far astray if we accept the following conception of the behavior of the proteins in the animal organism. In the stomach, the albuminous substances are almost entirely broken down into a number of very complicated products by the action of pepsin and hydrochloric acid. These pass on to the intestine, where they are attacked further by trypsin. Under these influences, polypeptides are produced, a larger or smaller number of the amino acids entering into their composition. A large number of free amino acids are split off at the same time, first, tyrosine, tryptophane, and cystine. Then follow

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[1] A. Ellinger: Z. physiol. Chem. **29**, 34 (1900); Ber. **31**, 3183 (1899); **32**, 3542 (1900).

[2] J. Liebig: Ann. **86**, 125 (1853). R. E. Swain: Am. J. Physiol. **13**, 30 (1905).

[3] A. Ellinger: Z. physiol. Chem. **43**, 325 (1904).

alanine, leucine, glutamic acid, aspartic acid, lysine, arginine, histidine, etc. Phenylalanine and proline are undoubtedly present in combination, for they are unacted upon by trypsin. They remain unattacked, combined with other amino acids in the form of polypeptides. All these cleavage-products are absorbed. The albumin synthesis starts in the intestinal walls, the serum-albumins being first formed. The rapidity of this synthesis is dependent on the presence of certain amino acids, for, as recent investigations have proved, the relative amounts of the amino acids in the serum-albumins are very constant. The albumin synthesis would necessarily be regulated by the amount of that amino acid which is present to the smallest relative extent. There is always the possibility that one amino acid may change into others. This is known to be true only of amino acids of the aliphatic series, among themselves; but in the same manner we can imagine the production of aromatic amino acids from one another. On the other hand, it is hardly probable that relations exist between these two groups or at most only in the sense that an aromatic amino acid might give rise to an aliphatic one, or at least, a fatty acid. Our knowledge of the formation of one food-stuff from another,[1] e.g. the production of fat from the carbohydrates, forces us to admit the possibility of several amino acids being produced from a single one.

If such transformations take place then naturally the extent of the albumin synthesis is considerably widened. Those amino acids which are not utilized for the production of new proteins are evidently already broken down in the walls of the intestine; i.e. the amino group is first split off and the residue consumed. The ammonia thus formed probably is utilized in the production of urea. From these conceptions, we should expect to find *a priori*, that the various albuminous substances behave differently; i.e. under certain conditions an influence upon the extent of the synthesis of albuminous substances in the intestines might affect the entire metabolism. This is well shown in the case of gelatin, which not only lacks whole groups of amino acids, but at the same time possesses combinations of such, which affect unfavorably the breaking down of the molecule and indirectly the formation of new proteins. The other proteins might also, according to the amino acids in their composition, be more or less favorable to the synthesis of protein, and especially for the formation of the serum-albumins. It could even be expected that it would not be possible to maintain the same nitrogen balance with every albuminous substance. Here it is assumed that the amino acids which are already broken down in the intestines are to a certain extent eliminated from the albumin metabolism.[2] We have intentionally dwelt on these

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[1] Cf. Lecture XIV.

[2] Cf. Lecture XI, p. 225.

relations somewhat more in detail, because they have hitherto received but little attention in experimental investigation.

The albuminous substances now produced, together with the ammonia that has been split off, and, possibly, other cleavage-products from the amino acids, pass from the intestine to the liver, and from here into the general circulation. We will mention that the liver is quite generally considered as the place where the above-mentioned aromatic products of putrefaction are conjugated with sulphuric and glucuronic acids. We have already mentioned the fact that urea is formed in the liver on a large scale.

As a consequence of the extensive decomposition and reconstruction of proteins in the alimentary tract, only those albuminous substances circulate in the organism which correspond to its entire construction. Every cell continually receives the same nourishment in the same composition, through the instrumentality of the blood. The whole mechanism of the cell is thereby greatly simplified. The cell is, in the widest sense, independent of the nature of the food in the exercise of its functions. It is, in its entire metabolism, adjusted to a nourishment of quite definite composition, which is always available to satisfy its demands. A prominent part is taken by the intestine in the total metabolism. The nutritional relations of the entire cell-material depend in the widest sense upon its activity. Its functions are simplified, in proportion as the proteins from the food are prepared by the combined action of hydrochloric acid and pepsin and of trypsin. The cells of the intestines will be built up more quickly the better the material available for synthesis is suited for the new proteins. Although any derangement in the secretion of the ferments would undoubtedly affect the processes in the intestinal tract, the disturbance in this tract must affect the entire metabolism even more seriously.

The individual body-cell removes from the blood such protein substances as it requires. It breaks them down in the same manner that trypsin does. Amino acids result, which, on further decomposition, produce, on the one hand, urea, and, on the other, carbon chains free from nitrogen, of whose nature we still know but little, and which, perhaps, enter into relations with the carbohydrates, fats, and, possibly, other organic components of the tissues. The breaking down of the amino acids in the cells is still an obscure process. We only know that the total nitrogen soon appears in the urine. It is questionable whether the total combustion of the amino acids is immediately effected after the elimination of the amino or $CO \cdot NH_2$ groups, or whether the surviving carbon chains in their further decomposition are independent of the above process. The formation of the nitrogenous end-products, whether it be urea or uric acid, is also but imperfectly explained. If we assume that the

liver is practically the only organ in the body producing urea, we must conclude that the nitrogenous cleavage-products, whether ammonia or any compound containing the $CO \cdot NH_2$ group, formed by the cell functions, would have to be transported to the liver, and there acted upon. Here is another large gap in our knowledge concerning the decomposition of albumin in the tissues, which we do not seem able to bridge over at present. Hypotheses have, therefore, been advanced here, which we have already discussed under the formation of urea and uric acid.

If we accept the foregoing explanation of the decomposition of albumin in the tissues, we must naturally expect that the presence of some of the amino acids which have been designated as representing transition stages may be detected. As a matter of fact, certain observations do indicate the presence of amino acids in the intermediate metabolism of albumin. We will, moreover, state, in order to prevent any misapprehension, that when the proteins are broken down by the cells into the amino acids, further decomposition need not necessarily immediately follow, any more than that the muscles must immediately consume any dextrose which may be presented to them. Just as the muscles produce their glycogen from dextrose, so the cells undoubtedly utilize the decomposition-products according to their requirements, at one time decomposing them further, and at another time linking them together into chains, thus utilizing the proteins thereby formed as building material for the contents of their own cells, or for forming new cells. Every individual cell must build up its own albumin, in the same manner as the intestine, and form its own peculiar albumin from its own particular nourishment, the serum-albumin. This probably takes place in much the same way as in the intestine, with its digestive ferments furnished by the glands. The body cells, also, probably supply themselves with the necessary materials for their cell requirements by breaking down the proteins into simpler portions. Polypeptides and amino acids very likely appear as transition products in the intermediate metabolism of albumin. Just as the intestines do not decompose the albumins entirely into the lowest cleavage-products, so we need not expect the tissue-cells to decompose all of the albumin into its simplest components. These cells also probably only decompose the proteins to the point where they can be utilized to reconstruct new albumins.

The assumption that cell-metabolism also produces amino acids, has been supported by the fact that it is possible to protect certain of these amino acids from being acted upon further by the administration of specific compounds which possess the faculty of uniting with them, and thus to recover them. In this way the presence of cystine in dogs has been verified by introducing phenyl halides (brom-, chlor-, or iodo-benzene) into the animal. By feeding benzoic acid to mammals, we obtain a com-

pound of glycocoll, while from birds we get one of ornithine. Some of the amino acids, such as cystine, have been directly isolated from normal organs.

The question whether amino acids are normal constituents of urine, has recently been raised repeatedly. Various answers have been given. If we examine critically the investigations so far published, we shall have to admit that there is no positive proof yet brought forth indicating the presence of amino acids in urine under normal conditions. Glycocoll is the only one that has been identified positively, and this was only accomplished after the urine had been liberally treated with alkali for many hours; in fact, several days. We can easily imagine that the glycocoll may have been split off from some compound. Until it is possible to show that the amount of this product depends upon the extent of albumin decomposition in the organism, we cannot regard this discovery as proof of the appearance of glycocoll in cell-metabolism. It is noteworthy that only glycocoll has so far been isolated. This amino acid is utilized to a considerable extent for conjugation with aromatic substances, especially benzoic acid. We can easily imagine that the glycocoll found in urine originated from this source. It is very probable that the organism maintains a supply of glycocoll for just this coupling process. When we consider in addition that the kidneys are active producers of hippuric acid, we can appreciate the possibility of glycocoll being flushed into the urine under certain conditions. From the investigations at hand, we are not at all justified in stating that amino acids are normal constituents of urine.[1]

Amino acids are, however, often present in urine in large amounts under certain pathological conditions. This, for instance, occurs in the case of acute atrophy of the liver, a disease in which the albumin decomposition is very rapid. The liver, in this case, is flabby and emaciated. The contents of the capsule of Glisson are quite soft, and, in part, semi-fluid. An extensive destruction process has taken place in all the liver cells. Amino acids can be found in the liver-paste, leucine and tyrosine being easily isolated on account of their insolubility. The remaining elements of the albumin molecule, especially those easily split off, are also probably present. The two amino acids mentioned, often crystallize out directly on the liver itself, in the form of a white coating. Leucine and tyrosine have been found in the urine at such times. A very analogous condition arises in phosphorus poisoning. Here, again, we find amino acids in the urine; tyrosine, leucine, and glycocoll[2] having been isolated. Undoubtedly

[1] E. Abderhalden and A. Schittenhelm: Z. physiol. Chem. **47**, 1906. G. Embden and H. Reese: Hofmeister's Beitr. **7**, 411 (1905).

[2] E. Abderhalden and P. Bergell: Z. physiol. Chem. **39**, 464 (1903). E. Abderhalden and L. F. Barker: *ibid.* **42**, 524 (1904).

there are other albuminous decomposition-products present in the urine of animals which have been poisoned by phosphorus.

We will add that the sudden destruction of cell-albumin as it occurs in acute atrophy of the liver, phosphorus poisoning, and many other conditions, has been compared to the autolysis of dead tissues. By autolysis, we mean "self-digestion" of the organs, which follows in a short time, when these are preserved in a sterile condition. A gradual solution and liquefaction of the whole organ takes place. Among the end-products of this process we find, for one thing, decomposition products of albumin, — arginine is rarely present, as it is further decomposed by the arginase, — then again cleavage-products from the nucleins, and finally also compounds arising from the remaining elements of the tissue. We obtain the impression, that all cell-ferments become active immediately after death, and then, when all the regular functions have ceased, indiscriminately tear everything to pieces. It is correct to assume from this conception of autolysis, that an analogous fermentation occurs in the cells. It would be, however, a grave error to conclude that the autolytic decomposition is to be regarded as the normal breaking down of the cell. The living cell unquestionably does not permit all its ferments to act at one time. It regulates its metabolism most carefully. One fermentation process is carried out, another then follows. By one of these processes a certain cleavage-product is formed, while the action of another ferment breaks it down further. All these processes coöperate with one another. The reconstruction proceeds uninterruptedly together with the decomposition. In the dead tissues all this regulating mechanism is wanting. Decomposition alone takes place. We are not justified in considering the severe destruction which occurs in the above-mentioned liver tissues as parallel to autolysis. It is possible that the whole process is a similar one, that the solution of the cell structure precedes the death of the cells; on the other hand, it must be remembered that we have, as yet, only established a restricted decomposition of cell proteins, while an absolute confirmation of the total dissolution of the cell tissues, which characterizes autolysis, is missing. The cell destruction, under the pathological conditions mentioned, is also much more rapid in autolysis than under normal conditions.

Autolysis also seems to play a part in the living organism, — in fact, assisting in the removal of dead matter; for example, of the fibrin produced by pneumonia in the lungs;[1] in the reduction of the uterus after childbirth; very probably in the absorption of copious exudations of corpuscular elements and the decomposition of decaying neoplasms, which have been cut off from the circulation, etc. It is questionable whether we are justi-

[1] F. Müller: Verh. XX, Kon. Med. Wiesbaden, 1902.

fied in calling these processes autolytic. We only know that the organism is capable of mobilizing ferments which take care of foreign material, and by decomposing and reducing the complex molecules, prepare it for assimilation. In fact, in pneumonia, during resolution the bronchial tubes seem to possess functions very analogous to those of the intestine. It would be better, for the present, to restrict the term " autolysis " to the ferment action of the cells in the tissues, which follows some time after death. It is like the works of a clock, whose spring has been released and suddenly runs down.

Amino acids have recently been found in the urine during various diseases. If we summarize these observations, we will obtain the impression that the metabolism has been deranged by lack of oxygen. Thus, tyrosine is found in the urine after prolonged, deep narcosis, during the coma of a diabetic, etc.[1]

While these cases represent merely isolated cases of the appearance of individual amino acids, due to temporary derangements, and which are not at all permanent, we, however, also know of a derangement in metabolism in which a greater or less amount of an amino acid is always present in the urine. This occurs during cystinuria. Cystine[2] is found in the urine during this rather rare disturbance in the decomposition of albumin. Small amounts of this compound seem to be always present in urine.[3] In cystinuria, however, the quantity is very largely increased, and often leads to the formation of calculi. There is not the least doubt but that this cystine originates from albumin. It, like the albuminous cystine, is an α-diamino-β-dithiodilactylic acid. Emil Fischer and Umetaro Suzuki[4] have recently established the identity of the two substances.

The significance of cystinuria was long in doubt. The discovery of L. von Udransky and E. Baumann[5] that other di-amines (putrescine and cadaverine) are present in the urine during cystinuria, for a long time led to the assumption that cystinuria is caused by an increased intestinal putrefaction. Cystine, according to this assumption, is split off from albumin in the intestines and absorbed as such. To-day we know that the formation of amino acids is a normal function of the alimentary tract, in no case causing their elimination in the urine. The di-amines mentioned are by

[1] E. Abderhalden: Z. physiol. Chem. **44,** 17 and 40 (1905); **45,** 468 and 471 (1905).

[2] W. F. Löbisch: Ann. **182,** 231 (1876). A. Niemann: Deut. Arch. klin. Med. **18,** 232 (1876). W. Ebstein: **19,** 138 (1877); **30,** 594 (1882). B. Mester: Z. physiol. Chem. **14,** 109 (1890). A. Loewy and C. Neuberg: *ibid.* **43,** 338 (1904). C. Alsberg and O. Folin: Am. J. Physiol. **14,** 54 (1905). E. Abderhalden: Z. physiol. Chem. **38,** 557 (1903). E. Abderhalden and A. Schittenhelm: *ibid.* **45,** 468 (1905).

[3] Stadthagen: *ibid.* **9,** 29 (1885). E. Goldmann and E. Baumann: *ibid.* **12,** 254 (1888).

[4] E. Fischer and U. Suzuki: *ibid.* **45,** 405 (1905).

[5] L. v. Udransky and E. Baumann: Z. physiol. Chem. **13,** 562 (1889).

no means found in all cases of cystinuria. It is far more probable that the disease is to be regarded as a disturbance in the breaking down of albumin, on the part of the tissues. That the cystine from the albuminous substances in the food is absorbed and assimilated is evident from the fact that the albuminous material in the tissues of a patient afflicted with cystinuria, certainly contains cystine; and that no diminution in the amount of this amino acid can be detected. Cystine is evidently produced in the decomposition of proteins during cell-metabolism, and is not further worked over. It is difficult to say why, in these cases, cystine is not decomposed. A patient afflicted with cystinuria consumes any cystine administered to him, and does not eliminate all the cystine-sulphur as such. It would be easy to imagine some change in the cystine molecule, such that the cell ferments are unable to find any point of attack. We have seen that the cystine from albumin, and that of the urine, are identical. This question must be left unsettled for the present. It may, of course, be possible, that the ferments capable of decomposing cystine are absent from some cells, and that this substance is, therefore, eliminated unchanged. Such an assumption has not, however, been experimentally confirmed. It would start with the hypothesis that each cell possessed a distinct ferment to produce each different amino acid, or group of amino acids. We must say that we have absolutely no proof of such a condition of affairs. We can imagine that cystine might occupy an isolated position on account of its difficult solubility. It is, however, possible that conditions may exist in the cells of a person afflicted with cystinuria, which may cause cystine to be thrown out. That cystine may, in time, accumulate in the tissues to large proportions, has recently been proved in the case of a boy 21½ months old.[1] He died with indications of gradual inanition. A post-mortem examination showed all the organs permeated with crystals of cystine. The spleen, for example, was saturated with cystine, and from this organ the pure amino acid could easily be isolated in large quantities. It was interesting that this was a case of inherited cystine diathesis,—in fact, in a progressive form. Perhaps some light may be shed upon this rare derangement in metabolism by the observation that other amino acids, besides cystine, may be found in the urine during this disease.[2] Thus, in one case, cystine, leucine, and tyrosine were found. Apparently from this discovery cystinuria corresponds to a more general disturbance in the breaking down of albumins than is usually assumed, and that, to a certain extent, this disease is to be considered as the simplest form of such derangement. We must again state, however,

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[1] E. Abderhalden: *ibid.* **38,** 557 (1903).

[2] E. Fischer and U. Suzuki: Z. physiol. Chem. **45,** 405 (1905). E. Abderhalden and A. Schittenhelm: *ibid.* **45,** 468 (1905).

that it is impossible to give a perfectly clear picture of the metabolic disturbance in question, which shall be based upon our present experimental knowledge. If we base our judgment concerning this anomaly in albumin-metabolism not only upon the observations made upon those afflicted with cystinuria, but upon our general knowledge of the total metabolism of albumin in the tissues, it then appears as most probable that we have in cystinuria a disturbance in the decomposition of proteins in the cell-metabolism. Conversely, we can consider the appearance of cystine in this disease as further evidence of the formation of amino acids from albumin in the intermediate metabolism, always remembering that one supposition is dependent on the other, and thus it is not a definitive proof.

Our insight into the intermediate decomposition of albuminous bodies is by no means limited to the discovery of amino acids in the urine under specific conditions, and to the recognition of the final albumin cleavage-products, — urea, in the case of mammals, and uric acid in birds and fishes. There are other products present in the urine, as yet largely unknown, which contain nitrogen and sulphur, and are undoubtedly closely related to albumin-metabolism. We will disregard the fact that there are albuminous substances in urine which have been variously interpreted. They are probably not simple substances. They belong partly to the mucins, and in part to the group of nucleo-albumins, and probably originate in the urinary passages. They have no bearing on the subject of albumin-metabolism. This applies especially to the large quantities of albumin which appear in the urine under pathological conditions, and especially in diseases of the kidneys. These only affect the albumin-metabolism indirectly, inasmuch as they continually withdraw this valuable material from the body, thus depriving the organism of the energy contained therein. It is indeed possible, that an exact examination of these substances would give us an insight into the course of albumin decomposition in the tissues. It would certainly be of the greatest interest to know the origin of the albumin always present in the various forms of nephritis.[1] We usually assume that the serum-proteins (serum-globulin and serum-albumin) under pathological conditions pass into the urine. Although this assumption is very plausible, it must be said it does not necessarily explain all the cases arising. That albumin does not normally appear in urine, excepting, of course, in traces, is due to the fact that the epithelial cells of the kidney, or those of the glomeruli, will not permit the colloidal albumin to pass through. This simple explanation is not invariably true, as is shown by the appearance of a very well-defined albuminous substance, the so-called " Bence-Jones albumin," in the urine. It is

[1] E. Abderhalden: Z. exper. Path. u. Therapie, **2**, 642 (1905).

principally present in cases of sarcoma formations in the bone marrow (*Sarcomatosis ossium*). It is usually found only in the urine, the epithelia of the glomeruli permitting this substance to pass through, at the same time retaining all the serum-albumins. It might be thought that the Bence-Jones albumin represents a lower albumin, and consequently diffuses through the urinary passages.[1] This assumption is, however, incorrect, because this albumin contains all of the usual amino acids, which seems to indicate that the albumin has not in any way undergone much decomposition. Tyrosine is one of these amino acids, and this amino acid, as is well known, is very easily split off from the rest of the molecule. The objection might be raised, of course, that the tyrosine is linked in a different manner in the Bence-Jones albumin from that in other varieties of albumin. There is at present no ground for such an assumption. According to its content of amino acids, the Bence-Jones albumin does not correspond to either of the two serum-proteins, and may be considered as one of the tissue-albumins, which, without being broken down or changed into one of the serum-albumins, is transmitted to the blood, and then is probably eliminated as an albumin foreign to the blood although suitable for the body. It would be interesting to investigate other analogous albuminous excretions from the kidneys.

Although such products are only found under specific conditions, normal urine, nevertheless, contains other complicated compounds, whose nature has not yet been determined, but which, from their elementary composition, must be closely related to albumin-metabolism. Their high percentage of oxygen stamps them as albumin oxidation products. Their presence indicates the possibility that the disintegration of the albumin molecule may proceed in different ways, and that our assumption, that albumin is broken down in cell-metabolism through the amino acid stage, does not apply to all albumin decomposition. It is indeed possible that a part of the albumin is oxidized in a manner unknown to us without undergoing previous cleavage. It is not impossible that, for this kind of albumin decomposition, those complexes come into consideration which, as we have seen, strongly resist the action of the proteolytic ferments. At all events, we shall expect that when these products are explained, we shall receive further insight into the intermediate metabolism. Here we can only mention the names of these various compounds, and assert that no proof exists of their individuality. Bondńzyski and Gottlieb[2] distinguish

[1] E. Abderhalden and O. Rostoski: Z. physiol. Chem. **46**, 125 (1905). Cf. A. Ellinger: Deut. Arch. klin. Med. **62**, 255 (1899). A. Magnus-Levy: Z. physiol. Chem. **30**, 200 (1900). F. Reach: Deut. Arch. klin. Med. **82**, 390 (1905).

[2] St. Bondzyński and Gottlieb: Zentr. Med. Wiss. (**1897**) No. 33, 577. St. Bondzyński and Panek: Bull. de l'Acad. d. sciences de Crascovie, Oct. 1902. St. Bondzyński, St. Dombrowski, and K. Panek: Z. physiol. Chem. **45**, 83 (1905). F. Pregl: Pflüger's Arch. **75**, 87 (1899).

first of all between an *oxy-proteinic* acid and an *alloxy-proteinic* acid. *Antoxy-proteinic* acid has recently been added to these. All three acids contain sulphur, nitrogen, and large amounts of oxygen.

The following figures will give some idea of their composition. The antoxy-proteinic acid contains 43.21 per cent C, 4.91 per cent H, 24.40 per cent N, 0.61 per cent S, and 26.33 per cent O; the oxy-proteinic acid 39.62 per cent C, 5.64 per cent H, 18.08 per cent N, 1.12 per cent S, and 35.54 per cent O; and the alloxy-proteinic acid 41.33 per cent C, 5.70 per cent H, 13.55 per cent N, 2.19 per cent S, and 37.23 per cent O. We must also add that O. Thiele [1] has described a uroferric acid occurring in urine, which undoubtedly belongs to this group of compounds. By heating it with hydrochloric acid in a sealed tube it decomposes, producing melanin substances, carbon dioxide, ammonia, organic sulphur compounds, hydrogen sulphide, and aspartic acid. We must also add that these substances do not give any albumin reactions. The biuret test, Millon's reaction, and the remaining characteristic test for albumins and their closely-related cleavage-products, all give negative results. We must content ourselves for the present with the mere enumeration of these substances. It is possible that perhaps some light is thrown upon the formation of them by the fact that a difficultly-dialyzable body containing no amino acids [2] may be isolated from urine, in the same way as alloxy-proteinic acid and oxy-proteinic acid were obtained. Such acids may, however, be obtained from this substance by boiling it with concentrated hydrochloric acid. Glycocoll, leucine, and glutamic acid are then isolated, and the presence of phenyl-alanine and aspartic acid indicated. The substance did not contain any tyrosine. It is very probable that this product is a residue of a partially disintegrated albumin molecule, which has escaped further disintegration. We know nothing further about its relations with the other acids just mentioned.

In the discussion of the decomposition products of tyrosine and phenyl-alanine, we called attention to two hydroxy-acids which occur in the urine during the very rare metabolic disturbance known as *alcaptonuria*. **Alcapton** was the name which Bödeker [3] gave to a substance which he isolated from the urine of a man afflicted with diabetes. It gave to the urine two distinguishing characteristics. The urine showed a very appreciable reducing power, and had the property of turning dark brown or black, taking on oxygen, when alkali was added. This alcapton has been isolated from urine by M. Wolkow and E. Baumann,[4] and its composition ascertained

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[1] O. Thiele: Z. physiol. Chem. **37**, 251 (1903).

[2] E. Abderhalden and F. Pregl: *ibid.* **46**, 19 (1905).

[3] Bödeker: Z. rat. Med. **7**, 130 (1859); Ann. **17**, 98 (1861).

[4] M. Wolkow and E. Baumann: Z. physiol. Chem. **15**, 228 (1891). Here are given references to the older literature.

after Kirk [1] had previously shown that a crystallized acid could be obtained from the urine of three children in the same family. He soon recognized the fact that it was a mixture of *uroleucic acid* and *uroxanthic acid*. The latter is, undoubtedly, identical with the homogentisic acid of Wolkow and Baumann, who have established its constitution.[2] It is a di-hydroxy-phenyl-acetic acid. Uroleucic acid, on the other hand, is a di-hydroxy-phenyl-lactic acid. The latter has only occasionally been found in alcaptonuric urine, and is, undoubtedly, the antecedent of homogentisic acid. Wolkow and Baumann have discovered the source of these acids, and also the conception of the whole phenomenon. Alcaptonuria is not to be looked upon as a disease; it is more to be considered as indicative of an anomalous metabolism, which, without causing any noticeable derangement, may continue for the entire lifetime. It is of considerable interest to note its appearance in several members of the same family. As far as the origin of the homogentisic and uroleucic acids are concerned, it is natural to look to the aromatic groups derived from the albumin molecule. We have already called attention to the fact that a large number of decomposition products may be obtained directly from this source and appear in the urine.

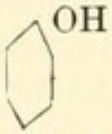
Tyrosine, until recently, was the only elementary, aromatic constituent of albumin, which was invariably found present and easily obtained. From it are derived *p*-hydroxy-phenyl-propionic acid, *p*-hydroxyphenylacetic acid, *p*-cresol and phenol. Wolkow and Baumann, by means of feeding experiments, showed that the acids of alcaptonuric urine were also formed from tyrosine. They found that the administration of tyrosine to a man afflicted with alcaptonuria caused an appreciable increase in the amounts of alcapton acids excreted. Wolkow and Baumann also indicate phenyl-alanine (phenyl-amino-propionic acid)[3] as another source of these alcapton acids. These investigators did not have a sufficient quantity of this aromatic acid, and they had to confine their researches to the relation of the alcapton acids to tyrosine. The methods recently introduced by Emil Fischer, for the isolation of the cleavage-products of proteins, made it possible not only to obtain phenyl-alanine in larger quantities, but also showed its universal distribution as an elementary constituent of albuminous substances. Very little albuminous material is free from it, and it is even more widely distributed than tyrosine. On the basis of this knowledge, and because of the recent discovery of the easy accessibility of phenyl-alanine, the relations between it and the alcapton acids have been investigated anew. W. Falta and Leo Langstein [4] found that when this amino

[1] Kirk: Brit. Med. Jour. **2**, 1017 (1886); J. Anat. and Physiol. **23**, 69 (1889).
[2] E. Baumann and S. Fraenkel: Z. physiol. Chem. **20**, 219 (1894).
[3] M. Wolkow and E. Baumann: *loc. cit.* p. 266.
[4] W. Falta and L. Langstein: Z. physiol. Chem. **37**, 513 (1903).

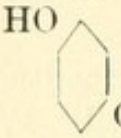
acid was administered to a man afflicted with alcaptonuria, the elimination of the homogentisic acid increased in the same manner as when tyrosine was used. Both of these elementary aromatic constituents of albumin are, therefore, to be considered as forming the basis for the formation of the alcapton acids.[1] It is very important to note that as far as our present knowledge is concerned, all the phenyl-alanine and tyrosine in the food materials are converted by persons afflicted with alcaptonuria, into the alcapton acids, so that the disturbance in the disintegration of these amino acids seems to be very complete.

A comparison of the constitution of tyrosine and of phenyl-alanine with that of the alcapton acids shows us that the formation of the latter from the former is not an altogether simple process.

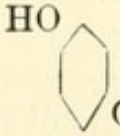
$CH_2 . CH . NH_2 . COOH$
phenyl-alanine



$CH_2 . CH . NH_2 . COOH$
Tyrosine



$CH_2 . CH(OH) . COOH$
Uroleucic acid



$CH_2 . COOH$
Homogentisic acid

Tyrosine is para-hydroxyphenyl-α-aminopropionic acid, and phenyl-alanine is a phenyl-α-aminopropionic acid. All the decomposition products of tyrosine which we have met with, partly as putrefactive products, and partly as products arising from intermediate metabolism, belong, as tyrosine itself does, to the para compounds. The constitutional formulæ of homogentisic and uroleucic acids, as shown above, indicate that this is not true of them. It is difficult to understand the formation of both of these alcapton acids from any of the known aromatic components of the proteins. In the transformation of tyrosine into homogentisic acid, the hydroxyl group must certainly be eliminated, either by being split off or by migrating. Two other places in the benzene ring are then oxidized, hydroxyl groups being formed para to one another. Altering the side-chain of the amino-propionic acid into an acetic acid residue presents nothing unusual, and can easily arise by merely removing the amino group.

It is very probable that the homogentisic acid is not directly produced from tyrosin, but from its derivative, *p*-hydroxyphenylacetic acid. It is here evidently that the anomaly in the further degradation of tyrosine occurs. The corresponding compound from phenyl-alanine is phenylacetic

[1] Cf. also A. C. Garrod and T. S. Hele: J. Physiol. **33**, 198 (1905).

acid. Embden [1] has shown that the alcapton acids are not produced when the latter is administered. This investigation does not, however, preclude the possibility that phenyl-acetic acid is one of the first degradation products of phenyl-alanine, even from persons affected with alcaptonuria, or that the disintegration of this amino acid does not proceed abnormally from the very beginning. Efforts have been made to establish homogentisic acid as a normal intermediate cleavage-product of phenyl-alanine and tyrosine.[2] From this point of view, alcaptonuria would be looked upon as a check on the complete combustion of the benzene nucleus. The formation of homogentisic acid would then be looked upon as an oxidation preceding the disruption of the benzene ring. The person afflicted with alcaptonuria would not be capable of carrying this process to the end, as a result of which, this anomalous metabolism produces a decomposition product in the intermediate metamorphosis which would otherwise have been lost to us.

There is something very attractive in the suggestion that alcaptonuria acts as a simple restraining influence in the normal disintegration of tyrosine and phenyl-alanine. We must admit that this assumption has received some support in the researches of Otto Neubauer and W. Falta. On the other hand, we must remember that the manner of formation of the alcaptonuric acids still remains a hypothesis, and that no absolute proof of its truth has as yet been presented.

The place of formation of the alcapton acids in the organism of a patient afflicted with alcaptonuria was for a long time very much in question. Wolkow and Baumann claimed that they were produced in the upper part of the intestine, by the aid of micro-organisms. We to-day believe that the alcapton acids are probably formed in the tissues themselves. This conclusion follows from the fact that phenyl-alanine, as far as known, is not set free by proteolytic ferments in the alimentary tract. The conditions are different with tyrosine. Large quantities of this are set free in the intestine. This, under normal conditions, is very largely assimilated, being even utilized by those affected with alcaptonuria in the production of albumin, which is indicated by the fact that the albuminous components of their blood show the same amounts of tyrosine and phenyl-alanine, as do those of normal persons.[3] A small portion of the tyrosine is undoubtedly attacked by bacteria in the intestine, in this way producing the various decomposition-products, until phenol is reached. The intermediate products, *p*-hydroxyphenylpropionic acid, and *p*-hydroxyphenylacetic acid, may also act as sources for the pro-

[1] H. Embden: Z. physiol. Chem. **18**, 304 and 317 (1894).

[2] L. Garnier and S. Voisin: Arch. physiol. Ges. **5**, 224 (1892). O. Neubauer and W. Falta: Z. physiol. Chem. **42**, 81 (1904).

[3] E. Abderhalden and W. Falta: Z. physiol. Chem. **39**, 143 (1903).

duction of the alcapton acids. The largest amounts are, however, produced in cell-metabolism, after the cell has disintegrated the proteins into their components, and now completes the decomposition of the amino acids. This is where the anomaly, or restriction, occurs.

If we combine all our knowledge of the proteins, their composition, and their decomposition, with what we know experimentally about the digestion, absorption, and assimilation of the albuminous materials of our food, together with the information gleaned from our study of their changes and their final disintegration in the tissues, we shall find that we are obtaining a very good idea of the whole subject of albumin-metabolism. To be sure, many bridges have been built purely provisionally from analogous conclusions and probable relations, to enable us to pass from one well-founded principle to another, and hypotheses still permeate all of our views. We, however, do not doubt that the progress of albumin chemistry will strengthen one position after another, and that eventually facts will supplant our assumptions.

LECTURE XIII.

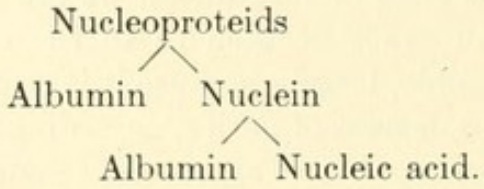
THE NUCLEOPROTEIDS AND THEIR CLEAVAGE-PRODUCTS.

In discussing the proteins, we have only briefly referred to those which do not occur by themselves, in the tissues, but are united to a second atomic complex. To these compound proteins, also called **proteids,** belong the **nucleoproteids.** They occupy an important position, not only in animal economy, but in that of the plant cell as well. They are widely distributed, and are mainly found in the nuclei of cells. It is at present very difficult to decide whether the materials classified under the name of nucleoproteids are of an individual nature, within certain limits. They are purified with difficulty, and are mainly characterized by their cleavage-products. From what we know, it appears that the albuminous component may vary widely in character. For example, we find histones and varieties of protamines. The other component, which we shall shortly consider, also shows differences in composition according to the nature and derivation of the nucleoproteid. When we recollect all that has been said regarding this class of substances, we are involuntarily forced to the conclusion that an exact decision as to the construction of the nucleoproteids is not possible, largely because it is certain that these proteids are obtained in various degrees of purity, according to the method used for isolating them; or, perhaps better expressed, because in certain investigations products have been worked with which had already undergone considerable change. The albumin component shows all the characteristics common to the proteins. Above all, it possesses the property of "denaturizing," which often serves to impart an entirely new property to an isolated product, thus apparently indicating a new compound. We are forced to obtain the nucleoproteids from the cells themselves, i.e., from a very complex mixture of proteins. The fact that in the different nucleoproteids the two components are combined with different degrees of firmness, may likewise lead to errors, and prevents, more than anything else, any energetic attack in the attempt to purify the isolated products.

When we take all these facts into consideration, there is little wonder that the existence of the nucleoproteids should be repeatedly questioned. We have to thank F. Miescher for much of our knowledge about these bodies. The non-albuminous component, *nucleic acid,* will precipitate albumin. It is conceivable that it shows its precipitating power during the isolation of the proteids, and is thus brought to our attention as

apparently combined with albumin. Nucleoproteids have, however, been obtained by salting them out. Although we have no doubt that such compounds with nucleic acid exist, especially of the basic proteins, like the histones and protamines, still we must admit that no convincing proof has yet been presented that there is such a state of combination in the cells themselves. We are accustomed to look upon substances which we always find in given localities, and are never absent, as being particularly important for the functions of the cells and tissues, especially when we find these in the parts of cells to which we assign great importance. Although such an assumption is probably true, we should be concealing the actual state of our knowledge, if we failed to mention the fact that the exact significance of the nucleoproteids is still unknown to us, and that we do not, at present, understand their relations in cell-metabolism.

We have already stated that the nucleoproteids are composed of two constituents, one of which is an albumin, and the other nucleic acid. It is not yet clear to us how we shall conceive the formation of the proteids from these two components. It has been shown that the decomposition into albumin and nucleic acid portions does not always take place as if it were a simple process. We obtain the impression that the nucleic acid is united with two parts of albumin. One part can be easily split off, the other with much more difficulty. The following scheme expresses this conception:



When albumin is split off from the nucleoproteid, a part of the protein remains combined with the nucleic acid. This product is called nuclein. This was first observed by Miescher on digesting a nucleoproteid with pepsin and hydrochloric acid. The albuminous portion, which is most easily split off, is decomposed, while the nuclein precipitates. More recent investigations have shown that active pepsin may even disintegrate the nuclein, thus leaving the pure nucleic acids behind.

We are especially interested here in the nucleic acids. We have already considered the protein constituent as far as it is known. All the nucleic acids contain phosphorus. When decomposed, they produce phosphoric acid and nuclein bases. Other products are also formed when the nucleic acids are decomposed. A carbohydrate group has been split off in some cases, while from others pyrimidine groups have been obtained. We shall here first consider all of the known cleavage-products of the different nucleic acids, and not pay any attention at present to the composition of these different acids. All of these nucleic acids which have been studied,

except inosic acid (from extract of beef) which forms crystalline salts,[1] are amorphous and react acid. They are easily dissolved in water containing ammonia or alkali, and form insoluble salts with the heavy metals.

Phosphoric acid is, as we have said, quite generally found among the cleavage-products of the nucleic acids. We do not know how it is united in the molecule. The occurrence of representatives of the group of purine bases is especially important. They vary according to their origin, and the number of bases participating in the constitution of the nucleic acids is also a variable one. J. Piccard[2] early met with these compounds in his investigations of the nucleins. The numerous observations of A. Kossel[3] have indicated their wide distribution, and also the nature of the purine bases present.

We wish to state in advance that the purine bases are very closely related to an important metabolic end-product, uric acid, not only from a purely chemical point of view, but also because recent experiments have indicated intimate biological connections. We wish, therefore, to describe briefly the most important points with regard to the constitution of this class of bodies. It will then be easier for us to follow the individual purine bases in their course through the organism, and to judge of the part they play in metabolism.

Uric acid, the earliest known member of this series, was discovered in urine and bladder stones, as far back as 1776, by Scheele[4] and Bergmann.[5] We will add that Pearson[6] has shown the presence of uric acid in "chalk-stones," and that Fourcroy and Vauquelin[7] shortly after proved it to be an essential constituent of the excrement of birds. Finally William Prout,[8] in 1815, found that the excrement of the boa-constrictor contained as much as 90 per cent of uric acid.

Uric acid was thoroughly investigated by Liebig. A large number of its important decomposition products also became known, without, however, indicating the true constitution of the acid itself. Wöhler and Liebig[9] mention its close relation to *allantoine*, even then regarded as a component of the allantoic fluid. They obtained urea and alloxan by treating uric acid

[1] J. von Siebig: Ann. **62,** 317 (1847) and Haiser: Monatsh. **16,** 190 (1895).

[2] J. Piccard: Ber. **7,** 1714 (1874).

[3] A. Kossel: Z. physiol. Chem. **4,** 290 (1880); *ibid.* **7,** 7 (1882); *ibid.* **10,** 248 (1886); **12,** 241 (1888).

[4] K. W. Scheele: Examen chimicum Calculi urinarii, Opuscula II, 73 (1876).

[5] T. Bergmann: Opuscula IV, 232 (1876). Cf. E. Fischer: Ber. **32,** 435 (1899). Cf. also Synthesen in der Purin- und Zuckergruppe, F. Vieweg & Sohn, Braunschweig, 1903, and Untersuchungen in die Puringruppe (1882-1906), J. Springer, Berlin, 1907. K. Bunte: Inaug. Diss. Berlin, 1905.

[6] Pearson: Phil. Trans. of the Royal Soc. London, **15,** 1798.

[7] Ann. de chim. **56,** 258 (1905).

[8] Ann. Phil. **5,** 413 (1815).

[9] Ann. **26,** 241 (1838).

with nitric acid. From alloxan they obtained a large number of closely related compounds. We are indebted to Adolf Baeyer[1] for establishing the constitution of alloxan and its closely allied derivatives. We will also mention the discovery of A. Strecker,[2] that heating uric acid with concentrated hydrochloric acid in a sealed tube to 170 degrees, produces glycocoll, carbon dioxide, and ammonia, water entering into the reaction:

$$\underbrace{C_5H_4N_4O_3}_{\text{Uric acid}} + 5\ H_2O = \underbrace{CH_2 \cdot (NH_2) \cdot COOH}_{\text{Glycocoll}} + 3\ CO_2 + 3\ NH_3.$$

From this fact, Strecker regarded uric acid as a glycocoll united with cyanic acid, and assumed that the uric acid first broke down into glycocoll and cyanic acid; the latter then being further decomposed into carbon dioxide and ammonia:

$$C_5H_4N_4O_3 = CH_2 \cdot (NH_2) \cdot COOH + 3\ HCNO.$$

This decomposition of uric acid was of great significance, because, using it as a basis, Horbaczewski[3] next produced uric acid by fusing glycocoll with urea at 220°–230° C.:

$$3\ CO\begin{matrix}/NH_2\\ \backslash NH_2\end{matrix} + CH_2 \cdot NH_2 \cdot COOH = C_5H_4N_4O_3 + 3\ NH_3 + 2\ H_2O.$$

On heating urea, ammonia is set free, cyanic acid also being formed, which can then act further on the glycocoll. Another synthesis was accomplished by melting urea with tri-chlor-lactamid:

$$2\ CO\begin{matrix}/NH_2\\ \backslash NH_2\end{matrix} + C_3Cl_3O_2H_2 \cdot NH_2 = H_2O + NH_4Cl + 2\ HCl + C_5H_4N_4O_3.$$

These syntheses did not lead to an exact conception of the constitution of uric acid. It was only through the carefully-planned, systematic investigations of Emil Fischer, that light was suddenly thrown on the whole group of the purines and their derivatives. Not only does the entire chemistry of all the compounds of this group depend on his work, but also all biological research in this field.

We cannot at this place trace the development of all of Emil Fischer's work, but will merely single out the points which are most important in our study.[4] It is necessary in the first place to establish the relations which exist between the various members of this group. Emil Fischer based his work upon the relations of all the members of this group to purine. He finally succeeded in obtaining purine itself, thus laying the

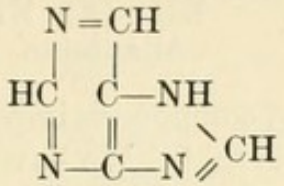
[1] Cf. his complete works, F. Vieweg & Sohn, Braunschweig, **1905**, vol. i, p. 57.

[2] Ann. **146**, 142 (1868).

[3] Monatsh. **3**, 796 (1882); **6**, 356 (1885).

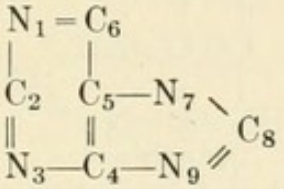
[4] Cf. E. Fischer: Ber. **30**, 549, 1839, 2226 (1897), and **31**, 104 (1898).

cornerstone for his whole investigation of the uric acid group. Purine is a strong base, readily soluble in water. Its constitution is evident from its preparation. It has the following structure:



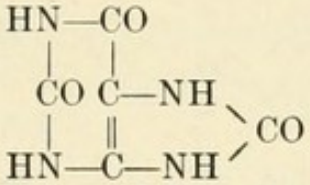
Purine

In order to make it possible to have a uniform nomenclature for the numerous representatives of this group, Emil Fischer numbered the purine ring in the following manner:



We shall make use of this scheme.

Uric acid itself has the following structural formula:

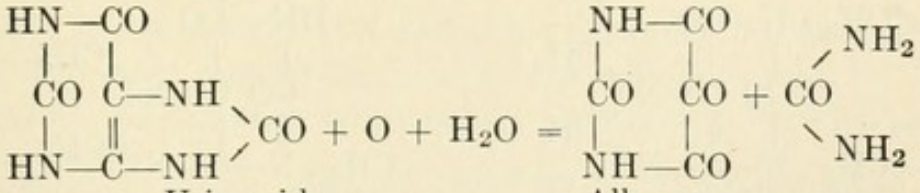


Uric acid (2, 6, 8-trioxypurine)

This formula harmonizes with the following important transformations of uric acid.

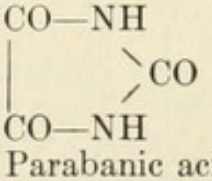
By heating with hydriodic acid and fuming hydrochloric acid in a sealed tube, glycocoll, carbon dioxide, and ammonia are produced.

Oxidizing with nitric acid or chlorine produces *alloxan* and *urea*. Alloxan is mesoxalyl urea:



Uric acid Alloxan

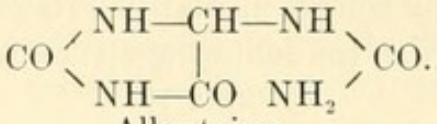
By oxidation of alloxan we obtain *parabanic acid*, which is oxalyl urea:



Parabanic acid

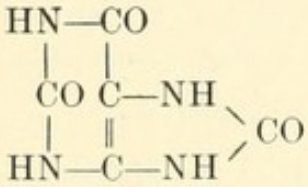
which decomposes, on boiling with water, into urea and oxalic acid.

The transformation of uric acid into *allantoine* by oxidation is very important:

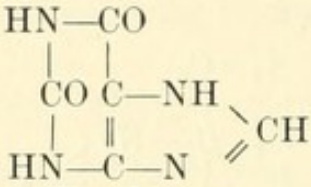


Allantoine

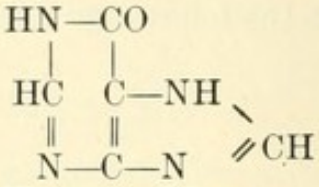
There are a large number of compounds important to the biologist which are very closely related to uric acid. We have mentioned that the nucleic acids, when decomposed, produced purine bases; in fact, the following: *xanthine*, *hypo-xanthine*, *adenine*, and *guanine*. Their structural formulæ are as follows:



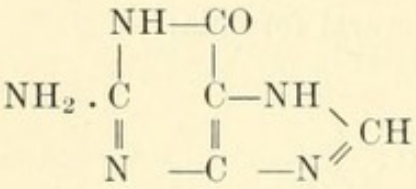
Uric acid =
2, 6, 8-trioxypurine



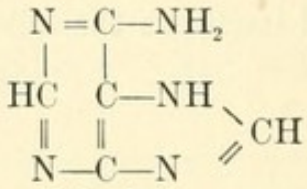
Xanthine =
2, 6-dioxypurine



Hypoxanthine =
6-oxypurine

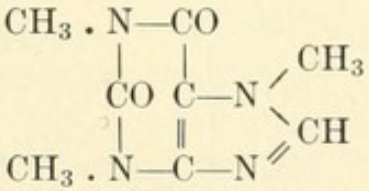


Guanine =
2-amino-6-oxypurine

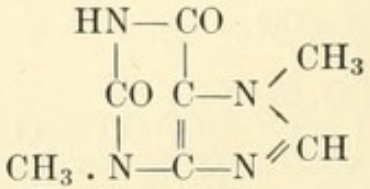


Adenine =
6-aminopurine

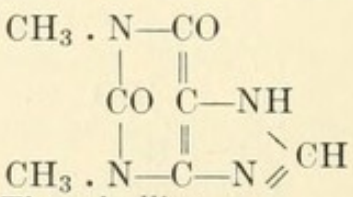
In this connection we will add that substances closely related to the purines have also been separated from the vegetable kingdom. These are caffeine, theobromine, and theophylline. The first two are found in table accessories, caffeine being present in coffee and tea, while theobromine is a constituent of cocoa. The relation of these three compounds to one another is indicated by their formulæ:



Caffeine =
1, 3, 7-trimethyl-2, 6-dioxypurine

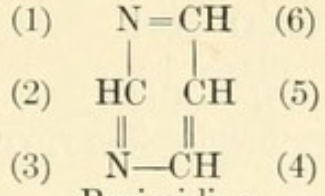


Theobromine =
3, 7-dimethyl-2, 6-dioxypurine



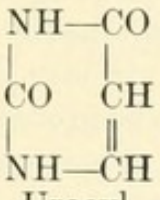
Theophylline =
1, 3-dimethyl-2, 6-dioxypurine

Very closely related to this group of nucleic acid cleavage-products are other compounds, which, instead of having a purine ring, contain one of pyrimidine:



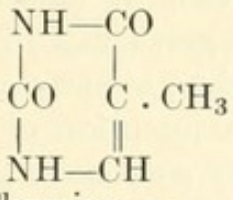
Pyrimidine

The syntheses of the members of this series and their constitution indicate their close relation to the purine derivatives. We are mainly indebted to A. Kossel for their discovery. Ascoli [1] first found *uracyl* in yeast-nucleic acid. It has the following constitution:



Uracyl =
2, 6-dioxypyrimidine

Emil Fischer and Georg Roeder [2] succeeded in synthesizing it. These same authors also established the constitution of another pyrimidine base, *thymine*. It is a 5-methyluracyl,



Thymine =
5-methyluracyl (5-methyl-2, 6-dioxypyrimidine)

This compound was first isolated from thymus nucleic acid by A. Kossel and Neumann.[3]

Finally, we are acquainted with a third pyrimidine derivative, *cytosine*, which was also separated from thymus nucleic acid by Kossel and Neumann.[4] It has been synthetically prepared by Wheeler and Johnson,[5]

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[1] A. Ascoli: Z. physiol. Chem. **31,** 161 (1900–01). A. Kossel and H. Steudel: *ibid.* **37,** 245 (1902).

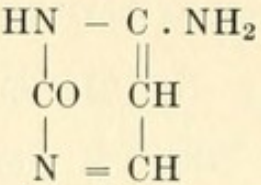
[2] E. Fischer and G. Roeder: Ber. **34,** 3752 (1901).

[3] A. Kossel and A. Neumann: *ibid.* **26,** 2753 (1893); Z. physiol. Chem. **22,** 188 (1896). H. Steudel and A. Kossel: *ibid.* **29,** 303 (1900). H. Steudel: *ibid.* **30,** 539 (1900); **32,** 241 (1901). W. Jones: *ibid.* **29,** 20 (1899); **30,** 461 (1900). W. Gulewitsch: *ibid.* **27,** 292 (1899); **27,** 368 (1899). O. Gerngross: Ber. **38,** 3408 (1905).

[4] A. Kossel and A. Neumann: Ber. **27,** 2215 (1894). A. Kossel and H. Steudel: Z. physiol. Chem. **37,** 177 (1902); **37,** 377 (1903); **38,** 49 (1903).

[5] H. L. Wheeler and T. B. Johnson: Am. Chem. J. **29,** 492 and 505 (1903).

and has the following structural formula:



Cytosine = 6-amino-2-oxypyrimidine

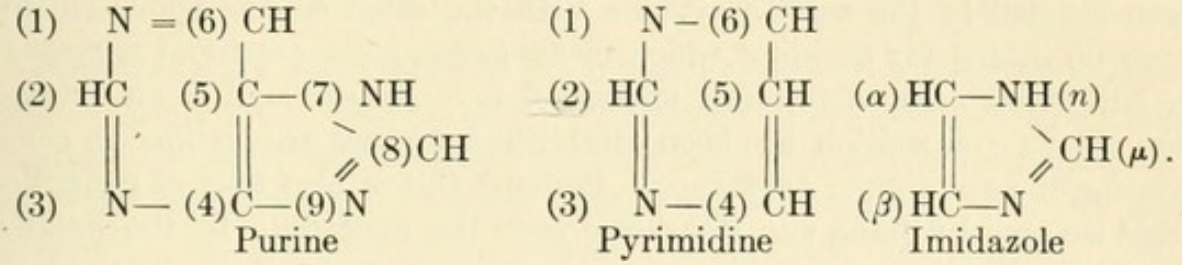
Carbohydrates — in fact mostly pentoses — have also been obtained from the nucleic acids in conjunction with the purine, and pyrimidine bases, and phosphoric acid. Such a typical pentose is xylose. Yeast nucleic acid is supposed to contain an hexose. It is also assumed that an hexose participates in the constitution of the thymus nucleic acid. Lævulic acid is obtained therefrom by an energetic decomposition.

It has recently become questionable whether all of the above compounds are to be regarded as primary cleavage-products of the nucleic acids. Steudel[1] has shown it to be probably true that adenine and guanine are the only primary building stones of the purine bases, and thymine and cytosine of the pyrimidine bases. Hypoxanthine, xanthine, and uracyl are formed secondarily by oxidation in the breaking down of the nucleic acids. This discovery lessens the value of the numerous investigations concerning the purine and pyrimidine bases in the different nucleic acids. It also explains why different authors have obtained divergent results in the study of nucleic acids from the same source. Although this indicates a great gap in our knowledge concerning the amount of individual building stones present in the nucleic acids, which can be filled only by the assumption of secondary transformations, still on the other hand, it is very good news to find that Neuberg and Brahn[2] and Bauer[3] have succeeded in clearing up the constitution of inosic acid. From the latter, one molecule each of hypoxanthine, phosphoric acid, and xylose or arabinose is always obtained. It is at present an open question whether the purine bases here observed are to be regarded as of primary or secondary formation, and whether perhaps adenine is not here also the primary building stone. The question that next arises is with regard to the way the components of the nucleic acids are held together in the molecule. Undoubtedly this again will only be answered when synthesis has established the relations.

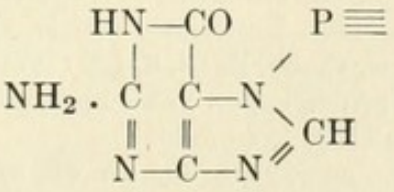
Burian[4] attempted to decide how the purine bases are held in the nucleic acid molecules. In one case he based his observations upon the fact that the purine bases, unlike the other components of the nucleic

[1] H. Steudel: Z. physiol. Chem. **49**, 406 (1906).
[2] C. Neuberg and B. Brahn: Biochem. Z. **5**, 438 (1907).
[3] Friedrich Bauer: Beitr. chem. Physiol. Path. **10**, 345 (1907).
[4] R. Burian: Ber. **37**, 696 and 708 (1904); Z. physiol. Chem. **42**, 297 (1904).

acid molecule, are very easily split off. Purine bases can be detected even when the material is merely dissolved in water at 60 degrees. Boiling with water for ten minutes is sufficient to separate practically all the purine bases. We are justified in looking upon the purines as being primary cleavage-products of the nucleic acid molecule, rather than resulting from secondary causes. We may consider them as being composed of a condensed nucleus, consisting of a pyrimidine and an imidazole ring, as may be seen by the following structural formulæ:



Corresponding to this assumption, the purines show reactions, which are characteristic of one or the other component. Imidazole possesses the property of reacting with diazo-benzene chloride, forming a red, crystalline product, which is (*n*) diazo-benzene-imidazole. This reaction also applies to those imidazoles whose α, β, or μ positions have been substituted; but not to those in which the *n*-position is substituted. The purines act in a very analogous manner. Substitution in the pyrimidine ring is without influence on the appearance of this reaction. On the other hand, the reaction is negative when the imide hydrogen of the imidazole ring, in position 7, is replaced. The reaction is positive in xanthine, hypoxanthine, guanine, adenine, theophylline; it fails with theobromine (3, 7-di-methyl-xanthine) and with caffeine (1, 3, 7-tri-methyl-xanthine). The nucleic acids, however, even if liberally endowed with bases, do not react with diazo-benzene-sulphonic acid. This last reaction only takes place when purines are being split off at the same time. Taking the above facts into consideration, we arrive at the conclusion that the purine bases are united with the nucleic acid residue through its nitrogen atom in the seventh position. Another series of observations indicate that the purine bases are linked in the first place to the phosphoric acid portion. It is a very striking fact that those purine bases, which are so easily split off by boiling water, are only released with great difficulty by boiling with sodium hydroxide. Certain organic phosphoric acid amides behave similarly. Burian believes that guanine is linked in the nucleic acid molecule in the following way:



The proof that such a state of combination exists in the nucleic acid molecule is not satisfactory. Other possibilities exist. Burian has, nevertheless, shown that such a linkage is probable.

In discussing the proteins, we found that a knowledge of the amino acids participating in the constitution of the individual albumins often gave us valuable suggestions regarding their behavior in the animal organism. The relations of most nucleic acids, on the other hand, are as we have already stated, not so clear, because they have not all been studied in the same way, nor with the same care. Above all we have no means for deciding which cleavage-products are to be regarded in any given case as primary or secondary. Inosic acid is an exception, as its composition has been established, and we are justified in considering it as a simple substance. Perhaps this is also true of **guanylic acid** [1] isolated by Bang and Raashon [2] from the pancreas. In the breaking down of this nucleic acid, guanine is the only purine base that could be detected in the presence of phosphoric acid and a carbohydrate. As to whether the remaining nucleic acids are simple substances or mixtures, we have no means of knowing. It will be best here to mention merely the most important nucleic acids. They are almost always designated by the name of the organs from which they were obtained.

The nucleic acids from the spermatozoa have been longest known. Since F. Miescher [3] first called attention to them, they have been the subject of frequent investigation.[4] It appears that the nucleic acids obtained from the various kinds of spermatozoa are closely related to one another. Certain observations indicate a far-reaching similarity. Great care should be taken, however, in drawing any conclusions regarding the identity of the various nucleic acids from analytical values or knowledge of the cleavage-products. According to the grouping of the cleavage-products, many differences may appear which are as yet hidden from our view. It is noteworthy that the percentage of phosphorus present in the nucleic acids isolated from the cells of ripe semen is, in general, a very constant one. The values range between 9.11 per cent and 9.62 per cent. Salmon nucleic acid, according to Schmiedeberg,[5] has the following composition: C, 37.42 per cent; H, 4.19 per cent; N, 15.24 per cent; and P, 9.64. All the known purine bases have been obtained in the cleavage of the spermatozoa nucleic acids. The values are given on the following page.

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[1] I. Bang Z. physiol. Chem. **26**, 133 (1898–99); **31**, 241 (1900–01).

[2] I. Bang and C. A. Raaschon: Hofmeister's Beitr. **4**, 175 (1903).

[3] F. Miescher: Verh. naturf. Gesell. Basel, **6**, 138 (1874). Cf. also the complete works of F. Miescher: *loc. cit.* **2**, 55; Arch. exper. Path. Pharm. **37**, 100 (1896).

[4] R. Burian: Ergeb. Physiol. **3**, 1, 48 (1904).

[5] O. Schmiedeberg: Arch. exper. Path. Pharm. **43**, 57 (1900).

	100 g. Dry Substance Contains			
	Adenine.	Guanine.	Hypoxanthine.	Xanthine.
Nucleic acid from the testes of the bull[1]	0.736	...	1.962	6.039
Spermatozoa of the bull	0.126	0.248	0.207	0.352
Spermatozoa from the testes of a boar[1]	1.181	0.187	0.635	2.057
Spermatozoa of the carp[2]	0.360	...	0.309	2.278
Salmon nucleic acid. 1st preparation[1]	...	0.127	0.664	2.924
Salmon nucleic acid. 2d preparation	...	0.193	1.208	3.914
Pancreas[1]	...	...	0.154	0.740

We must once more state that too much reliance should not be placed upon these figures. Burian and Walker Hall[3] called attention to the fact that, in the preparation of the purine bases, oxypurines (xanthine and hypoxanthine) might be formed from the aminopurines (adenine and guanine). As we have seen, Steudel has proved this directly. This probably accounts for the fact that Schmiedeberg, in investigating salmon nucleic acid, found guanine and adenine, but no xanthine nor hypoxanthin.

Thymo-nucleic acid from the thymus gland is another nucleic acid which has been investigated considerably.[4] Phosphoric acid, thymine, cytosine, guanine, adenine, lævulic acid, and ammonia were obtained by hydrolytic decomposition. *Guanylic* acid, obtained from the pancreas, broke down into guanine, phosphoric acid, and a pentose. A nucleic acid has also been isolated from the spleen. We may add, finally, that analogous products can also be obtained from the plants. Triticonucleic[5] acid, obtained from the embryo of wheat, has been most studied. It yields by hydrolysis: guanine, adenine, cytosine, uracyl, phosphoric acid, and a pentose. Nucleic acids have also been isolated from tubercle-bacilli and from yeast.

We cannot at present say anything regarding the nucleic acids and their cleavage-products, and shall confine ourselves in the following to the discussion of their participation in metabolic processes. We will state, at the start, that there can no longer be any doubt that the nucleoproteids are disintegrated in the cell-metabolism, and participate to the same extent in the reconstruction. There is scarcely any question but that the animal cell obtains the components of the nucleoproteids already formed in the food. It does not seem probable that the purine and pyri-

[1] Y. Inoko: Z. physiol. Chem. **18**, 57 (1894).

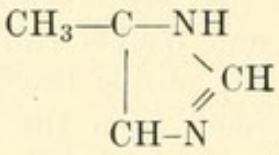
[2] S. Schindler: *ibid.* **13**, 432 (1889).

[3] R. Burian and W. Hall: *ibid.* **38**, 366 (1903).

[4] H. Steudel: *ibid.* **42**, 165 (1904); **43**, 402 (1904); **46**, 332 (1905); P. A. Levene, **32**, 541 (1901); **37**, 402 (1902–03); **38**, 80 (1903); **39**, 4 (1903); **39**, 479 (1903); **43**, 199 (1904); **45**, 370 (1905); Am. J. Physiol. **12**, 213 (1905).

[5] T. B. Osborne and J. F. Harris: Z. physiol. Chem. **36**, 85 (1902).

midine bases are synthetically formed by the cells for this purpose. To be sure, the animal organism — at least, that of the birds and the reptiles — is capable of building up uric acid. From the whole manner of its formation it would seem very improbable that the purine bases have the same origin. In this connection we must indeed recall the work of F. Knoop and A. Windaus,[1] from which we can easily assume a synthetic production of a compound which is important to the cell-metabolism. These two investigators have shown that the action of ammoniacal-zinc-hydroxide on grape-sugar in the cold produces an oxygen-free base in large quantity; namely, methyl-imidazole:

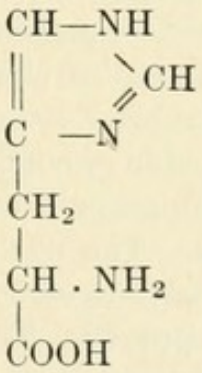


This gives us a connecting link between the carbohydrates and the purine bases. It is possible that analogous reactions may occur in the plant organism. The animal cell would hardly look to the carbohydrates as a source for the production of nitrogenous material; at least, nothing at present known would indicate that it does. We will state here that, in spite of the large number of recent observations in the field of purine metabolism, it is still impossible for us to give an exact account of the influence of this class of bodies on the total metabolism, and even less so in the case of the nucleoproteids and nucleic acids in the individual cells. We know that the animal organism utilizes materials containing purines for its requirements. There are nutrient substances, like meat, rich in purine bases, and others, again, containing less of these. Milk belongs to the latter class. The animal cell undoubtedly requires nucleic acid and also purine bases for the purpose of building up nucleoproteids. They constantly decompose — as we shall soon see — those constituents which contain purine and replace them again. From this we can easily imagine that the cell utilizes the nucleic acids either in their original or converted form to replace wasted material or to construct new cells. We shall see later that the animal organism continues to break down material containing purines, even in cases of extreme starvation, some of the derivatives formed by this process appearing in the urine. There is a far-reaching analogy here to the behavior of the proteins in the organism. It is possible — in fact very probable — that the nucleoproteids in the form of nucleic acids are utilized by the cells, at least in part, in the manner just indicated. We must not forget, however, that we have no absolute proof of this. In fact there are some observations which point to the probability that the animal organism, like the plants, is also capable of directly synthesizing the purine

[1] F. Knoop and A. Windaus: Ber. **36,** 1166 (1905). Hofmeister's Beitr. **6,** 392 (1905).

bases. Tichomiroff [1] has shown that hibernating insect eggs contain only traces of purine bases, while the maturing eggs show a much larger percentage of these. A. Kossel [2] finally showed that the yolks of unincubated eggs contained practically no purine bases. After fifteen days' incubation larger amounts of guanine and hypoxanthine could be detected. We must also refer to the work of Burian and Schur.[3] They estimated the amounts of bases present in new-born animals, and compared these values with those obtained from older sucklings. Although the latter had received almost no purine bases with their nourishment, the milk, the amount of these substances continually increased. Finally, we must remember the work of F. Miescher,[4] who found that even the salmon, during the fasting period when it remains in fresh water, not only builds up nucleins from the simplest components, but newly forms the purine bases as well. All these important observations show us what difficult syntheses the animal cell is capable of effecting. There does not seem to be any reason for doubting that the most varied constituents of the tissues of the animal organism are built up of the simplest components. It is still an open question regarding the manner in which the growing organism carries out this process.

It is, at present, impossible to make any definite statements concerning the products utilized in the synthesis of the purine bases. It is indeed possible that further investigations with histidine and its behavior in the animal organism may give us some clew to this process. This albuminous cleavage-product, as we have seen, is very probably an α-amino-β-imidazole-propionic acid:



If this be true, we have another bridge from the proteins to the purines.

We specifically call attention to our ignorance of the relations of the nucleic acids to the general metabolism, because recent investigations on the disintegration of these substances in the tissues have indicated the ease with which these gaps in our knowledge may be overlooked. They immediately become apparent when we attempt to explain the

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[1] A. Tichomiroff: Z. physiol. Chem. **9**, 518 (1885).

[2] A. Kossel: Z. physiol. Chem. **10**, 248 (1886).

[3] R. Burian and H. Schur: *ibid.* **23**, 55 (1897).

[4] F. Miescher: Arch. exper. Path. Pharm. **37**, 100 (1896).

causes of the well-known metabolic derangement, gout, which is undoubtedly closely related to a disturbance of the purine assimilation. Our uncertainty begins when we proceed to follow the behavior of the nucleic acids in the alimentary tract, although we are a little better informed regarding the decomposition of the nucleoproteids themselves. The latter are, at the start, vigorously attacked by pepsin and hydrochloric acid in the stomach. The loosely-linked albuminous component is split off and converted into peptones. Nuclein then separates in an insoluble form, but later on is partially dissolved. Trypsin likewise separates the albuminous component from the nucleoproteids. The nucleic acids, however, seem to remain entirely unaltered. They must, therefore, occupy a class by themselves among the food materials, because all the others so far discussed are largely disintegrated in the intestine in order to supply the tissue cells, and partly those of the intestine itself, with the necessary material for their individual needs. We would expect, *a priori*, that the nucleic acids would also have to be disintegrated to make them available. Up to the present time but one ferment has been isolated from the tissues capable of separating nucleic acids into their components. This is the so-called *nuclease*.[1] Trypsin destroys this ferment. Nuclease has been found in the pancreas of the dog and the thymus gland of the calf. Undoubtedly, such ferments must be widely distributed in the tissues. They account for the first stages of the cleavage and degradation of the nucleic acids. Recent investigations [2] have shown that neither the active nor the inactive pancreatic juice is able to decompose the nucleic acids into their components; both, however, are capable of so altering them that their entire characteristics are changed, thus making them more easily dialyzable. Even the cell walls of the intestine possess ferments which are capable of completely decomposing the altered nucleic acids. The animal organism evidently treats this valuable material in a very economical manner. The nucleic acid cleavage-products are difficultly soluble in water, and not easily absorbed, as feeding experiments with purine bases have proved. These experiments indicate that the complete disintegration of these compounds takes place only in the walls of the intestine. Material, foreign to the organism, is there prepared for its requirements. We are unacquainted with the exact manner in which the pancreatic juice changes the nucleic acids. It may possibly be that it acts as the beginning of a hydrolytic decomposition. The disintegration proceeds in stages, and we must expect to meet complexes analogous to the peptones, and dextrins. We have not the least doubt but that the nucleic acids very closely resemble the albumins in their entire construction and the way they are broken down.

[1] F. Sachs: Z. physiol. Chem. **46**, 337 (1905).

[2] E. Abderhalden and A. Schittenhelm: *ibid.* **47** (1906).

A part of the nucleic acid of the food is undoubtedly decomposed by bacteria in the intestines. Purine bases are present in the fæces.[1] Martin Krüger and Schittenhelm[2] have shown that only a small part of the purine bases in the fæces could originate in this manner. It has been found that the quantitive distribution of the various purine bases in the fæces corresponds very closely to that in the different organs, and that the main source of supply is undoubtedly the degenerating intestinal epithelium and dead bacteria. Only small amounts of bases are introduced into the intestines by means of the pancreatic and intestinal juices.

Until recently, we knew but very little about the relation of the nucleoproteids, or, rather, of the nucleic acids and their cleavage-products, to the decomposition products of the general metabolism. Indeed, many scientists, largely from purely chemical considerations, were strongly in favor of assigning to the nucleins a relation to the formation of uric acid. It remained, however, for recent experiments to show that in man, and mammals in general, the greater part and perhaps all of the uric acid results from the decomposition of the nucleic acids and their cleavage-products, especially the purine bases.

For a long time the attempt had been made to show that uric acid was the antecedent of urea in the breaking down of proteins. In fact, the amount of uric acid in the urine was even considered to be a direct expression of the activity of oxidations in the animal organism. The more extensive these oxidation processes were, the less uric acid would be found in the urine. Evidence against this assumption was brought forward from time to time, and, above all, it was always claimed that in no case could any direct relationship be shown between the uric acid excreted and the disintegration of the albumins, and that there was no evidence that it indicated the extent of the oxidation processes. Scientists always came back to the above view, however, because it was not found possible to show positively that there was an increased elimination of uric acid after the administration of nucleic acids and purine bases. It was only by the experiments of Horbaczewski[3] that the problem was cleared up.

Horbaczewski showed with mammals that if the pulp or extract of organs were digested for several hours out of contact with the air, purine bases were formed, while exposure to the air gave rise to uric acid. On adding nucleoproteids a better yield of uric acid was obtained. It was

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[1] A. Schittenhelm and F. Schröter: Z. physiol. Chem. **39**, 203 (1903). A. Schittenhelm and C. Tollens: Z. innere Med. **25**, No. 30 (1904).

[2] M. Krüger and A. Schittenhelm: Z. physiol. Chem. **45**, 14 (1905); **35**, 153 (1902). A. Schittenhelm: Dent. Arch. klin. Med. **81**, 423 (1904).

[3] Horbaczewski: Monatsh. **10**, 624 (1889); **12**, 221 (1891). P.Giacosa: Att. R. Acc. Scienze di Torino, **25**, 726 (1891). W. Spitzer: Pflüger's Arch. **76**, 192 (1899). H. Wiener: Verh. xvii, Kong. innere Med. **1889**; 622 Arch. exper. Path. Pharm. **42**, 375 (1899).

also shown by feeding experiments that the administration of nucleoproteids and of purine bases increased the elimination of uric acid. Similarly the formation of uric acid is increased when a food rich in purine bases — e.g., meat, liver, thymus, etc. — is added to a definité diet.[1]

Horbaczewski himself believed that the uric acid was derived in the first place from the nucleic acids, or their purine bases, obtained from leucocytes, and his work was followed by a large number of investigations concerning this question. It was, in fact, found that there was a certain relation between the amount of uric acid eliminated and the number of leucocytes. A particularly pregnant example, according to this view, is given in leucæmia, a disease which in its entire ætiology is but little understood. One of its most prominent symptoms is a more or less extensive increase in the number of leucocytes. The observation that the increased elimination of uric acid, so often noted during this disease, is dependent on the destruction of leucocytes, is undoubtedly true. The generalization that the uric acid of urine could only result from the destruction of the above-mentioned cells was not, however, correct.[2] All the other cells of the organism must be considered in the same manner. The most important result of the investigations of Horbaczewski is that the purine bases, in minced organs and tissue extracts, can, in the presence of oxygen, be converted into uric acid.

To prevent any misunderstanding, we will state at this point, that the increased elimination of uric acid can, in no case, be looked upon as evidence of an increased cell destruction. It may just as easily arise from an increased cellular metabolism; i.e., from the breaking down and reconstruction of the cell-body, and especially of the nuclei.

Before discussing the mechanism of the conversion of purine bases into uric acid, we wish to devote a little space to an important investigation of Burian and Schur.[3] These two scientists showed that by a diet containing no purine bases it is possible to diminish appreciably the excretion of uric acid, but not to prevent it entirely. It is noteworthy that the amounts of uric acid then excreted remain practically constant for each individual, but vary with different individuals.[4] Burian and Schur desig-

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[1] R. Burian: Med. Klin. **1,** 131 (1905). E. Salkowski: Virchow's Arch. **117,** 570 (1889). C. v. Noorden: Lehrbuch d. Path. d. Stoffwechsels, **54,** Berlin, Hirshwald, 1893 (new ed. 1906). C. Dapper: Berliner Klin. Wochsch. **30,** 619 (1893). W. Cammerer: Z. Biol. **28,** 72 (1891); Z. physiol. Chem. **33,** 139 (1896). A. Schittenhelm: Z. Stoffw. u. Verdauungskrankheiten, **5,** 226 (1904). H. Wiener: Ergebnisse Physiol. I, **1,** 555 (1902).

[2] Mareš: Monatsh. **13,** 101 (1892).

[3] R. Burian and H. Schur: Pflüger's Arch. **80,** 241 (1900); **87,** 239 (1901). Cf. E. W. Rockwood: Am. J. Physiol. **12,** 38 (1905).

[4] Schreiber and Waldvogel: Arch. exper. Path. Pharm. **32,** 69 (1899). Cf. M. Kaufmann and L. Mohr: Arch. klin. Med. **74,** 141 (1902).

nate the amount of uric acid eliminated with a diet free from purine bases as *endogenous* uric acid, and contrast this part of the total uric acid elimination during an ordinary diet with that obtained from the purines in the food. The amount of the latter, which they designate as *exogenous* uric acid, naturally varies, and is dependent upon the quantity of purine bases ingested and absorbed. The amount of endogenous uric acid remains constant, even from a diet rich in purine bases. The uric acid arising from the purine bases is added to that of endogenous origin. Burian states that the amount of endogenous uric acid excreted daily by a normal adult ranges between 0.3–0.6 gram. The endogenous uric acid value is a direct expression of the extent of cell activity, which, as we know from various observations, is very carefully regulated and adjusted for each individual.

Although we may look upon this conception of endogenous and exogenous uric acid, in the sense suggested by Burian and Schur, as a distinct advance in our knowledge of the course of cell-metabolism, we must, nevertheless, emphasize the fact that this separation between the two sources is merely a superficial one. In no case are we justified in concluding that the total purine metabolism is sharply divided into two phases; i.e., that the purines in the food are immediately converted into uric acid, nor that cell-metabolism, in the narrowest sense of the word, takes place independently. We have no doubt that the purine bases, and the other components of nucleic acid, replace continually material used in the building up of cells, and particularly their nuclei, and thus take part in the formation of endogenous uric acid, of a later period. Although we know that the total nitrogen of the food, as a rule, soon reappears in the urine, we nevertheless assume that the albumins, at least to some extent, participate in cell-metabolism, and in the construction of cells partly supplying material, and partly acting as a source of energy. Similarly, the purine bases in the food undoubtedly participate in cell-metabolism. The endogenous purine value may perhaps be compared, within certain limits, with that amount of albumin which the cells require even during starvation. We do not mean to represent that this comparison is an absolute one. It merely suggests the similarity between the metabolism of albumin and purine — both are very closely related to cellular metabolism. We must be especially cautious not to differentiate sharply the endogenous and exogenous uric acid with regard to the total purine metabolism of the cells.

The question arises, What sources have we to assume in particular for the endogenous uric acid? In general it may be said that the endogenous uric acid may be traced primarily to the decomposed nuclear material, and also to the cells which have been completely destroyed. R. Burian [1]

[1] Z. physiol. Chem. **43**, 494 (1905).

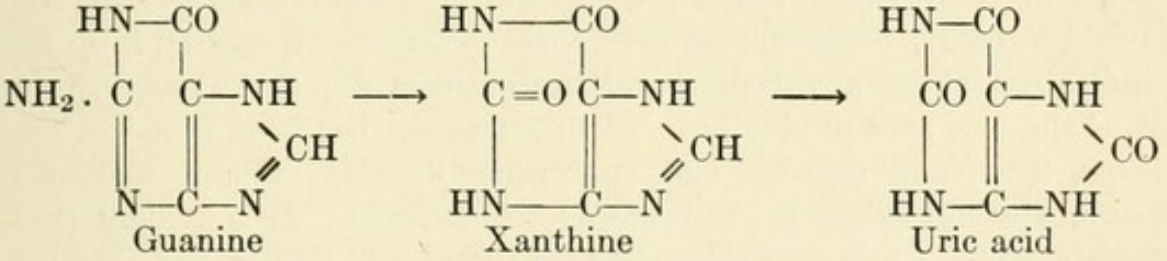
has recently called our attention to the importance of considering the muscles as a source of purine bases, and, consequently, they are related to the formation of uric acid. He showed in the first place that the endogenous uric acid elimination in human beings during twenty-four hours was not appreciably influenced by muscular activity, whereas hourly values were distinctly changed. Vigorous muscular exertion is followed by an hour of increased elimination of urinary purine. This increase, during the time of work, does not influence the uric acid, as such, but principally the purines. The noticeable increase in uric acid elimination is only evident some time later. By a subsequent diminution of the uric acid and purine eliminations, the daily uric acid and purine values are practically unaffected by periods of rest or of activity. Naturally such investigations can be carried out only when no food is eaten, or at least none containing purine, or the amount of purine bases in the food must be definitely known. Burian finally, in order to establish more closely the relations of the muscles to purine metabolism, caused blood to flow through the surviving muscles of a dog. It was found first of all that the liquid which was originally perfectly free from uric acid always contained it after a short time. If the muscle was stimulated, purine bases appeared in considerable quantity, and chiefly hypoxanthine. The discovery is also very important that the amount of hypoxanthine in the muscle itself is greatly increased when it is tetanized. From this we must assume that the muscle, when at rest, constantly oxidizes hypoxanthine to uric acid. When its metabolism is increased by greater demands upon it, the muscle cells are no longer able to oxidize all of the purine bases, and especially the hypoxanthine, so that then unchanged hypoxanthine is given up to the blood. It is very important that according to these observations the muscle cells are constantly forming hypoxanthine. These investigations are not to be regarded, however, as perfectly conclusive. We have mentioned them here because from them we may perhaps expect to obtain the first explanation of the part played by the purines of the food in cellular metabolism, and concerning the extent of the synthetic formation of purine bases.

We have been informed recently concerning the breaking down of the individual purine bases to uric acid, and their subsequent fate in the animal organism, by a series of valuable experiments by A. Schittenhelm.[1] They have been confirmed by further observations by Richard Burian.[2] We have already mentioned the fact that Horbaczewski could detect the formation of uric acid in the pulp of organs, or in extracts of them, in the presence of oxygen. It was now found possible to follow carefully the

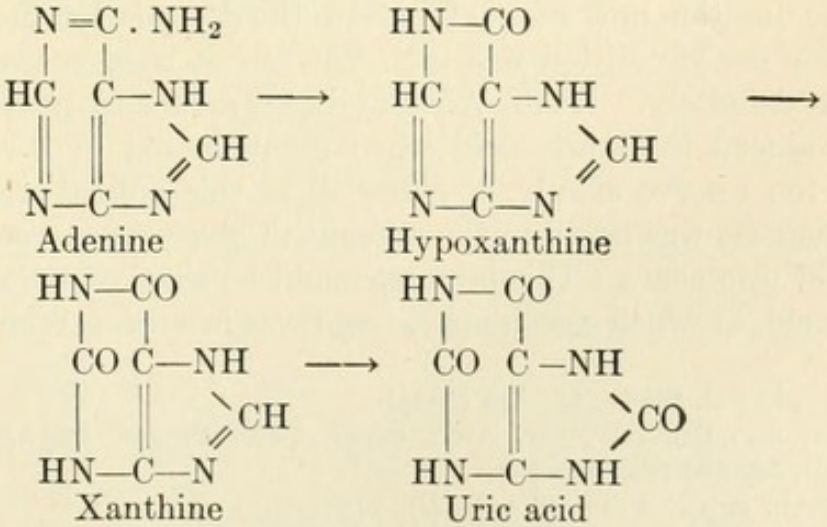
[1] A. Schittenhelm: Z. physiol. Chem. **42**, 251 (1904); **43**, 228 (1904); **45**, 121, 152, 161 (1905); **46**, 354 (1905).

[2] R. Burian: Z. physiol. Chem. **43**, 497 (1905).

various phases of this conversion. We have also called attention to the fact that the tissues of many organs contain ferments which are capable of breaking down the nucleoproteids into their components, and finally disintegrating the separate cleavage-products, such as the nucleic acids, into their simpler components. The individual purine bases are changed eventually by ferments into uric acid, as has been unquestionably proved by the above investigators. Schittenhelm has proved that when the pulp, or extract of organs, is first boiled, no uric acid can be obtained. He finally succeeded in isolating the ferment producing uric acid from the organs. Instead of employing organ extracts, we can now utilize active ferment solutions for the experiments. The advantage of this method is evident when we appreciate the fact that the pulp, or extract of organs, necessarily contains varying amounts of purine bases, which are practically absent from the isolated ferment or in its solutions. Schittenhelm obtained xanthine on adding the ferment from beef-spleen to guanine out of contact with the air, but, by conducting air through the liquid, uric acid resulted. The transformation of guanine into uric acid accordingly takes place with the intermediate formation of xanthine. The following formulæ indicate these changes:



Guanine is converted by hydrolysis into xanthine by the loss of an NH_2 group. By the oxidation of xanthine, uric acid is formed. Two ferments, therefore, participate in the conversion of guanine into uric acid. In the same manner adenine goes over into hypoxanthine, which is then converted into xanthine, and the latter into uric acid:



The ferment which converts guanine into xanthine, and adenine into hypoxanthine, is widely distributed. It is evidently found in all organs. The oxidizing ferment, on the other hand, which finally produces the uric acid, seems to be restricted to individual organs. It has been found in the spleen, lungs, liver, intestine, muscles, and the kidneys of cattle. Further investigations have disclosed the remarkable fact that the same organs of different kinds of animals vary considerably in this respect, so that a generalization of results obtained with different species of animals is not permissible. Schittenhelm and Bendix [1] showed that the transformation of purine bases into uric acid not only takes place in this way in glass vessels, but also in the organism itself, by injecting guanine subcutaneously and intravenously into a rabbit. They found a considerable increase in the uric acid of the urine, and detected the presence there of a purine base corresponding to xanthine, which is evidently to be regarded as an intermediate product in the formation of uric acid from guanine.

Up to this point we have not mentioned an important fact which makes it difficult to trace the quantitative relations in the production of uric acid from the purine bases. There are ferments present in many tissues of the animal organism which are capable of further decomposing the uric acid formed. Schittenhelm calls them *uricolytical* ferments, in order to indicate that we are dealing with an entirely different process from that of the uric acid production. Such a ferment has been found in the kidneys, liver, and muscles, and very probably also in bone marrow.[2] Schittenhelm has succeeded in isolating this ferment. It is evident that if a destruction of uric acid takes place in the animal tissues, the old-time conception that the amount of uric acid excreted is an index of the quantity of uric acid formed in the organism, can no longer be accepted. An increased excretion of uric acid in the urine may, of course, be due to a greater production thereof; it may, however, also indicate a lessened destruction.

The question now arises, What are the degradation products of uric acid when a decomposition sets in? We can at once answer that we do not know definitely. Hugo Wiener [3] has practically proved that glycocoll is produced from uric acid when administered to a rabbit. He found that the reserve supply of glycocoll in this animal was fairly constant. An increase was noted in the amount of glycocoll excreted after the injection of uric acid. This increase could be checked by administering benzoic acid, in which case hippuric acid was formed. Wiener has also shown

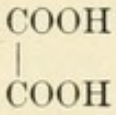
[1] Z. physiol. Chem. **43**, 365 (1905).

[2] Cf. also Hugo Wiener, Arch. exper. Path. Pharm. **42**, 375 (1899); also Zentr. Physiol. **18**, 690 (1905).

[3] Arch. exper. Path. Pharm. **40**, 313 (1897).

that the amount of glycocoll in beef kidneys could be appreciably increased by digesting them with uric acid.[1] It seems probable that the decomposition of uric acid may result in the formation of glycocoll; but we, nevertheless, wish to state that Wiener's investigation is not entirely convincing, for his method of estimating the glycocoll was not an exact one, and, above all, its production from other sources was not absolutely excluded. As the proteins participate largely in the production of glycocoll, it is necessarily difficult to estimate the amount of glycocoll originating from the uric acid.

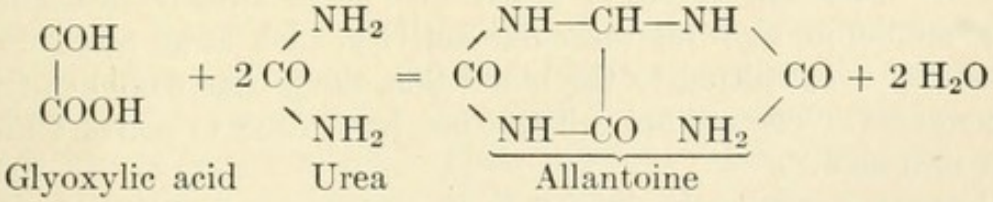
Urine also contains oxalic acid:



A part of this undoubtedly originates from the food. Another portion is unquestionably produced in the tissues. The assumption has often been made that oxalic acid may be a decomposition product of uric acid, although no satisfactory proof has been presented to substantiate this view. From a chemical standpoint such a formation of oxalic acid seems perfectly possible. We know that uric acid can go over into alloxan, parabanic acid, oxaluric acid, and finally into oxalic acid. It is impossible to say anything further about a source of the oxalic acid which does not come from the food.

Allantoine has often been regarded as a decomposition product in the hypothetical formation of oxalic acid from uric acid. It was first found in the allantoic fluid, and later recognized by Wöhler [2] as a normal constituent of the urine of suckling calves. Gusserow [3] has recently isolated it from the urine of new-born children. Allantoine is also found in the urine of various full-grown mammalia; for instance, dogs, cats, and rabbits.

Allantoine is the diuride of glyoxylic acid. It can easily be obtained by melting glyoxylic acid with urea:



The question of the origin of allantoine has been answered in various ways. It is generally believed to be related to the purine bodies. This assumption was strengthened by the discoveries of Salkowski [4] and Min-

[1] Arch. exper. Path. Pharm. **43**, 375 (1899).

[2] Ann. **26**, 244 (1838); **70**, 229 (1849); **88**, 100 (1853).

[3] Arch. Gyn. **3**, 270 (1872). G. Pouchet: Beiträge zur Kenntnis der Extraktivstoffe des Urins, Paris, **1880** (pp. 28 and 37).

[4] E. Salkowski: Zent. Med. Wiss. **36**, No. 53, 929 (1898).

kowski,[1] who showed that when a dog was fed on material rich in purine bases, such as thymus or pancreas, the amounts of allantoine eliminated appreciably increased. Cohn [2] found that the administration of hypoxanthine to this animal had the same effect. Mendel and White [3] have proved that intravenous injection of uric acid into cats and dogs, as well as the injection of salts of nucleic acids, caused an increase in the allantoine elimination. Wiechowski [4] has furnished a better proof that uric acid is decomposed into allantoine. He succeeded in showing that in surviving beef-kidney and dog's liver, uric acid is changed quantitatively into allantoine. This shows one way in which uric acid may be decomposed, but it does not necessarily follow that in the decomposition of uric acid allantoine is formed in every case; perhaps the latter is further decomposed into urea and glycocoll, and it is quite possible that normally the decomposition of uric acid may follow an entirely different course. On the other hand, we can hardly assume that the uric acid is all decomposed in one way. It is conceivable that, for example, a part is decomposed with the intermediate formation of glycocoll, while another part gives rise to allantoine.[5]

This would give us a clear conception of the formation of uric acid in mammalia. It can undoubtedly be traced to the degradation of nuclein substances, and finally to the purine bases. It has never been decided whether in birds and reptiles (animals in which, as we have seen, the uric acid in its entire significance and formation takes the place of urea) a part, if only a very small part, is formed in the way we have just indicated. It is hardly to be doubted that entirely analogous processes take place in the organisms of birds and reptiles. Similarly we have no reason for denying that a synthetic formation of uric acid may also take place in the tissues of mammals.

Before considering the behavior of the remaining cleavage-products of the nucleic acids in the animal organism, we must pay some attention to the purine bases appearing in urine. We have already mentioned, that, for example, in vigorous muscular activity, such large amounts of purine bases are transmitted to the blood that the purine content of the urine is increased. The animal cells do not have time to convert these bases into uric acid.

Purine bases are constantly present in the urine, some of which are to be regarded as direct decomposition products of the nucleic acids, while

[1] O. Minkowski: Zentr. innere Med. **19**, No. 19 (1898).

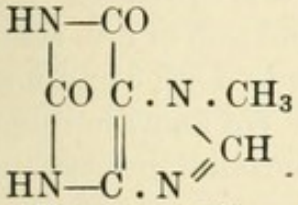
[2] T. Cohn: Z. physiol. Chem. **25**, 507 (1898).

[3] L. B. Mendel and B. White, Am. J. Physiol. **12**, 85 (1905).

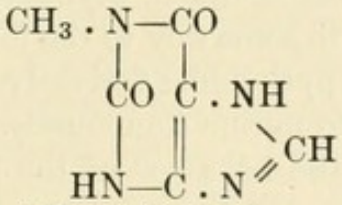
[4] Wilhelm Wiechowski: Hofmeister's Beitr. **9**, 295 (1907), **10**, 247 (1907.)

[5] It is interesting to know that allantoine has been found in the bark of tree-branches and in buds. Cf. E. Schulze and J. Barbieri: Ber. **14**, 1602 (1881); J. prakt. Chem. **25**, 145 (1882); Z. physiol. Chem. **11**, 420 (1886).

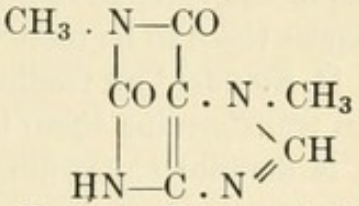
others are only indirectly related to the decomposition products of nucleins. The amount of such substances present in urine is small and varies. It may be increased by a diet rich in purine bases. An increased elimination of purine bases may also result from a greater destruction of leucocytes. Heteroxanthine,[1] paraxanthine,[1] and *l*-methylxanthine[2] are purines which bear no relation to nuclein metabolism. They represent the most important constituents of the so-called alloxuric bases of urine, and arise from the caffeine, theobromine, and theophylline present in our table accessories. The following formulæ will give us an idea of the relations between these substances:



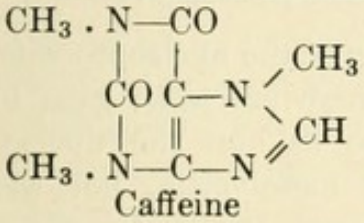
Heteroxanthine = 7-Monomethylxanthine



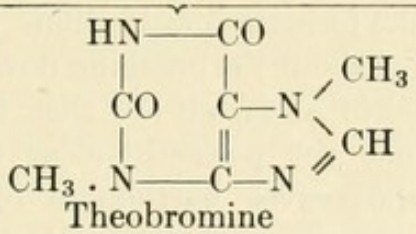
l-Methylxanthine



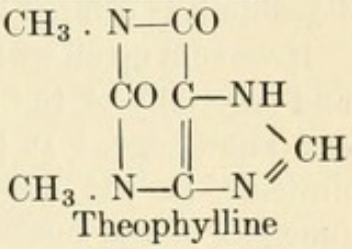
Paraxanthine = 1:7-Dimethylxanthine



Caffeine



Theobromine



Theophylline

The relation of these alloxuric bases to the purine bases of tea, coffee, and cocoa has been established by means of feeding experiments.

Xanthine, hypoxanthine, guanine, and adenine are among the purine bases which can be derived directly from the nucleic acids. The two latter are not invariably present. They evidently appear only when there is an increased decomposition of material containing nucleins with relatively less oxidation. Usually they are evidently converted into the corresponding oxypurines. Xanthine occasionally participates in the formation of renal calculi. Pure xanthine calculi rarely occur. Uric acid is usually a constituent of these bladder stones. These may occur as small concretionary masses, or as large stones. They are often stratified, in which cases uric acid layers alternate with those of calcium oxalate.

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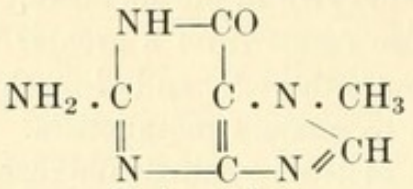
[1] G. Salomon: Arch. Anat. Physiol. **1882,** 426; **1885,** 570. M. Krüger and G. Salomon: Z. physiol. Chem. **21,** 169 (1895); Ber. **16,** 195 (1883); **18,** 3406 (1885).

[2] M. Krüger: Arch. Anat. Physiol. **1894,** 553; M. Krüger and G. Salomon: Z. physiol. Chem. **24,** 364 (1898).

Two other alloxuric bases, the so-called episarkine [1] and epiguanine,[2] have also been isolated from urine. The latter is 7-methylguanine:



Epiguanine

We must now attempt to answer the question as to what becomes of the remaining constituents of the nucleic acids. We are especially interested in the fate of the three pyrimidines, — uracil, thymine, and cytosine. We should expect them to be related in some way to the formation of uric acid, although H. Steudel,[3] on feeding pyrimidine derivatives to dogs, could not succeed in transforming them into purine compounds. We know nothing else that is definite concerning the behavior of the pyrimidine bases in animal economy.

As regards the phosphoric acid which is obtained by the cleavage of nucleic acid, we can only surmise what its relations are in metabolism. Possibly it is utilized in the formation of lecithin.

If we sum up all we know about the breaking down of the nucleoproteids, and especially of the nucleic acids, we see that there are large gaps in our knowledge. It is not yet perfectly clear to us how the nucleoproteids in the cells themselves participate in metabolism, nor the function of the nucleus in cell-metabolism. Although the study of the uric acid formation has given to us a fairly clear picture of the transformation of purine bases, on the other hand it has not been found possible from this knowledge to shed much light into the obscurity enveloping the metabolic disturbances which occur in gout and uric acid diathesis. We can indeed imagine that in these diseases either the production of uric acid is increased for some reason, or that there is not so much decomposition of this acid as takes place normally. Now uric acid is very difficultly soluble in water, and its occurrence in certain tissues — especially in cartilage — is to be traced to this fact. His[4] found that one part dissolved in 39,000 of water at 18° C. We shall study these relations at another place, and will here merely mention the fact that of the four hydrogen

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[1] Balke: Zur Kenntnis der Xanthinkörper, Inaug. Diss. Leipzig, **1893**. Georg Salomon: Z. physiol. Chem. **18,** 207 (1894).

[2] M. Krüger: *loc. cit.* Arch. Anat. Physiol. **1894,** 553; Z. physiol. Chem. **24,** 364 (1898); **26,** 389 (1898–99).

[3] Z. physiol. Chem. **32,** 285 (1901).

[4] Verh. xvii, Kong. Med. p. **315** (1899); Deut. Arch. klin. Med. **67,** 81 (1900); Verh. xviii, Kong. Med. **425** (1900) and Zent. Stoffwechsel-und Verdauungskrankheiten, **1,** 61 (1900); **3,** 434 (1901); His and Paul: Z. physiol. Chem. **31,** 64 (1900).

atoms which are replaceable by radicals in the uric acid molecule, only two take part in the formation of salts. Uric acid is, therefore, a dibasic acid, and forms two series of salts, the acid or monobasic salts, and the neutral or dibasic salts. Thus we have *acid sodium urate*, also called *monosodium urate*, and *neutral* or *disodium urate*. Many speculations have been brought forward regarding the deposition of uric acid in the tissues, especially those of the joints, in gout, basing them upon the difficult solubility of uric acid. On the one hand, an increased formation of uric acid may account for its elimination, and on the other hand the composition of the blood, lymph, and other constituents of the tissues may be such that the uric acid is even more insoluble than under normal conditions. All of these hypotheses have failed to be very fruitful. They have no good foundation. For example, we do not know in what form the uric acid is transported in the blood and tissues. The assumption has been made that it circulates, not in a free state, but combined with albumin, nucleic acids, and other substances, although no positive proof has yet been presented that such is the case. No conclusions can be drawn from the deposits themselves, which consist of monosodium urate. Great stress was formerly laid on the increased presence of uric acid in the blood. We know to-day, that other conditions may result in an increase of uric acid in the blood without causing the appearance of the symptoms of gout. Weintraud [1] has in fact shown that in a normal individual there is an increased amount of uric acid in the blood after a diet rich in purine bases. Again, great stress was laid upon the increased elimination of uric acid in gout until it was positively shown that it is permissible to speak of an increased elimination only during an acute attack. Otherwise — aside from the fact that in gouty diseases the purine values of the urine vary more than under normal conditions — the amount of purine present in the urine is practically the same during a long period of time.

It is very noticeable that the deposition of uric acid, especially in gouty inflammations, seems to be confined to specific locations, such as the smaller joints of the extremities. The suggestion has been made that the primary cause of the whole ailment is not a disturbed metabolism of the purine substances, but an alteration of the tissues at the place in question. Just as it has been assumed that the formation of gall-stones is due primarily to an inflammation of the biliary passages, and likewise that renal calculi may originate from some organic lesion, the assumption has also been made that the circulating uric acid was deposited at a given spot on account of some change in the tissues there.

This is not the place to consider the pathology of gout, nor to enter into any discussion of the various theories concerning it. We can only attempt

[1] Wiener klin. Rundschau: **10**, Nos. 1 and 2, pp. 3 and 21 (1896). For further literature see H. Wiener: Ergeb. Physiol. (Asher and Spiro) Jg. **2**, 1 Abt., p. 377 (1903).

here to explain pathological processes in the light of physiological-chemical investigations, and on the other hand obtain from pathological research new points of view for physiological-chemical work. Doubtless the essential part of purine metabolism will find its full explanation only by means of further studies in pathology and in physiology. We must be satisfied with merely sketching the existing situation, and bring forward the fact that for the present there is plenty of room for hypotheses, a sure sign that the investigations have by no means solved the problem. It seems certain that gouty diseases will not all be referred to a single cause, nor to a single disturbance in cellular metabolism. Here, as in diabetes, various disturbances may take place in different stages of the total purine metabolism, which will all finally result in the same symptoms.

LECTURE XIV.

THE MUTUAL RELATIONS BETWEEN FATS, CARBOHYDRATES, AND ALBUMINS.

I.

In our previous treatment of the three most important classes of organic food-stuffs, the fats, carbohydrates, and albumins, we have considered each individual group by itself, as regards the way in which it is absorbed, the place where it is assimilated, and the relations of the group to specific functions of the body. We have found that carbohydrates are the most important source of muscular power, while in the fats we have the principal source of heat. The significance of the albumins is much less certain. We know that they are absolutely unreplaceable, and also that they act as building material for the cells, being necessary to replace the parts which have been used up. We do not at present understand why the animal organism requires so much albumin under all conditions, nor why it so quickly decomposes, up to a certain limit, the ingested albumin.

By more closely following the metabolism, we quickly encounter results which do not harmonize, for example, with the assumption that the muscles are only capable of acting by means of the energy given to them by the carbohydrates. Again, it was noticed early that a portion of the carbohydrates disappeared when the diet was rich in these substances, — i.e., this part could not be detected in the form of glycogen, — and, moreover, an exact study of the respiratory exchange showed that no increased combustion of the sugars had taken place. According to this we can only assume that the extra carbohydrate is retained in some form other than glycogen.

Before discussing those facts, which force us to the assumption that a transformation of one group of food-stuffs into another may take place in the animal organism, we will first of all call attention to the relations existing between fats, carbohydrates, and albumins, according to our present knowledge regarding the chemical composition of these substances. Of course, this will only show us the *possibility* of such transformations, and indicate the manner in which they *may* take place. The organism itself may, naturally, choose an entirely different course, and utilize other

compounds, e.g., certain decomposition products, as bridges from one food-stuff to another.

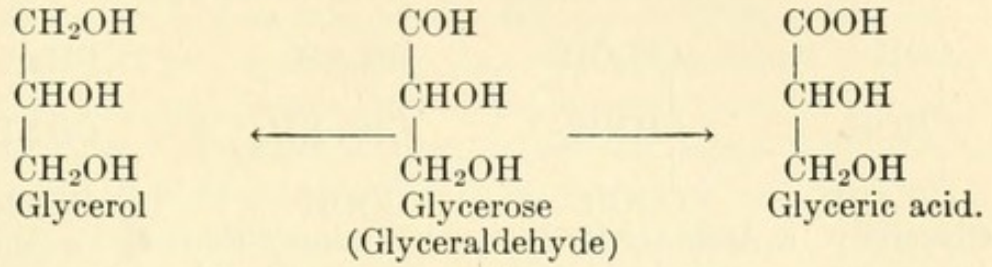
If we follow the formation of the most varied carbon compounds in plants, which are the prime source of all carbon combinations in living organisms, we are forced to the assumption that the first product arising from the assimilation of carbon dioxide under the influence of chlorophyll and the sun's energy, is a carbohydrate, or at least some compound very closely related to this class of substances.[1] The carbohydrates undoubtedly assume a central position in plant metabolism, while the albumins and fats are of minor importance. But it is not impossible that the assimilation of carbon dioxide may proceed in various ways, and form different primary assimilation products. It is indeed possible that the carbohydrates, i.e., the large amount of starch present, may conceal other compounds.

Up to the present time, because starch is detected so easily and so positively, this and analogous substances have been of chief interest to us. On the other hand, the carbohydrates impart to the plant organism their individuality. We find them in every direction; and when we consider the numerous polysaccharides (the more simple ones like starch, which arise from the condensation of a single kind of sugar, and the other innumerable, more complicated sugars, consisting of unlike components, such as arabinose, xylose, dextrose, etc.), we will recognize the fact, immediately, that the carbohydrates occupy the same predominating position in plants that the albumins do in animal organisms. At all events, each of the three groups of food-stuffs — fats, carbohydrates, and proteins — is ultimately derived from the carbonic acid of the air, for that is the only source worth mentioning of the carbon in plants.

In considering the assimilation of carbon dioxide by the plants, we referred to Baeyer's hypothesis, that formaldehyde is to be looked upon as the first condensation product. We stated then that we could easily imagine the various different kinds of sugars to result from the condensation of several molecules of formaldehyde. On the other hand, we also mentioned Emil Fischer's hypothesis, which includes the possibility that the glycerose, discovered by him, may occupy the primary stage in the whole process of carbonic acid assimilation. This assumption has much in its favor; and if glycerose, perhaps, does not actually appear at this first stage, we must remember the possibility that it may result from the disintegration of carbohydrates. At any rate it is interesting to think that glycerose may perhaps occupy an intermediate position in the further syntheses of the plant organism. From glycerose on, it is easy to build bridges to the group of proteins as well as to that of the fats.

[1] Cf. Lecture IV.

The relations to the fats are evident from the following formulæ:



Glycerose is simply the aldehyde of glycerol.[1] Its preparation from glycerol was accomplished by Emil Fischer. Conversely, we can imagine one of the fat components, glycerol, as originating from glycerose. The relations become much more complicated if we attempt to derive the fatty acids, the other components of fats, from sugar. Emil Fischer[2] suggests their formation in the following manner: To produce oleic and stearic acids, three molecules of grape-sugar (or six molecules of glycerose) may unite at their aldehyde groups, thus forming a chain of $3 \times 6 = 18$ carbon atoms. By rearrangement of the atoms and withdrawal of oxygen, i.e., by reduction, the above-mentioned acids may be formed. The other fatty acids, e.g., palmitic acid, can be formed perhaps in a similar manner, except that sometimes only hexoses are used for the synthesis, while, at other times, hexoses and pentoses are both utilized, according to the number of carbon atoms in the acid molecule. Thus we can imagine palmitic acid, with its 16 carbon atoms, being produced from one hexose and two pentose molecules. Pentoses are very widely distributed in the vegetable world, and in large amounts. They play a far less important part in the animal body, although they may be formed by decomposition. We have already learned that a pentose, arabinose, may be very easily derived from glucose, or from gluconic acid; and on the other hand, we have seen that an oxidation product of glucose, glucuronic acid, will easily go over into a five-carbon sugar by splitting off carbon-dioxide.

Thus, while we are in a position to derive one of the components of the fats, glycerol, from the carbohydrate group, we have but little foundation for the formation of fatty acids from the same group. We are at present confined to hypotheses. We have not yet succeeded by any chemical means in converting sugars into fat.

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[1] The original glycerose, with which Fischer worked, really contained chiefly dihydroxyacetone. von Lippmann and others, however, prefer to call glyceraldehyde (of which there are two stereo isomers) glycerose rather than the dihydroxyacetone.—TRANSLATORS.

[2] Die Chemie der Kohlehydrate und ihre Bedeutung für die Physiologie, Berlin, A. Hirschwald, 1894, p. 28.

Let us consider the relations of glycerose to the proteins, or to their building materials. The following formulæ will be helpful:

COH | CHOH | CH_2OH	CH_2OH | $CHNH_2$ | COOH	CH_2SH | $CH \cdot NH_2$ | COOH	CH_3 | $CHNH_2$ | COOH
Glycerose	α-Amino-β-Hydroxy-propionic acid =Serine	α-Amino-β-thio-propionic acid =Cysteine	α-Amino-propionic acid =Alanine

These formulæ show at once what slight differences exist between these apparently entirely distinct groups. It would not be at all difficult to imagine the above three amino acids as being derived from glycerose. Leucine might be produced from one molecule of a hexose or two molecules of glycerose, by the addition of ammonia and partial reduction;[1] and by oxidation, the dibasic glutamic and aspartic acids may be formed from leucine. We meet with certain difficulties when we attempt to account for the formation of these last compounds. Leucine, which is isobutyl-aminoacetic acid, contains a branched chain. The difficulties increase when we come to proteins containing an aromatic ring, such as phenyl-alanine and tyrosine. To produce the benzene nucleus from the carbohydrates is a highly complicated process. Such transformations undoubtedly do occur in the organism of plants, although the formation of these substances is, probably, not a direct one.

We must also consider the close relation of alanine to lactic acid, which is so easily produced by the action of alkalies or ferments.

CH_3 | CHOH | COOH	CH_3 | $CHNH_2$ | COOH
Lactic acid	Alanine

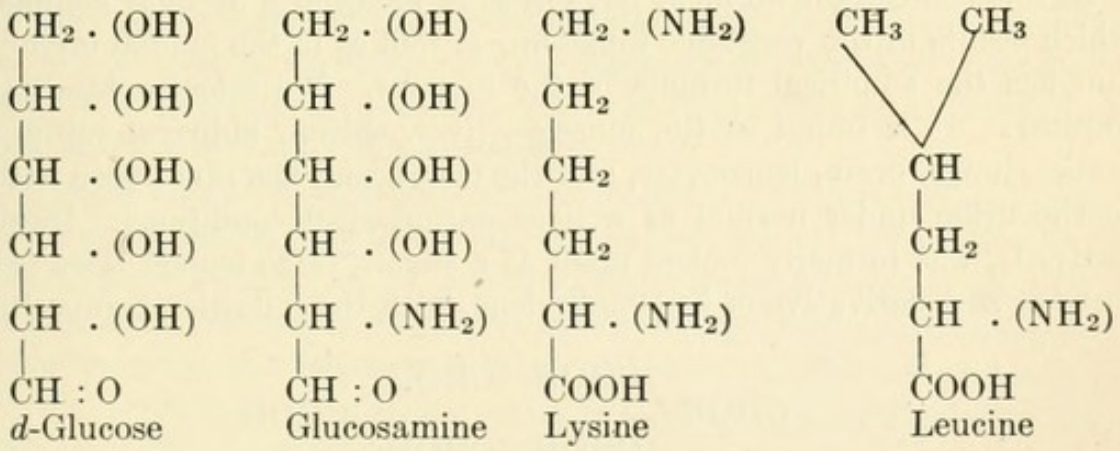
Plants could also form their albumins or individual amino acids in this manner. Conversely, lactic acid may be easily formed from alanine, serine, and cysteine by disintegration; from alanine, for example, simply by splitting off ammonia. In fact, such processes must take place in the animal organism to a considerable extent. Lactic acid, therefore, may arise from two sources in the animal organism. It may come from carbohydrates or from albumin.

The formation of albumin in the animal organism from the other kinds

[1] Cf. E. Fischer and E. Abderhalden: Z. physiol. Chem. **36**, 268 (1902).

of food is of little consequence, or at least all of our present knowledge of albumin metabolism speaks against it. We need only think of the possibility of fats and carbohydrates being produced from albumin. Now we have already seen that a large part of the elementary components of the proteins are very closely related to the lower members of the normal fatty acid series. We know further, that only a part of the carbon leaves the organism in combination with the nitrogen of the individual amino acids. A portion of the carbon chain must remain behind. What becomes of this is not yet apparent. Right here the investigation must start concerning the transformations of the albumins into fats and carbohydrates, for all such changes must result from these carbon chains. We have, to be sure, no positive proof that such transformations do take place.

Perhaps the intermediate product, glucosamine, may throw some light upon the formation of albumin from carbohydrates in plants, and, conversely, the production of carbohydrates from albumins in the animal organism. It is a derivative of glucose (or of mannose), and is closely related on the one hand to the sugars, and on the other, to the oxyamino acids. It is certainly not without significance, that nature has provided such connecting links. We recall the following formulæ:



The carbohydrates, as we have already seen, are related to the polyhydric alcohols on the one hand, and to various acids of different character on the other. We can thus, either directly or indirectly, connect large groups of different compounds with the sugars. We are still entirely in the dark regarding the method of formation of innumerable tannins, ethereal oils, alkaloids, etc., which occur in the plant organisms. All of these must ultimately be referred to the carbon dioxide of the air. What relations they possess to the carbohydrates is still entirely beyond our knowledge; in fact, owing to their great complexity, we are almost forced to the conclusion that they are not at all related. C. Harries[1] has

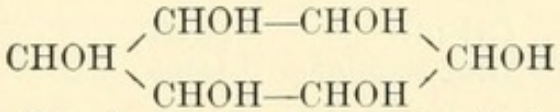
[1] C. Harries: Ber. **38**, 1195 (1905).

shown, however, that products of the vegetable kingdom which apparently are not at all related to the carbohydrates, may, nevertheless, have been derived from them. He showed that caoutchouc, a compound containing an eight-carbon ring (1.5-dimethyl-cyclo-octadi-ine), on hydrolysis yielded lævulinaldehyde, $CH_3 . CO . CH_2 . CH_2 . COH$, and the ozonide of caoutchouc yielded similarly lævulic acid. Now, the sugars readily go over into lævulic acid, while, on the other hand, it is also possible that caoutchouc may be built up of pentoses. Their reduction to C_5H_8, and subsequent condensation into

$$\left(\begin{matrix} CH_3 . C . CH_2 . CH_2 . CH \\ \| \qquad\qquad\qquad\quad \| \\ HC . CH_2 . CH_2 . C . CH_3 \end{matrix}\right)_X$$

could account for the formation of caoutchouc. Perhaps the α-methyl-furan, discovered by Atterberg [1] from beech-wood tar, which easily goes over into lævulinaldehyde,[2] may be even more closely related to the caoutchouc synthesis. At all events, this gives us a new support for the assumption that the whole large group of terpenes may likewise be derived from the carbohydrates.

In this connection we must not forget to mention a peculiar compound which occurs in the vegetable kingdom, as well as in the animal organism, and has the empirical formula of the hexoses. We refer to inositol (or inosite). It is found in the muscles, liver, spleen, kidneys, suprarenal bodies, lungs, brain, leucocytes, and the testes, and has often been noticed in the urine under normal as well as pathological conditions. Inositol, $C_6H_{12}O_6$, was formerly looked upon as a sugar. Maquenne [3] later recognized it as a derivative of hexamethylene, with the following composition:



Hexahydroxybenzene = Inositol

It is indeed possible that we have in this case, one of the first stages in the conversion of a carbohydrate into the benzene ring, so that this compound which is of so much biological interest opens up further possibilities for the formation of the numerous aromatic compounds in the plant organism. Although chemical investigation has often served to give us better understanding concerning many obscure biological processes, we must not forget that a clear conception is lacking in regard to the most

[1] A. Atterberg: *ibid.* **13**, 879 (1880).
[2] C. Harries: *ibid.* **31**, 37 (1898).
[3] Maquenne: Compt. rend. **104**, 1719 (1887); *ibid.* **109**, 812 (1889).

important changes. We are obliged to resort here almost exclusively to experiments with animals. Certain observations are also a result of Nature's physiological experiment, pathology. In all such investigations, we are dealing with *indirect* methods of proof. Experiments with animals rarely lead to other than indirect results. These are not entirely conclusive; they are merely more or less convincing, and in most cases are dependent upon the personal interpretation of the investigator. This is a weak point in nearly all biological investigation, which undeniably gives it a certain amount of fundamental uncertainty. For this reason, it is difficult to form a positive decision from the numerous experiments which have been performed in the attempt to decide the question as to the conversion of one class of food-stuffs into another. We can merely enumerate here the more important and best substantiated conceptions and those experiments which have been carried out in the most convincing manner. On the other hand, we would be committing a grave error if we were to consider only those processes and changes in the organism as proved for which we have a purely chemical explanation, and which we are able to repeat, where possible, outside of the living cell. We would thus be making a restriction, which would hinder the further development of biology, and we should then be forgetting that biology has a distinct field of its own, with its own peculiar methods of investigation. Ultimately, of course, we must always depend upon the exact sciences, chemistry and physics, and consider a biological problem as fully settled when these sciences supply the key-stone.

Let us begin first of all with the carbohydrates and their conversion into the other two groups of food materials, fats and albumins. The formation of albumin from carbohydrates in vegetable organisms comes into consideration only to the extent that the plant cells utilize the carbon chains of the sugars in syntheses together with the nitrogen, which is either assimilated by the roots from the ground, or, as in rare cases, is obtained directly from the air. We do not know anything definite about these processes. In the animal organism, we can deny the possibility of such transformations. It is otherwise, however, as regards relations of the carbohydrates to the fats. One of the first observations in this connection was the formation of fat in ripening rape-seeds, and in the pulp of the olive. We know that unripe rape-seeds contain large amounts of carbohydrates, but practically no fat. During the ripening of the seeds we find that the carbohydrates gradually diminish, while fat takes their place. This observation is very significant, for the seed is unable to give off its carbohydrate externally, or to take up fats. The changes in nature of a food material are also attested by the metabolism. The respiratory quotient changes. Gerber[1] has shown that the ratio of carbon dioxide produced to the oxygen

[1] C. Gerber: Compt. rend. **125**, 658 and 732 (1897).

consumed, in the unripe seeds, is less than one. More oxygen is taken up than is given off as carbon dioxide. During ripening the ratio becomes greater than one, only to fall below one again after the fruit has become completely ripe. Analogous results have been observed in the ripening of olives, which in their unripe condition contain mannitol. The following figures published by A. Roussille[1] will give us an idea of the process which accompanies the ripening of olives:

	Raw Fat.	Albumin.
	Per cent.	Per cent.
June 20	1.40	...
July 30	5.49	...
August 30	29.19	14.619
September 30	62.30	4.189
October 30	67.21	4.411
November 25	68.57	4.329

Leclerc du Sablon[2] found the following amounts of carbohydrates and fats in nuts per 100 parts of the dry substance:

	Water.	Oil.	Glucose.	Saccharose.	Amylose.
July 6	837.	3.	7.6	0.	21.8
August 1	535.	16.	2.4	0.5	14.5
August 15	274.	42.	0.	0.6	3.2
September 1	48.	59.	0.	0.8	2.6
October 4	10.	62.	0.	1.6	2.6

The changes of carbohydrates into fat in the almonds were as follows on a basis of 100 parts of dry substance:

Date.	Water.	Oil.	Glucose.	Saccharose.	Amylose.
June 9	896.	2.	6.0	6.7	21.6
July 4	716.	10.	4.2	4.9	14.1
August 1	219.	37.	0.	2.8	6.2
September 1	117.	44.	0.	2.6	5.4
October 4	12.	46.	0.	2.5	5.3

It is interesting to note that fatty acids — evidently as intermediate products — have been observed during the formation of the fats.[3] How the transformation, as a whole, is effected, has never been explained.

We also know that the reverse process, i.e., the formation of sugars from the fats, also takes place, and this has been followed in the case of germi-

[1] A. Roussille: *ibid.* **86**, 610 (1878).

[2] *Ibid.* **123**, 1084 (1896).

[3] von Rechenberg: Ber. **14**, 2216 (1881).

nating rape-seeds. If we permit these to develop away from the light, we observe the fat disappearing from the cotyledons. In its place we find carbohydrates: starch, cellulose, gum, sugar. If we allow seeds, rich in starch, to germinate in glass tubes sealed under mercury, no change in the volume of gas can be observed. During the germination of seeds containing oil, on the other hand, we notice that gas is being consumed, from the fact that the mercury rises. This corresponds to the amount of oxygen which is required to convert the fats into carbohydrates which are richer in oxygen. These important observations were made by Julius Sachs and Wiesner [1] in 1859.

There is, therefore, no doubt that the plant cell is capable of producing carbohydrate from fat, and, conversely, fat from carbohydrate. If we assume with Kassowitz [2] that the protoplasm absorbs, or assimilates, one of these compounds, e.g., the fat, in order subsequently to form in this case, the carbohydrate, in such a way that there is no direct connection between the two compounds, then we miss the point at issue, and the whole process is beyond our understanding, for it cannot be a matter of indifference for the functions of the protoplasm, whether at one time fat, at another time carbohydrate, and yet again albumin, is at its disposal. The question concerning the transformations of the separate food-stuffs is only postponed by such assumptions, and it becomes more involved and obscure.

A much disputed question is this: Does the animal cell possess the same abilities as the plant cells? Can the animal organism convert fats into carbohydrates, and, conversely, carbohydrates into fats? The last question has been answered in the affirmative. We know that with a diet rich in carbohydrates, an appreciable part of the ingested carbohydrate is not stored up in the form of glycogen, although there is no glucohemia. The fact forces us to the conclusion that carbohydrates can be stored away as reserve material in some other form than glycogen. Numerous feeding experiments have shown that an accumulation of fat follows a diet composed largely of carbohydrates.[3] This decision may be reached in two different ways: first, by determining the amounts of fat formed with a diet containing a definite quantity of fat, albumin, and carbohydrate; and secondly, by estimating the daily elimination of carbon dioxide. The first proof has been carried out, as a rule, in the following manner: Two

[1] J. Sachs: Bot. Zeit. **1859**.

[2] Cf. M. Kassowitz: Allgemeine Biologie (3 vols.).

[3] Cf. also B. Schulze: Landw. Jb. **1**, 57 (1882). F. Soxhlet: Z. Landw. Vers. Bayern. August (1881). St. Chaniewski: Z. Biol. **20**, 179 (1884). H. Weiske and E. Wild: *ibid.* **10**, 1 (1874). I. Munk: Arch. Path. Anat. **101**, 91 (1886). E. Meissl and F. Strohmer: Sitzber. Akad. Wiss. Berlin, **88**, **III** (July, 1883), and Monatsh. **4**, 801 (1883). E. Meissl, F. Strohmer, and N. v. Lorenz: Z. Biol. **22**, 63 (1886). C. Voit: Sitzungsber. Münchener Akad. **1885**, p. 288. M. Rubner: Z. Biol. **22**, 272 (1886).

dogs from the same litter and about the same weight, are permitted to fast for quite a length of time. One of the animals is then killed, and the amounts of albumin and fat contained in the carcass are determined. The other animal, which is assumed to possess approximately like quantities of the above substances, is then fed for a time on a definite diet, the composition of which in fat, albumin, and carbohydrate is known. The unabsorbed part of this material can be estimated by an analysis of the fæces. After several days, this animal, which has now gained in weight, is killed, and the amount of its fat and albumin determined. In this manner it can be shown that as much as 85 per cent of the ingested starch has been converted into fat. We must not place too much confidence in these values, for the assumption that the one animal, at the termination of the fasting period, contained exactly the same amounts of fat and albumin as the other, is open to question. It is remarkable, nevertheless, that there should be such great differences between the organisms of the two animals, and that corresponding results have been obtained in a number of experiments.

Thus, Tscherwinsky [1] obtained the following values:

	Albumin.	Fat.
Animal fed 4 months with barley contained	2.52 kg.	9.25 kg.
Animal used as a "control"	0.96	0.69
First animal, therefore, gained	1.56 kg.	8.56 kg.
The food contained	7.49	0.66
Difference	−5.93 kg.	+7.90 kg.

The animal used in the experiment, a young pig, had, therefore, gained 7.9 kilograms fat, which could not have come from the fat in the food, and certainly not from the albumin. Carbohydrates, therefore, were transformed into fat.

We can, as we have said, also follow the respiratory exchange in an animal fed on a diet rich in carbohydrates. If we know the amounts of carbon, albumin, and carbohydrate present in a food poor in fat, but rich in carbohydrates, we can, on the one hand, by determining the nitrogen in the urine, estimate the amount of albumin retained in the body, and, on the other hand, we can estimate the quantity of carbon remaining in the organism, by determining the amount exhaled as carbon dioxide in addition to that eliminated as urea. In this way it has been found that the amount of carbon retained may be so large that the only possible explanation is that the ingested carbohydrates have been converted into fat.

The production of fat from the sugars has, *a priori*, much in its favor.

[1] N. Tscherwinsky: Landw. Vers-sta. **29**, 317 (1883).

The animal organism has only a certain amount of room in its tissues for the carbohydrates. The quantity of glycogen which can be stored up in the liver, muscles, and the other organs, is very limited. The animal organism is often provided with larger amounts of carbohydrate than it is able, at the moment, to utilize. It is here that the depots for fat storage are utilized. Large amounts of carbohydrates after having been transformed into fat can be held in reserve until needed. We have not the slightest idea where, in the animal organism, or in what organ, this transformation takes place. It is possible that the liver executes this complicated process.

Although it is generally admitted that the animal cell is able to convert carbohydrates into fats, the reverse process is a much disputed problem. We are accustomed to look upon most chemical reactions as reversible. We also know that the animal cells are capable, directly or indirectly, of performing characteristic processes. They build up and they tear down material only to reconstruct it, and, finally, by its complete destruction, they utilize the energy contained in the food. We have seen representatives of all three classes of nutrient materials break down in the intestine, and, again, we have traced their reconstruction into more complicated compounds. The cells of the liver produce glycogen from grape-sugar, permitting it to become glucose again as it is required. Another problem is this: Is the animal organism, under normal circumstances, capable of satisfying its carbohydrate requirements from fats. This would hardly be the case under ordinary conditions, for, *a priori*, as we shall later on see more in detail, there is no reason at hand why the fat should first go over into carbohydrate, in order that the organism may utilize its energy for specific purposes. On the other hand, the possibility of such a transformation unquestionably exists. We can imagine that each cell can only utilize certain compounds for specific functions. Thus, we may assume that the muscle cells operate only by means of carbohydrates. The selective action of the ferments would support such an assumption. We know that, in many cases, they are unable to act upon substances closely related to compounds that they can decompose. Every ferment seems peculiarly fitted to attack only certain definite compounds. Thus it would be easy to understand that the muscle cells, which are especially adapted to act upon carbohydrates, can utilize only such potential energy as is presented to them in this form. On the other hand, we must remember that the cleavage and combustion of the food materials are not the source of the liberated energies, but only act as a loosening momentum, or as a shock. The true cause is the chemical energy of the food substances — ultimately the sun's energy, transformed sunlight. We cannot understand, *a priori*, why the energy liberated by the combustion of the fats could not be just as well utilized by the muscle cells as that which arises from the

carbohydrates. If the muscle cell possesses the ability of consuming the fats, it hardly seems probable that it must first convert the fats into carbohydrates in order to abstract the energy from them.

If the fats were first changed into carbohydrates before they could be utilized for work, then, according to Zuntz, a diet of fats exclusively would require about 30 per cent more energy to perform a given amount of work than would be required after a diet of carbohydrates. This, in fact, is not the case.[1]

Until recently the above discussion would have been quite unnecessary. The general conception was that the different food materials in the organism, i.e., in the tissues, and finally in the cells, were burned up directly by the aid of oxygen. It was only necessary that the food material and the oxygen should both be present in such form that they could react together.

The whole problem thus became a very simple one. The conviction is, however, becoming more and more pronounced, that the relations are much more complicated. The cells themselves participate far more actively in the combustion of the food materials than we have hitherto imagined. They prepare the substances for disintegration and combustion. The fact that our foods are not attacked by the oxygen of the air, has led to the assumption that the oxygen is " activated " in some manner in the organism, and that this activated oxygen effects the combustion. This problem of the activation of oxygen has been the main topic of interest, whereas the behavior of the food materials themselves, in the oxidation process, has been entirely neglected. Now it has only recently been shown, as we have seen, that the diabetic possesses, as far as our present knowledge is concerned, an absolutely normal capacity for oxidation. Grape-sugar alone he consumes more or less imperfectly. As soon, however, as any slight attack has been made upon the glucose molecule, — as soon as it becomes "opened up," — the diabetic is able to consume completely, and to utilize, the energy contained therein.[2] We must consider the possibility of a change occurring in the food by means of a cell influence — either directly or indirectly by means of ferments — before the oxidation takes place by the oxygen, which is carried to the tissues with the blood. The material is first of all " opened up " so that it can be oxidized. How this takes place, we do not yet know. We can imagine, however, that the oxygen is first attached to the " opened " molecule, which is then destroyed. We know, from the investigations of C. Harries,[3] that caoutchouc, under the action of ozone, goes over into a compound rich in

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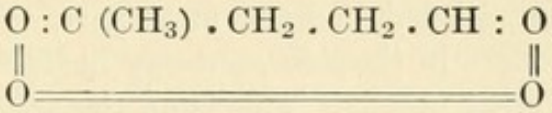
[1] H. N. Heinemann: Pflüger's Arch. **83**, 44 (1901). J. Frentzel and F. Reach: *ibid.* **83**, 477 (1901).

[2] Cf. Lecture V, p. 101.

[3] C. Harries: Ber. **37**, 2708 (1904); *ibid.* **38**, 1195 (1905).

oxygen, the so-called "ozonide," which can be looked upon as a peroxide of lævulinaldehyde.



It decomposes into lævulinaldehyde, lævulic acid, and hydrogen peroxide, on prolonged boiling with water. It is clear that, in the animal organism, such an energetic action of oxygen can hardly take place on the unaltered food materials, although it is possible that the cell might so change the food as to make it more susceptible to attack. Such a conception corresponds more nearly to the actual functions of the cells. It consumes the substances which it needs from time to time; it must, therefore, have a direct influence on these processes. Under normal circumstances, oxygen is invariably present. The cell, i.e., its protoplasm, either activates the oxygen, or else it seizes the separate food materials as it needs them, and offers them to the oxygen for oxidation. At any rate, the decompositions, which have been observed directly, e.g., that of the carbohydrates, may be traced back directly to cell-activity. The cell is, therefore, able in two ways to regulate exactly its requirements of energy: by the activation of the oxygen, or by the preliminary preparation of the food for combustion.

We have intentionally gone into this matter somewhat in detail, in order to show that the combustion of the food materials in the tissues is not necessarily such a simple process as we have heretofore assumed. For these reasons it is, as yet, impossible to decide whether fats are normally converted into carbohydrates. Such a conversion is hardly probable. It is more natural to imagine a direct utilization of the energy in fats by the animal cell, and especially by the muscle cells.

Experiments have been made with animals in the attempt to settle the question whether fats are converted into carbohydrates. Before discussing these we must show in general what deductions can be safely drawn from such investigations.

Investigations on the formation of sugars from other compounds than carbohydrates, and especially from fats, have, as a rule, been carried out in one of two ways. We can make the animal chosen for experiment free from glycogen, by fasting, hard work, or strychnine convulsions, and then feed it the compound which is to be tested as a glycogen-former. By subsequently determining the glycogen content of the whole animal, it is possible to find out whether any glycogen has been formed. Only in exceptional cases, however, have these experimental conditions been satisfied. Rarely was it satisfactorily proved that the animal was free from glycogen at the real beginning of the experiment, and such a source of error is serious on account of the small amount of glycogen which is found

at the end of the test. Again, the methods employed in determining the amount of glycogen were inaccurate in many cases. Finally, the experimenters have been satisfied, for the most part, with merely estimating the amount of glycogen in the liver, entirely neglecting that which might be retained in the other organs. This is on the assumption that the liver is the place where the most glycogen is formed. We do not know, however, how the animal organism behaves when it has been deprived of its carbohydrate stores. It is perfectly possible that the sugar will first go to those organs which need it the most. The chief objection to all these experiments is always that the ingested compound may have had an indirect effect, i.e., when we add fat, to determine whether fat can produce glycogen, and we do, as a matter of fact, find that this causes an increase in glycogen, then the objection can be raised that perhaps the fat, which is itself consumed, has acted as an albumin-sparer, so that the glycogen may have been produced from albumin. The method of proof is, in every case, an indirect one, and this makes it far more difficult to arrive at the correct conclusion. In one and the same experiment, the formation of sugar may be traced back to either the fats or albumins, according to the point of view. The same may be said of the second method of carrying out the experiment. In this case, glucosuria is first produced, and then the influence of various substances on the elimination of sugar is studied. Thanks are due to E. Pflüger [1] for critically examining all of the investigations which have been made up to the present time that have any bearing on this subject, thereby showing with great clearness that the whole problem is still in a very uncertain state.

First of all, glycerol was tested with reference to its ability to produce sugar; i.e., in other words, the problem was to decide whether the animal cell is capable of synthesizing sugar from glycerol. This compound, as we have already seen repeatedly, is related to sugar in its composition. We have mentioned the hypothesis that glycerose is the starting-point in the formation of glycerol by the plant cell, and in the same way we can regard glycerose as resulting from glycerol when the plants convert fat into sugar. Now the old idea that only the plant cells are capable of effecting synthesis, has long since been set aside. We know that the animal organism is also able to build up. There is, therefore, no reason why sugar could not be formed from glycerol. Of the many experiments which have been performed in this direction, we will refer to those of Cremer [2] and Lüthje.[3] They fed dogs, whose pancreas had been removed, with glycerol, and determined the increased elimination of sugar in the urine. Lüthje

[1] E. F. W. Pflüger: Das Glycogen u. s. Beziehungen z. Zuckerkrankheit, 2d ed. Bonn., M. Hager, (1905). Cf. E. Pflüger: Pflüger's Arch. **103,** 1 (1904).

[2] M. Cremer: Sitzber. Gesel. Morph. u. Physiol. München, May 27, 1902.

[3] H. Lüthje: Deut. Arch. klin. Med. **79,** 498 (1904); **80,** 101 (1905).

administered as much as 350 grams per day. The animal under experiment, a dog, weighed 15 kilograms. If, in accordance with common experience, we assume that this animal had stored up 11 grams of glycogen per kilogram of weight, at the beginning of the experiment, we obtain, as the total amount of glycogen present, 165 grams, corresponding to 183 grams sugar. If we take the maximum value, 40 grams of glycogen per kilogram weight, we have 600 grams glycogen, corresponding to 664 grams sugar.[1] The animal under experiment, however, excreted 1408.4 grams sugar. With the first assumption, 1225 grams, and with the second, 744 grams, of sugar thus remain unaccounted for. The ingested albumin and glycerol must be regarded as producing this sugar. The animal excreted 209.8 grams nitrogen during the whole period of the experiment. Lüthje calculated that, at most, 630 grams of sugar could have been produced from the conversion of the albumin. This leaves considerable sugar still unaccounted for unless we admit that it came from the glycerol. Lüthje's experiment is the only one which really proves that glycerol can be converted into sugar.[2] All of the other investigations, namely, those which showed an increase in the glycogen content of the liver, are not above criticism. The discovery that the animal cell is capable of transforming glycerol into sugar is another connecting link between the animal and plant cells. We must not, however, deceive ourselves with the thought that the conversion of glycerol into sugar takes place in normal metabolism to any considerable extent. We know it is true that fats, before being absorbed, are more or less disintegrated into their components, glycerol and fatty acids. We also know that neutral fats are formed again in the intestine. A small amount of free fatty acids remains, and likewise some glycerol. There is but very little, however. It might be possible for fat to undergo combustion without previous hydrolysis, and the glycerol therein converted into glucose instead of being consumed with the rest of the fat molecule. The amount of such glycerol would be only 11 per cent of the neutral fat, and this is relatively little.

The next question that arises is whether the fat itself, i.e., the fatty acid component also, can go over into sugar. Direct experiment indicates that this is not the case. Fat itself does not cause any increase of sugar in the urine, even in the most severe cases of diabetes. E. Pflüger, who has recently come to the conclusion that a source of sugar other than the carbohydrates themselves must be looked for in bad cases of glucosuria and diabetes, nevertheless holds to the opinion that fat can produce sugar. He explains the fact that fat does not cause any increased excretion of

[1] E. Pflüger calculates as a maximum 615 grams sugar. (See Glycogen, *loc. cit.* p. 537.)

[2] More recently Karl Grübe, Plüger's Arch. **118**, 1 (1907), has shown that glycerol after having been passed through the liver of a turtle caused an increase in the glycogen content of this organ.

sugar during diabetes, by the behavior of fat materials in the general metabolism. In the first place, it is brought forward that the animal organism does not regulate its consumption of energy according to the quantity of food administered; that is, the combustion in the organism cannot be increased beyond certain limits by the amount eaten. The amount of energy utilized is dependent on the work that the organs have to do. This regulates the amount of material consumed. If more food is eaten than is utilized, the excess can be stored up. If all food is withheld from an animal, it will live largely upon its store of fat. An appreciable falling off in metabolism immediately follows, which cannot be entirely recovered simply by a diet of fat. This may, however, be accomplished if a little albumin is added. Voit has also shown that fat metabolism ceases when a sufficient amount of albumin is fed to an animal. It will then live solely at the expense of albumin. This observation applies, strictly speaking, only to the carnivora. The omnivora and herbivora are unable to handle as much albumin as the metabolism requires. They consume carbohydrate and fat in proportion as the albumin alone is insufficient to fulfill all of the requirements. The quantities of carbohydrate and fat changed over are regulated by the supply of albumin at hand. "The extent of albumin metabolism is dependent on the amount of albumin presented; while fat metabolism is independent of the fat supply."[1] Fat changes are regulated chiefly by the supply of albumin, and, secondarily, the amount of carbohydrates. Every excess of fat is deposited. The reason that a diet of fat does not cause any increased elimination of sugar in a diabetic patient, is due to the fact that the fat consumed cannot go beyond prescribed limits. By feeding fats we only produce an accumulation thereof. The formation of sugar from fat is regulated exclusively by the amount of work performed by the cells which oxidize fat to sugar. In order to trace the influence of fat upon the elimination of sugar, there remains the method proposed by E. Pflüger.[2] A dog, with extirpated pancreas, is fed exclusively on albumin. If the eliminated sugar is derived from the body fat, we would expect, as soon as the animal had been freed of its fat, that the elimination of sugar would cease. Pflüger's experiments in this direction led to no definite conclusions, for his animals all died at a time when the fat supplies were about exhausted.

Now what is the origin of the sugar which the animals in the experiments of Pflüger and others always eliminated, even when all carbohydrates were withheld from them? Lüthje, to whom much credit is due for his extensive investigations regarding the production of sugar during diabetes, is of the opinion that the albumins in the food are the source of the sugar in such experiments. This brings us to the relations of the albu-

[1] E. Pflüger: Glycogen, *loc. cit.* p. 329.

[2] E. Pflüger: Pflüger's Arch. **108**, 115 (1905).

mins to the carbohydrates. Clinical experience has long since decided that albumin will produce sugar during diabetes. In no case, however, is the proof clear and free from criticism. We are compelled to state that at present we know nothing definite concerning the way sugar is formed from albumin. It has been attempted to get around this problem by placing especial stress upon the carbohydrate groups present in the proteins. We have, however, already called attention to the fact that the amount of carbohydrate groups present in proteins is of little quantitative significance. To be sure, the carbohydrate content of the different albumins from a practical standpoint will vary greatly, for we do not feed animals with "pure" albumins, but albumin in the form of meat, etc. Even then, however, if we were to assume that these albuminous substances may contain as much as 10 per cent of carbohydrates, this would not explain the appearance of the large amounts of sugar which are eliminated by diabetics. E. Pflüger himself has brought forward the best proof in this direction, by feeding dogs with codfish, which is practically free from glycogen and sugar.[1] This flesh contains 0.55 per cent fat. The following table gives a summary of such an experiment:

Period of Time, 1905.	No. of Days in Each Period.	Average Weight of the Dog in Grams.	Daily Intake of Nitrogen in Grams.	Daily Elimination of Nitrogen in Grams		Daily Average of Sugar in Grams.	Nitrogen Balance.	$\frac{D^2}{N}$
				in the Urine.	in the Fæces.			
1. Jan. 14–17 . .	4	8580	15.2	13.2	4.2	27.8	− 2.2	2.10
2. Jan. 18–30 . .	13	8350	27.9	24.6	2.5	55.9	+ 0.8	2.27
3. Jan. 31–Feb. 15	16	8300	36.1	30.8	4.1	69.8	+ 1.2	2.26
4. Feb. 16–Feb. 20	5	8200	36.8	31.1	6.3	71.2	− 0.6	2.29
5. Feb. 21–Feb. 26	6	8150	39.1	31.0	6.3	47.3	+ 1.8	1.52
6. Feb. 27–Mar. 4	6	7070	8.8	16.2	4.5	35.5	−11.9	2.20

Here the case is perfectly clear. The question to decide is: Does the eliminated sugar arise from fat or from albumin; i.e., to be more precise, from the amino acids in the albumin? Only these two classes of food-stuffs can be taken into consideration, and not the insignificant amount of carbohydrate groups. The reverse process, the production of amino acids from carbohydrates, undoubtedly takes place in plants. We have already become acquainted with the close relations existing between glycerose (as well as lactic acid, a derivative of the carbohydrates) and alanine, serine, and cysteine, and have learned incidentally that the relations between the other known albumin decomposition products to the carbohydrates still remain unknown. The plant cells could hardly be considered as producing carbohydrates from albumin. Conversely, the animal cell unques-

[1] E. Pflüger: Pflüger's Arch. 108, p. 136. [2] Cf. page 321.

tionably does not synthesize albumin from carbohydrates. It does not follow that the reverse process may not take place. We must also remember that the proteins contain large complexes, the nature of which we know but little. It is possible that more complicated hydroxy-acids, such as diamino-tri-hydroxy-dodecoic acid,[1] are present, the conversion of which into carbohydrates would be easy to understand. At all events, we must admit that the conversion of amino acids into sugars is no more difficult to understand than is the transformation of fatty acids into carbohydrates, and conversely. The formation of sugar from amino acids is connected with the question as to what becomes of the carbon of the amino acids which does not leave the organism in the form of urea. When the function of these nitrogen-free carbon chains is explained, the problem of the sugar formation of sugar from albumin will be less difficult to solve. This is the vital point of the whole question, and from this standpoint the whole subject must be considered.

We might think that feeding amino acids alone would show, in the first place, whether they have any effect upon the formation of glycogen; and secondly, whether they effect the elimination of sugar. Such experiments have, in fact, been made. Alanine and leucine, on account of their having respectively three and six carbon atoms in the molecule, seemed especially suited for the experiment, although the latter has a methyl side chain. It is well known that normal carbon chains of the carbohydrates can easily go over into branching chains,—for example, in the formation of saccharine; and so, on the other hand, we can imagine the possibility of the reverse process taking place in the transformation of leucine into a sugar. The experiments which have been performed in this direction are very contradictory, and have led to no positive conclusions,[2] although apparently the feeding of individual amino acids, in particular leucine and alanine, does not cause any accumulation of glycogen.

This does not by any means preclude the possibility of sugars being produced from amino acids. We can easily imagine that the disintegration of the amino acids from a protein proceeds in its intermediate stages in a different manner from that which takes place when we feed large amounts of the individual amino acids, as such, to the organism. We know very little as yet about the intermediate disintegration of albumin, and are unable to state whether the amino acids, as such, are set free, or whether the disintegration of the proteins does not take place after the removal

[1] E. Fischer and E. Abderhalden: Z. physiol. Chem. **42,** 540 (1904).

[2] Cf. E. Pflüger's criticism (Glycogen, *loc. cit.*). R. Cohn, Z. physiol. Chem. **28,** 211 (1899), found that feeding rabbits with leucine caused an increase of over 400 per cent of glycogen, whereas O. Simon, *ibid.* **35,** 315 (1902), found no glycogen formation to take place. Grübe (Pflüger's Arch. **118,**1 (1907)) also came to the conclusion, from experiments with the livers of turtles, that leucine and alanine did not increase the glycogen content.

of the amino groups and oxidation. We should undoubtedly be making a great mistake if we were to draw the conclusion that all such compounds are decomposed in the same way, merely because in mammals the greater part of the nitrogen from ingested protein, amino acids, and polypeptides appears in the urine. We must not forget that the formation of urea represents only one phase in the disintegration of the amino acids. It certainly does not explain the intermediate albumin metabolism.

The formation of sugar from amino acids has been regarded as a safe conclusion on account of the fact that these acids are closely related to the fatty acids, and for this reason it may seem superfluous to make such a sharp distinction between the formation of sugar from fats and from albumin. On the other hand, the objection may be raised that the amino acids which have been studied are derivatives of the lower fatty acids. If we assume that the amino groups are removed from the amino acids while they are in the tissues, thus forming fatty acids, it would be expected that an accumulation of glycogen would result on feeding animals with the fatty acids in question. L. Schwarz [1] has carried out such experiments, and found that the fatty acids administered did cause an increase in the acetone bodies eliminated, but not in the sugars.

Embden and Salomon [2] have recently investigated the influence of individual amino acids (alanine, glycocoll) of asparagine and of lactic acid on the elimination of sugar in dogs whose pancreas had been removed. They found that an increase resulted. It is possible that the sugar was formed, in this case, from the above compounds, and that this kind of experiment is more suitable for establishing the formation of sugar than the method of studying the glycogen formation. It is certain that sugar will be formed from albumin or its amino acids only in proportion as sugar is needed by the organism. Further experiments will be necessary to determine whether the administered amino acids have acted in a direct or indirect manner.

The experiments with the amino acids themselves, therefore, are not as yet such as lead to definite conclusions. We are thus brought back to our former question: Is sugar produced from albumin itself? Claude Bernard made experiments on the formation of glycogen after feeding albumin. He showed that a dog, which had been fed for months only on meat, contained large amounts of glycogen in its liver. He also raised fly-maggots upon boiled egg-albumin or extracted meat, and found large amounts of glycogen.[3] E. Külz [4] repeated these last experiments. He divided 72 fresh eggs of *Musca vomitoria* into two equal groups, and

[1] L. Schwarz: Arch. klin. Med. **76** (1903).
[2] G. Embden and H. Salomon: Hofmeister's Beitr. **6**, 63 (1904); *ibid.* **6**, 507 (1904).
[3] C. Bernard: Leçons sur le Diabète, p. 464 (1877).
[4] E. Külz: Pflüger's Arch. **24**, 71 (1881).

immediately determined the amount of glycogen in one of these, while the remainder were cultivated upon the albumin from hens' eggs. He could not observe any formation of glycogen in this case, although positive results were obtained when the maggots were nourished with meat. These experiments do not furnish a direct proof that sugar is formed from albumin. We know now that neither the albumin from hens' eggs nor from meat is free from sugar. It is possible that the glycogen produced by the maggots results from the sugar in the food. Numerous feeding experiments with animals fed exclusively on albumin in the form of meat, etc., have, without exception, led to the conclusion that glycogen is produced from albumin.[1] It would take too long to dwell upon all of these experiments. Many of them can be thrown out because there was sufficient carbohydrate present in the food to account for the glycogen found. On the other hand, in many cases it was not shown that the animals undergoing experiment contained no glycogen at the beginning of the test. Finally, we must add that improvements in the methods of estimating glycogen have shown the unreliability of values formerly obtained. Thus, 8.5 grams of glycogen was assumed as an average content per kilogram weight of a dog. Pflüger increased this value to 11 grams, while to-day we regard 41 grams as more nearly correct.

It will be sufficient if we compare the two series of experiments of E. Pflüger and H. Lüthje,[2] which are free from criticism. We have already given Pflüger's figures, and will add merely that he in one experiment, for example, found the following balance:

Total sugar formed	3097.1 grams
Explainable as residual glycogen	422.3 grams
Sugar formed from other sources	2674.8 grams

This result corresponds with that of Lüthje, and proves positively that sugar must have been formed from some other source than carbohydrates. Lüthje also fed casein to a dog whose pancreas had been removed. The animal weighed 5.8 kilograms. It eliminated 1176.7 grams of sugar between October 2 and November 24. E. Pflüger [3] subtracts 650.6 grams from this as being due to sugar present in the food and from the glycogen originally present in the body. It must be said that this value subtracted by Pflüger is, if anything, too high rather than too low.[4] Thus, at least,

[1] Cf. E. Pflüger: Glycogen, *loc. cit.* p. 240, etc.

[2] H. Lüthje: *loc. cit.* Deut. Arch. klin. Med. **79,** 4999 (1904). Pflüger's Arch. **106,** 160 (1904).

[3] E. Pflüger: Pflüger's Arch. **106,** 168 (1904).

[4] E. Pflüger calculates 109 grams sugar from 328 grams of serum-albumin, basing this on the values obtained from mucin by F. Müller. Serum-albumin and serum-globulin contain, at the most, 2 per cent sugar. If we take out 7 grams sugar as due to the serum-albumin, we would undoubtedly be placing the sugar value too high rather than too low. Lüthje administered 4100 cubic centimeters serum. Since 1000

526 grams of sugar remain unaccounted for. Lüthje assumes that this means that albumin forms sugar. This assumption of Lüthje corresponds to the fact that the elimination of nitrogen, and likewise that of sugar, increases uniformly when the administration of albumin is increased. The ratio of the sugar (dextrose) eliminated to that of the nitrogen in the urine is usually designated by the symbol $\frac{D}{N}$.

This ratio is assumed to have a constant value, namely, 2.8.[1] E. Pflüger calls attention to the fact that the value is not as constant as is generally supposed, but that very appreciable deviations may arise. Thus, in some of his experiments, Pflüger found the value to be less than 1, and in other cases it rose as high as 14.6. Furthermore, it must be remembered that every increase in the amount of albumin administered results in the saving of a corresponding amount of fat and carbohydrate. Every addition of albumin lessens the extent to which carbohydrates are oxidized. Now diabetics, as well as animals afflicted with glucosuria, have not lost all of their ability to consume sugar. Some work is performed in both cases at the expense of energy present in sugar. If now albumin be fed in large amounts, this can be burned up in place of the carbohydrates formerly required. The result of this will be that more unconsumed sugar circulates in the blood, and is, therefore, eliminated. This also would account for the parallel elimination of sugar and of nitrogen.[2]

If we consider all that we know with regard to the formation of sugar from substances other than carbohydrates, we arrive at the conclusion that it is at present impossible to decide whether fats or proteins must be drawn upon as a further source of supply. We know merely that sugar can be produced from one of these two classes of compounds. From a chemical standpoint, the transformation of fatty acids into sugar is just as complicated as is that from the amino acids; yes, we may say, that the conversion of the oxyamino acids into carbohydrates is easier to understand, because glucosamine may be regarded as a compound intermediate between the two latter groups. On the other hand, we must not forget that a very large part of the albumin molecule is composed of simple amino acids.

The question as to whether sugar is produced from fats or from albumins is probably an unnecessary one. We can see no particular reason why both should not be utilized in this way, according to the conditions. Such an assumption would explain the observed irregularities, and enable us to understand why the quotient $\frac{D}{N}$ should at one time be less than 1, and

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grams serum would not have over 1.5 grams sugar in a free form, 4100 cubic centimeters must contain less than 6.15 grams.

[1] O. Minkowski: Arch. exp. Path. Pharm. **31**, 85 and 97 (1892).

[2] E. Pflüger: Glycogen, *loc. cit.* p. 325. Cf. also Lecture I, pp. 6 and 7.

again more than 2.8. There is apparently no reason why we should say that either the fats or albumins do not produce sugar; and when we find one author regards fats, and another albumin, as the sole producer of sugar other than carbohydrates, it only means that there is no direct proof of a formation of sugar from either one of these substances, but there is merely an indirect proof of their influence. Too much depends upon the interpretation of the results. Whether sugar is formed normally from fat or albumin has not been proved by any of the experiments. It is possible that the organism of the diabetic and that of the dog suffering from glucosuria, may behave in entirely different ways.

We must consider the possibility that the glucosuria produced by different causes may have different effects. We do not know the organs in which the transformation of albumin or fat into sugar takes place. This does not preclude the possibility that the process is carried on for both substances in the same place, nor that the transformation of one material is more affected than another in any given case of glucosuria. An observation of G. Rosenfeld [1] may have a bearing on this subject. He caused a dog weighing between 3 and 5 kilograms to fast for five days, and then on the sixth and seventh days injected 2 or 3 grams of phloridzin. Carbohydrates were fed to the dog at the same time. The dog was killed on the eighth day. The liver showed no indication of fat infiltration. If, on the other hand, there is no carbohydrate in the food, but fat, or nothing at all, is fed, a decidedly fatty liver is obtained. While the amount of fat in the liver of a fasting dog is about 10 per cent of the dry substance, the livers of animals used for the last experiment have shown from 25 to 75 per cent of fat. The glycogen had shrunk to small proportions. The fatty liver disappeared two days after the injection of the phloridzin. In these cases, as was shown by microscopic examination, the fat was not deposited in the connective tissues, but directly in the liver cells. Here it is a transference of fat from other organs of the body, as was shown by Rosenfeld, and not a transformation of glucose or albumin into fat.

It is possible that this phenomenon has some connection with the conversion of fat into sugar, although it need not apply to glucosuria due to other causes. No fatty liver results, for example, in glucosuria caused by extirpation of the pancreas. It, therefore, appears, *a priori*, wrong, to place the various forms of glucosuria and diabetes upon the same basis, simply from the fact that they have in common the predominating symptom of glucohemia and the resulting glucosuria. The widely different causes of the different kinds of glucohemia cannot be sufficiently emphasized. It is possible that a study of an individual case in different directions, rather

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[1] G. Rosenfeld: Verh. Kong. innere Med. 359 (1893).

than following solely the elimination of sugar, would more likely lead to a solution of the problem.[1]

In this connection, the question arises whether all carbohydrates are able to form glycogen. We have already seen that glucose and fructose are glycogen-formers. We also know that milk-sugar and cane-sugar,[2] on being introduced into the blood, are eliminated unchanged in the urine. Cane-sugar is normally disintegrated in the intestine. The usefulness of milk-sugar is evidently dependent on the presence of lactase, as E. Weinland [3] has shown. Glycogen does not seem to be formed from pentoses.[4] There is a large amount of uncertainty concerning these experiments on account of the fact that a compound which causes an accumulation of glycogen, need not necessarily itself participate in its production. The combustion of the substance may indirectly shield, for example, glucose from oxidation, thus causing deposition of glycogen. Although this objection may seem uncalled for, there is much in its favor. Perhaps only those sugars are glycogen-formers which are capable of going over into glucose; for grape-sugar is, probably, the only building material of glycogen. All of those compounds which can be changed into it, must be looked upon as producers of glycogen.

We now reach the problem as to the relations of albumins to fats. Is albumin converted into fat? There is, *a priori,* no reason why such a change should be impossible. We know that the nitrogen in urea only carries with it a part of the carbon present in the albumin, while the larger part of the carbon in albumin is transformed in the body in some other way. It is conceivable that these other carbon chains may be deposited in the form of fat under certain conditions, in which case a direct fat accumulation might result from a diet of albumin. The assumption of the formation of fats from albumin would be of considerable value with reference to the production of sugar from albumin or fat, for those intermediate compounds which lead to the synthesis of fat from albumin may also be closely related to the formation of sugar. We should, as a rule, be rather cautious in assuming such complicated changes, especially in view of the rapid progress of albumin metabolism. On the other hand, the old ban, which was so long placed on the animal cell as not being in any manner capable of effecting a synthesis, will gradually have to be withdrawn. It is desirable, therefore, to establish more proof from other points of view.

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[1] One might expect the problem to be solved by following the respiratory exchange. Unfortunately there are no convincing investigations at hand. Cf. E. Pflüger: Pflüger's Arch. **108,** 473 (1905); Magnus Levy: Z. klin. Med. **56,** 83 (1905).

[2] F. Voit: Deut. Arch. klin. Med. **58,** 523 (1897).

[3] E. Weinland: Z. Biol. **38,** 16 and 606 (1899); **40,** 386 (1900). Cf. also R. H. A. Plimmer: J. Physiol. **34,** 93 (1906).

[4] E. Salkowski: Z. med. Wiss. **11** (1893). Z. physiol. Chem. **32,** 393 (1901).

The conversion of albumin into fat was for a long time looked upon as an established fact, albumin being even considered as the main source of the body fat. This conception originated with Voit and Pettenkofer,[1] as an outcome of their experiments on metabolism. Since the time when E. Pflüger[2] critically examined the values given by the above authors, the belief in the production of fat from albumin has been more and more doubted, this being especially true since many observations, undertaken in order to show such effects, have indicated that the above conclusions were erroneous. Pettenkofer and Voit fed dogs with meat as free as possible from fat. They found all of the nitrogen of the ingested albumin present in the excretions, but only a part of the carbon. It was natural to conclude from this, as we have already indicated, that the portion of carbon which did not leave the organism in combination with the nitrogen, was utilized for the production of fat. Pettenkofer and Voit reached this conclusion owing to the fact that they had assumed 1 : 3.68 to be the relation of nitrogen to carbon in meat free from fat. Pflüger, after allowing for glycogen, reduces this value to 3.22, while Rubner places it at 3.28. If we apply these changed values to the results of Pettenkofer and Voit, we find that the assumption that fat is produced from albumin no longer has any support.

M. Kumagawa[3] has recently tried to solve the problem in the following manner. He caused two dogs from the same litter to fast for 24 days. One of the animals was then killed and analyzed. The second dog for quite a long period was fed a liberal supply of horse meat (49 kilograms in 49 days). The body weight rose from 6.08 to 10 kilograms. The fat content of this animal at the beginning of the experiment must have been about 120 grams. The other dog contained this amount. The animal under experiment showed 1087.7 grams of fat on being killed. The meat fed to this dog, however, contained 356 grams of glycogen and 1084 grams of fat. The amount of fat in the food, therefore, would of itself have been sufficient to cause this increase.

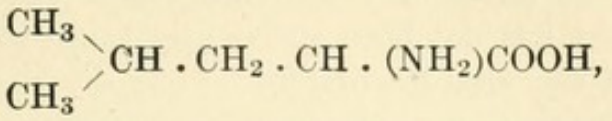
Although we must admit that no satisfactory proof has been obtained by experiments on metabolism as regards the transformation of albumin into fat, we must not forget, on the other hand, that these results merely give us a rough idea of the whole interchange of material, but never the finer details of cell activity. It is still a very remarkable fact that such a small portion of the carbon in albumin should leave the organism in com-

[1] M. Pettenkofer and C. Voit: Ann. Supp. 57, 361 (1862). C. Voit: Z. Biol. **5**, 106 (1869); **6**, 371 (1870); **7**, 433 (1871). Handbuch der Physiologie des Gesamtstoffwechsels und der Fortpflanzung, Leipsic, **1881**. Ueber die Ursachen der Fettablagerung im Tierkörper, Munich, **1883**.

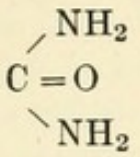
[2] E. Pflüger: Pflüger's Arch. **50**, 98 (330 and 396) (1891); **51**, 229 (1892); **52**, 1 (1892); **68**, 176 (1897); **77**, 521 (1899).

[3] M. Kumagawa: Communication from the University of Tokio (1890).

bination with the relatively large amount of nitrogen. This must certainly possess some deep significance. Let us consider leucine:



and urea:



The nitrogen united to C = O in the latter compound, is derived from two molecules of monoamino acid. What becomes of all of the rest of the carbon chain? We know nothing about this nitrogen-free residue of the amino acids. It is possible that it is immediately consumed. This has never been satisfactorily proved. On the other hand, we can understand the fact that nitrogen leaves the organism in the form of urea, from the standpoint that the organism has utilized the energy of the albumin to the fullest possible extent. An elimination of carbon chains containing nitrogen would indicate an incomplete combustion of available material. The fact that the disintegration of the albumin in this manner is a very economical one,—a loss of energy always occurs in the urea itself,—does not permit us to form any opinion regarding the utilization of the remainder of the carbon. We call attention to these relations, because the whole question of the relation of albumins to fats and carbohydrates is dependent upon this, and we wish to emphasize the point that the assumption of the albumin being rapidly consumed *in toto*, is not sufficiently supported by the facts.

Let us consider those investigations and observations which are held to prove that fat may be produced from albumin. We must state, at the start, that a large number of such investigations are worthless, owing to the fact that the methods employed for estimating the fat do not give any idea as to the actual fat content of any individual organ. The fat in the organs is evidently present in different forms. A part of this may easily be extracted by ether. If we examine a piece of tissue which has been freed of fat in this manner, we obtain the impression that all of the fat has been removed. This is, however, not the case; for if we digest the residue with pepsin-hydrochloric acid, or boil it with 2 per cent hydrochloric acid, we can extract some more fat with ether. Instead of using ether for extraction purposes, it has been recommended to employ alcohol and chloroform alternately.[1] The fact that the fat is not entirely extracted

[1] C. Dormeyer: Pflüger's Arch. **65,** 90 (1897). J. Nerking: *ibid.* **73,** 172 (1898); **85,** 330 (1901). O. Frank: Z. Biol. **35,** 549 (1897). E. Voit: *ibid.* **35,** 555 (1898). E. Bogdanow: Pflüger's Arch. **86,** 389 (1897). G. Rosenfeld: Z. in. Med. **33** (1900).

by the ether, is partly because some of the fat is inclosed in the cells, while another portion is undoubtedly present in combination with other substances. In many of the investigations dealing with this problem, this latter fact has not been sufficiently taken into consideration in the estimation of the total fat present.

The old observation of the formation of grave-wax, or adipocere, has been brought forward as evidence of the production of fats from albumin.[1] By this process we understand the production of a wax-like mass from a corpse. The albumin disappears little by little from the muscles, and fat takes its place. This characteristic change is especially noticeable in moist burying-places, where a slow decomposition proceeds in the presence of a minimum supply of oxygen. By carefully following this process, and especially by direct experiments, it has been proved that there is no such conversion of albumin into fat, but that the fat already present in the body is responsible for the production of adipocere. This is mainly due to the fat present already in a given locality, and to such infiltrated fatty masses as may be deposited there by the water. Moreover, if it had been proved that the fat arose from albumin in this process, it would possess no bearing on the production of fat from albumin in the living animal organism. It would be conceivable that lower organisms, such as bacteria, etc., are capable of effecting such conversions. The formation of fat from albumin during the ripening of cheese, for example, is attributed to the interaction of fungi.[2]

Hoffmann,[3] however, has carried out a notable investigation, which is considered a proof of the transformation of albumin into fat. He collected the eggs of flies, and determined the amount of fat present in a number of them, while the others were cultivated upon defebrinated blood. This contained 0.032 per cent of fat, and the fly-eggs 4.9 per cent. The maggots cultivated upon the blood finally showed at least 10 times as much fat as was present in the eggs and blood. Two objections can be raised, for, in the first place, the method used for estimating the fat is not entirely above criticism, while, in the second place, this conversion of albumin into fat may have been brought about by means of bacteria, which developed rapidly in the blood. O. Frank,[4] who repeated the experiment, using meat,

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[1] Cf. J. Kratter: Z. Biol. **16,** 455 (1880). Erman: Vierteljahresschrift gericht Med. **37,** 51 (1882). E. Salkowski: Festschrift für Virchow's Jubiläum, p. 23 (1891). K. B. Lehmann: Sitzber. physikal. med. Gesel. Würzburg (1888). E. Voit: Sitzber. Gesel. Morph. und Physiol. München, **4,** 50 (1888). F. Kraus: Arch. exp. Path. Pharmak. **22,** 174 (1887).

[2] K. Windisch: Arbeiten aus dem Kaiserl. Gesundheitsamte, **17** (1900). H. Jacobsthal: Pflüger's Arch. **54,** 584 (1893).

[3] F. Hofmann: Z. Biol. **8,** 153 (1872).

[4] O. Frank: *loc. cit.* Z. Biol. **35,** 549 (1897).

which had been freed as much as possible from fat, could not obtain any definite results. The fat formed may very easily have been derived from the fat of the meat. These experiments also are not suitable to establish the fact that fat is produced from albumin.

Finally, it may be mentioned that the fat contents of the secretions, those of the lacteal and sebaceous glands, have repeatedly been referred to albumin as their source. Direct experiments do not confirm this assumption. It is also difficult to decide this question, owing to the fact that the animal organism has stores of fat at its disposal, and is not dependent on the fat in the food. Moreover, the conversion of carbohydrates into fat must be taken into consideration.

Now we happen to know of a number of processes in which organs, containing, as far as the eye can see, hardly any fat at all, are suddenly permeated with it. This is especially noticeable in the case of the liver. We have already met with an infiltration of fat in this organ. We have seen, that, as the glycogen disappears after glucosuria has been brought about by phloridzin, fat will take its place, provided, of course, that no carbohydrates are present in the food. We have here a physiological infiltration of fat. It is especially noteworthy owing to the fact that it disappears again on discontinuing the poisoning by phloridzin. Normal liver cells, capable of performing their functions, will remain.

We know of a large number of poisons, such as phosphorus, arsenic, antimony, chloroform, alcohol, English oil of pennyroyal, etc., all of which are capable of causing a local accumulation of fat. Finally, pathologists are well acquainted with the appearance of a "fatty degeneration," which follows various disease processes (influence of toxins, etc.). We can very easily imagine that the appearance of acute accumulations of fat in places where we would hardly expect to find any, would lead to the conclusion that some substance had been changed into fat at that place. As the body cells are generally composed of albumin, it seemed to prove that a transformation of albumin into fat had taken place. This conception remained for a long time undisputed, until it was shown both macroscopically and microscopically, that this fat could have no connection with the true fat content of an organ. A tissue apparently free from fat may yet contain as much as 20 per cent, and an organ seemingly laden with fat may actually possess less than an apparently fat-free tissue. It was necessary first to trace the formation of fat by exact quantitative methods. A great advance was also made in the discovery that it was not sufficient to determine the fat content of any specific organ, but that the amount of fat in the entire animal must be taken into consideration. We can pass over the earlier experiments in this direction, which were characterized by questionable methods for estimating the fat, and confine ourselves to the more recent work.

Athanasiu [1] determined the fat content of 124 frogs. He then poisoned a like number of animals with phosphorus, and again estimated the amount of fat in the entire collection. He found no increase in the total quantity of fat. Taylor,[2] in fact, observed an actual decrease.

Experiments with mice gave the same results,[3] whereas control animals, which were fed in the same manner, showed from 13.8 to 29.3 per cent; those which had been poisoned with phosphorus gave only 4.13 to 7.9 per cent of fat. The organism had, therefore, been deprived of fat. The livers of those mice which had been poisoned with phosphorus showed from 7.4 to 37.4 per cent, while this organ in normal mice gave only 5.1 to 11.8 per cent of fat. All of the other tissues had lost fat while the liver had gained. From this it is easy to assume that the increased fat content of the liver must stand in direct relation to the diminution in the fat supply of the remaining tissues. Rosenfeld [4] has proved this to be so by direct experiment. He observed fat, from a deposit in a dog which had been fed with mutton tallow, to migrate directly into the liver, and the composition of this fat was that of the fat deposited in the dog's own tissues. He also showed that fasting dogs and hens after being poisoned with phosphorus gave no indication of any increase of body fat. Their fat deposits had already been drained. Thus, the old idea that albumin is converted into fat after poisoning with phosphorus must be discarded.

We have already called attention to the fact that the cleavage-products of albumin, such as tyrosine, leucine, glycocoll, etc., appear in the urine after phosphorus poisoning. This circumstance seems to support the old idea that albumin goes over into fat. In fact, a superficial observation could easily lead to that conclusion. Albumin is decomposed, while fat appears in its place. The decomposition of the albumin, however, may take place entirely independently of the fat infiltration. It is clear, on the one hand, that the great derangement in metabolism caused by phosphorus poisoning also affects the disintegration of the albumins, and causes destruction of albumin; while the observed amounts of amino acids, on the other hand, have no relation to the amount of fat present. Athanasiu could not observe any increased disintegration of albumin, although a noticeable infiltration of fat had taken place in the liver. The fattening of other organs, the muscles, heart, etc., can be explained in the same manner, while the infiltrations of fat due to other harmful influences in no case point to a formation of fat from albumin. In

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[1] J. Athanasiu. Pflüger's Arch. **74**, 511 (1899).

[2] Taylor: J. exp. Med. **4**, 399 (1899).

[3] F. Kraus and A. Sommer: Hofmeister's Beitr. **2**, 86 (1902).

[4] G. Rosenfeld: Verhandl. Kong. in. Med. (1894). Allgem. med. Zent. No. 60 (1897); No. 89 (1900). Cf. Fettbildung, Part II. Ergebnisse Physiol. (Asher and Spiro), Bergmann, Wiesbaden **2** (1903), p. 50 *et seq.*

every case there is enough fat already present to explain these fat accumulations.

Considering all the results of physiological and pathological experiments with regard to the formation of fat from albumin, we must admit that up to the present time no proof has been found which compels us to assume a transformation of albumin into fat. We must not neglect to remark that, although the fat present in the body is sufficient to explain the accumulations of fat, still, this fact does not preclude the possibility of its production from some other substance. We arrive at the above indirect conclusion as it seems most probable to us. We would, however, be making a grave error were we to consider the problem of the production of fat from albumin as finally solved. Our comments only go to show that the experiments and methods so far utilized are insufficient to confirm such transformation. New questions and new points of view are necessary for this problem.

We have now reached the end of our observations concerning the conversion of one substance into another. In the animal organism we are absolutely certain only of the production of fat from the carbohydrates. The reverse process, as well as that of the production of sugar from albumin, has not yet been sufficiently demonstrated to warrant any definite decision. At any rate, it will be necessary to look for some other source than the carbohydrates for an explanation of the elimination of sugar during severe cases of glucohemia. We have developed the assumption that both fat and albumin must be taken into consideration, and that the great differences of causes of glucohemia may correspond to different origins of sugar. Finally, we have seen that there is no absolute proof that the formation of fat is at all related to the presence of albumin.

We have intentionally avoided giving any definite opinion on these important questions, preferring to take the opposite stand corresponding to uncertainty of the facts in hand. Nothing can hurt the progress of knowledge more than the desire to reach conclusions on such complicated questions from purely theoretical considerations. We are greatly indebted to E. Pflüger, whose critical observations have been followed in every case where it seemed as if the question had been positively answered. He has considered all the previous experiments, and has to a certain extent repeated them.

The relations are evidently entirely different in the plant. It must manufacture its carbohydrate, fat and albumin, all from the same raw materials. It easily converts carbohydrates into fats, and fats into carbohydrates. It also undoubtedly synthesizes its albumin from sugar and its derivatives. The future can alone decide whether there is any marked fundamental difference between the activities of the plant and animal cells, or whether any difference between them may be only quan-

titative rather than qualitative. At any rate, the fact that the animal cells convert carbohydrates into fat indicates that they possess the same functions as do the plant cells. The transformation, whether it be from fat or from albumin into carbohydrate, is a very complicated process, and it is safe to assume that the animal cell is far more efficient than we have hitherto imagined. We shall have to take such relations more into account and synthetic processes will receive more attention in the future. It is only by reason of far-reaching changes, through disintegrations and reconstruction, that we are able to understand why every species of animal, in fact, every individual, should possess a specific composition in spite of the fact that each may have had the same food.[1] This alone is sufficient to warrant us in assuming complicated synthetic processes as taking place in the animal cell.

[1] Cf. Lecture XXIX.

LECTURE XV.

MUTUAL RELATION BETWEEN FATS, CARBOHYDRATES, AND ALBUMINS.

II.

The Law of Isodynamics.

So far we have considered only the most important organic nutrients from one point of view, namely, their chemical composition, and sought out those facts which led to the assumption that one class of substances goes over into another in the animal organism. In such cases extensive chemical changes take place — reduction, oxidation, analysis, and synthesis — before one substance can replace another. This, however, is not the only way in which one substance may appear in place of another. The substitution may be a purely physical one; i.e., the energy imparted to the body by the substances in question may be the most important factor. In other words, we can conceive that the different organs, e.g., the muscles, do not work with one single food material alone in performing their prescribed functions, but with representatives of all three groups of nutrients. We could indeed imagine, as we have already mentioned,[1] that every individual cell in the body is so adjusted that it works only with a definite material. We would then have to assume that the substitution of one of these combustible substances by another must be preceded by a transformation into the former. When we recall the facility with which the vegetable organism converts carbohydrates into fats, and fats or albumin, or both together into sugars, such a conception would, *a priori*, not seem so improbable. On the other hand, in such a case the organism evidently would not work economically, if it were called upon first of all to produce deep-seated and far-reaching transformations, before it could utilize the food materials. The entire metabolism would thus resolve itself into an extremely complicated process, and such transformations would make themselves felt especially in the case of a restricted diet with a definite kind of food, e.g., of fats, which are deficient in oxygen, or of carbohydrates, which are rich in oxygen. One might cite the diabetic as proof of the fact that an organ can perform its work with the most varied

[1] Cf. Lecture XIV, p. 311.

kinds of food, for the diabetic can perform muscular work, even though he is not able, in proportion to the severity of the disease, to utilize to advantage the most important source of energy, the carbohydrates. For him the carbohydrates scarcely come into consideration as a source of energy. Evidently the diabetic performs his work at the expense of other food material. We cannot look upon this proof as entirely convincing, for it is precisely those observations dealing with diabetics which have led to the assumption, that sugar is produced from the two other organic nutrients: albumin and fat. Why does the diabetic patient produce sugar from these? Why does he not utilize these according to their calorific value? Why does he carry out the extremely complicated chemical changes, some of which are so difficult to understand? Certainly not in order to eliminate more sugar. These transformations must have a deeper significance. Is this a normal process which comes to light because of an insufficient utilization of the products formed, or are we to look upon the production of sugar from fat and albumin as a derangement of the entire metabolism? These are enigmas, the solution of which still reaches into the far-distant future. At all events, we repeat once again, nowhere else does the question appear as vital as here, as to whether specific atomic groupings are not normally utilized for definite purposes. Nothing is of greater promise in the entire field of biological investigation than the clearing up of just such uncertainties and contradictions. Such work must lead to new problems and new results. We have, on the one hand, the claim that large amounts of sugar arise from fats and proteins, while, on the other hand, there are numerous exact statements which make it seem improbable that such conversions are, as a rule, necessary in normal metabolism for the accomplishment of definite work.

If we, for the moment, leave out of consideration the significance of the organic nutrients as building material for the worn-out or new cells, we find that their most important function is that of a source of energy. Chemical energy is transmitted to the animal organism by means of the food. Mechanical work is performed by the transformation of this energy. Only a part of the energy is utilized in this way. Another part, and in fact a very considerable one, is changed into heat. The animal cell can utilize these sources of energy in two ways: first, by cleavage, and second, by oxidation. Only a portion of the energy can be transformed into kinetic energy by the former method; oxidation alone furnishing the possibility for a complete utilization of the energy. Now the different organic food-stuffs (carbohydrates, fats, and albumins) possess different amounts of energy; i.e., they have different fuel values. The amount of energy possessed by the various food materials can be determined by the quantity of heat which they liberate when undergoing combustion. This is generally expressed in calories, a small calorie (cal.)

being that amount of heat required to raise 1 gram of water from 0° to 1° C. A large calorie (Cal.) is the amount of heat necessary to raise 1 kilogram of water from 0° to 1° C. We shall use the large calorie in every case to express the heat values of individual food materials.

Complete oxidation of one gram of each of the following articles of food in a calorimetric bomb gave the following values:

	Calories
Casein	5.86
Egg-albumin	5.74
Conglutin	5.48
Average value for protein	5.71
Animal tissue-fat	9.50
Butter-fat	9.23
Cane-sugar	3.96
Milk-sugar	3.95
Glucose	3.74
Starch	4.19

The values given for the carbohydrates and fats represent exactly the amount of heat which is liberated during combustion in the animal organism. The animal cells likewise oxidize the carbohydrates and fats to carbon dioxide and water. The physiological heat value of the fats is generally given as 9.3 calories, and of the carbohydrates 4.1 calories for each gram of substance. The values in the table do not apply to the albumins as oxidized in the living organism. The animal cell does not utilize completely the energy present in albumin. A portion of this energy goes to waste, usually in the form of urea. We are indebted to Rubner[1] for an exact estimation of the physiological heat value of albumin. He fed a dog exclusively on washed meat, whose heat value had been carefully determined. From this he subtracted the heat values of the urine and fæces, as well as that necessary for the swelling of the albuminous material and for dissolving the urea. In like manner Rubner determined the heat of combustion of the decomposed albumin in the body of a fasting rabbit. He found the following values for the physiological heat of combustion for each gram albumin:

One gram of dry substance —	Calories
Albumin from meat	4.4
Lean meat	4.0
Albumin during fasting	3.8

The physiological heat of combustion is not identical for the different proteins. The normal value for animal albumin is estimated as 4.23

[1] Max Rubner: Z. Biol. **21**, 250 and 337 (1885). Berthelot and Vielle: Compt. rend. **102**, 1284 (1886). Berthelot and Recoura: *ibid.* **104**, 875, 1571 (1887); Berthelot and André: **110**, 884 and 925 (1890). F. Stohmann: Z. Biol. **31**, 364 (1895).

calories; 3.99 for vegetable albumin; and 4.1 as an average value for proteins as a class.

Before discussing the significance of these figures, we must consider whether the law of the conservation of energy [1] applies entirely to the animal organism. We have seen that plants, with the assistance of the kinetic energy of the sun's rays, are able to liberate oxygen from water and carbon dioxide. They use up kinetic energy and form potential energy.[2] The reverse process takes place in the animal organism. In it the oxygen unites with the compounds poor in oxygen, the end products being water and carbon dioxide again. This applies, at least as indicated above, to the fats and carbohydrates. Potential energy is utilized and kinetic energy takes its place. This appears partly in the form of heat, partly as mechanical work. We may expect that the sum of the energies of the metabolized food materials will be exactly equivalent to the energy produced by the animal organism.

The first experiment in this direction was carried out by Lavoisier,[3] as early as 1780, with, to be sure, rather primitive methods. Neither he nor the two later investigators Despretz [4] and Dulong [5] were able to establish a satisfactory agreement between the amounts of energy received and that produced. We owe to Max Rubner [6] the first exact proof of this relation, while more recently W. O. Atwater [7] has repeated the experiments, eliminating all sources of error. Atwater compared the amounts of potential energy in the substances which were actually oxidized in the body with the amount of kinetic energy evolved by the latter. This appears in the form of heat in the rest experiments, and as heat and muscular work in the work experiments. Even in the latter case this was measured in heat units. The following table gives a summary of some of Atwater's results, the experimental details of which we shall discuss in another place.[8]

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[1] Cf. R. Mayer: Die Mechanik der Wärme. Stuttgart 1867 (2d ed. 1874); Die Erhaltung der Energie. Berlin, 1889.

[2] This, of course, only applies to the principal activity of the parts of the plants containing chlorophyll. These, also, require oxygen and give off carbon dioxide (Cf. Lecture IV).

[3] Lavoisier et de la Place: Mem. Acad. roy. sciences, p. 355 (1780).

[4] Despretz: Ann. chim. phys. **27,** 337 (1824).

[5] Dulong: *ibid.* (3) **1,** 440 (1841).

[6] Max Rubner: Z. Biol. **30,** 73 (1894).

[7] W. Atwater: Ergeb. Physiol. (Asher and Spiro) Jg. III, 1 Abt. p. 497 (1904).

[8] See Lecture XXVII.

COMPARISON OF INCOME AND OUTGO OF ENERGY IN 45 EXPERIMENTS ON METABOLISM, COVERING 143 DAYS. AVERAGE AMOUNTS PER DAY.

Subject and Kind of Experiment.	Number of Experimental Days.	Net Income (Potential Energy of Material Oxidized in Body).	Net Outgo (Kinetic Energy Given off from Body).	Difference (in Terms of Net Income).	
		Calories.		Calories.	Per cent.
Ordinary Diet.					
Rest experiments:					
7 experiments with E. O. . .	25	2268	2259	− 9	−0.4
1 experiment with A. W. S. .	3	2304	2279	−25	−1.1
3 experiments with J. F. S. .	9	2118	2136	+18	+0.8
1 experiment with J. C. W. .	4	2357	2397	+40	+1.7
Average for experiments . .	41	2246	2246	0	0
Work experiments:					
2 experiments with E. O. . .	8	3865	3829	−36	−0.9
4 experiments with J. F. S. .	12	3539	3540	+ 1	0
14 experiments with J. C. W. .	46	5120	5120	0	0
Average for 20 experiments .	66	4682	4676	− 6	−.01
Average for all rest and work experiments with ordinary diet	107	3748	3745	− 3	−0.1
Special Diet.					
Rest experiments:					
6 experiments with E. O. . .	17	2313	2319	+ 6	+0.3
3 experiments with A. W. S. .	6	2308	2356	+48	+2.1
1 experiment with J. F. S. . .	3	2124	2123	− 1	0
Average for 10 experiments .	26	2290	2305	+15	+0.7
Work experiments:					
1 experiment with E. O. . .	4	3922	3928	+ 6	+0.2
2 experiments with J. F. S. .	6	3583	3552	−31	−0.9
Average for 3 experiments . .	10	3719	3702	−17	−0.5
Average for all rest and work experiments with special diet	36	2687	2695	+ 8	+0.3
Average for all the above experiments	143	3481	3481	0	0

As the values show, it is evident that the law of the conservation of energy holds with surprising exactness for the whole animal organism. It was found possible to obtain such a close agreement between the sum of the amounts of energy introduced into the body and that produced by combustion within the organism, only by extending the experiment through quite a number of days.

This brings us to the important question as to whether the chemical energies introduced into the body by the individual nutrients are all equivalent, or, in other words, whether it is immaterial in what form the animal organism receives its chemical energy. After considerable investigation Max Rubner[1] was able to show that the different organic nutrients could replace one another in amounts corresponding approximately to their relative heat values. This principle comprises the Law of Isodynamics. According to this law we can represent each substance used as food in a common unit and give it a calorific value. Thus, for example, 100 grams of fat are isodynamic with the following weights of

	As Determined by Experiments with Animals.	Heat of Combustion by Calorimeter.
Syntonin	225	213
Dry meat	243	235
Starch	232	229
Cane-sugar	234	235
Grape-sugar	256	255

Strictly speaking, the Law of Isodynamics only applies to fats and carbohydrates. It fails with the albumins. These, to a certain extent, are absolutely necessary for the animal organism. It is indeed possible to keep a dog alive for a long time on albumin alone; i.e., the albumin itself may be looked upon as isodynamic with the fats and carbohydrates. It is, however, as we shall see later on, impossible to nourish a dog on fats and carbohydrates exclusively, even when more than sufficient calorific units are provided. Starvation metabolism begins in the absence of albuminous material; i.e., the animal draws on its own body albumin, for which it has no substitute.

Studies on metabolism have shown how much nourishment is required for the maintenance of a definite organism, and how to express this requirement in terms of calories. We shall consider these relations more in detail later. Here we shall only state, that the exact formulation of the total metabolism obtained by considering the foods solely as combustible material, although of great importance for the entire conception of meta-

[1] Cf. Max Rubner: Die Gesetze des Energieverbrauches bei der Ernährung. Franz Deuticke, Leipsic and Vienna, **1902**.

bolism, by no means tells the whole story. The calorific values serve merely as a skeleton, and give us an outline of the changes which take place in metabolism. These changes are always to be traced back to the individual cells. It is not the foods as such which determine in general the metabolism, but the cells themselves. These, naturally, require a certain amount of energy. We shall see later on, that metabolism varies in different individuals, and that the consumption of material, to a large extent, is regulated by the functional activity of the separate organs. The same work — e.g., a definite amount of muscular work — will require a greater quantity of energy the first time it is performed than when it is repeated. By practice, the organism adapts itself to its requirements. It learns how to perform a given amount of work with the least expenditure of energy. We must call attention even here to this fact in order to show that experiments in metabolism, and especially experiments dealing with the energy required for a definite amount of work, are not likely to give true values, unless they be carried out through an extended period of time. It will only then be possible to compare the fluctuations and irregularities of the separate daily periods, and it is only in this manner that we shall be able to obtain values which will be comparable with others which have been secured under different circumstances. In practical work, as we shall see later, we do not study the fats, carbohydrates, and proteins by themselves, but make use of those mixtures which are present naturally in meat and vegetables. The impracticability of laying too much stress upon the calorific values is very well shown by the significant discovery that the whole work of the digestive glands, and consequently digestion itself, is dependent to a great extent upon the nature of the ingested food material. It is only by combining the knowledge gained from investigations on the transformation of energy with that obtained in the study of cell activity that we are enabled to get a complete conception of metabolism in general.

We are first of all interested in the questions: What relations do the foods bear to one another, and what proofs do we have that certain organs are able to perform definite functions with all three classes of nutrients? Let us first take up the last question. In discussing the carbohydrates, we have already drawn the conclusion, from many experiments, that they form an exceptionally important source of muscular activity. Now, are the muscles also capable of performing their functions by utilizing the energy contained in representatives of the two other kinds of organic food, the fats and proteins?

The fact that protein may serve as a source of muscular energy was proved by Pflüger.[1] For over seven months he fed a dog exclusively on meat which contained only small amounts of fat and carbohydrates, — in fact, not enough to satisfy the requirements of the heart's work. Pflüger.

[1] E. Pflüger: Pflüger's Arch. **50**, 98, 330, 396 (1891).

furthermore, made this dog do heavy work for considerable lengths of time. The dog, therefore, had to perform all of its muscular work at the expense of protein. This proves that protein can also serve as a source of muscular energy. Under normal conditions, i.e., with a mixed diet, the muscle cells will first make use of the carbohydrates as a source of energy, and, if this is exhausted, then attack the protein.

A much discussed question is the value of the fats as a source of muscular energy. Chauveau [1] and others took the stand that fat, as such, can in no case be utilized as a source of energy by the muscle cells for the performance of work. The fat in every case must first be transformed into sugar before it can be used by the muscle cells. According to this assumption, the value of fats for the production of muscular energy could not be larger than that corresponding to the quantity of sugar which this amount of fat can form. Now 1 gram of fat is isodynamic with 2.56 grams of dextrose when the heat units of both are taken into consideration.

If we assume that the fats, before they can be utilized, must be changed into carbohydrates, it follows that 1 gram of fat would correspond to 1.6 grams of carbohydrate, if the fat is oxidized directly to sugar. We might expect to be able to determine by direct experiment how much of the potential energy in fats the body can transform into muscular force. This has not yet been satisfactorily accomplished. If we feed an animal with a mixture of albumin, fat, and carbohydrate, the calorific value of which we know exactly, we are unable to decide by which part of the energy the animal organism performs its different functions. We do not know whether such a selection actually does take place in the case of a mixed diet, or if it is not more probable that the organism takes all of the energy presented as such, and uses it for all of its functions. Atwater [2] justly calls attention to the fact that we have no means of differentiating internal from external muscular work. We must also remember that in every case only a part of the energy used for accomplishing work is shown by the work performed. A large part of this energy is transformed into heat. We can obtain an idea indirectly of the value of fats as a source of muscular work, if we regulate the conditions of the experiment so that an economical utilization of the available energy is guaranteed. If in the food only barely enough energy is supplied to the body to meet its requirements, or even less than enough, we would expect it to utilize all of the available energy in the most economical manner. Atwater has carried out such experiments. Particular stress is laid on the fact that the albumin in the food must be limited as much as possible in order to compare the fats and carbohydrates. Atwater, therefore, used only about as much protein in severe muscular work as he found necessary to maintain the nitrogen equilibrium in a

[1] A. Chauveau: Cf. Compt. rend. **121**, 26, 91 (1895); **122**, 429, 504, 1098, 1163, 1169, 1244, 1303 (1896); **123**, 151, 283 (1897).

[2] Atwater: Ergeb. Physiol. III, Abt. 1, p. 497 (1904).

previous "rest" experiment. This amount of albumin, with small quantities of fat and carbohydrate, was the starting ration of the "work" experiment. In the principal test, in one case a considerable amount of fat (butter, cream) was added, while in another experiment an equivalent amount of carbohydrate (milk-sugar and cane-sugar) was employed. The total energies received was somewhat less than the organism required; that is, some of the body substance was attacked. These experiments established the values of fats and carbohydrates as sources of muscular energy in two directions. In both cases the energy in the form of albumin and the total energy received were the same, the only differences being the predominance of fat in the one case, and of carbohydrates in the other. The external work was likewise the same in both cases. If the total energy utilized for the production of a definite amount of work was the same for a fat as for a carbohydrate diet, the fact would be established that fats and carbohydrates have the same value as a source of muscular energy. In the next place the amount of energy abstracted from the body itself — the quantity of energy received in the food was, as already stated, not quite enough to satisfy the requirements — must be a measure of the relative value of a diet in which either fat or carbohydrate predominates. If equal quantities of body substance were used up in both cases, we would have a further support of the equality of carbohydrates and fats as sources of muscular energy. The following table will give us an idea of the results obtained by Atwater in his experiments.

RELATIVE VALUES OF FATS AND CARBOHYDRATES IN THE FOOD FOR THE PERFORMANCE OF MUSCULAR WORK.

Name, Nature of the Experiment.	Time.	Energy in the Food.	Energy Equivalent to External Muscular Work.	Energy of the Oxidized Material.	Energy Equivalent to the Gain (+) or Loss (−) of Body Substance.
	Days.	Calories.			
No. 40 J. C. W. carbohydrate diet	4	4180	518	5251	−1071
No. 41 J. C. W. fat diet	4	4150	522	5304	−1154
No. 44 J. C. W. carbohydrate diet	4	4602	571	5125	− 523
No. 43 J. C. W. fat diet	4	4496	548	5155	− 659
No. 47 J. C. W. carbohydrate diet	4	4366	562	5173	− 807
No. 46 J. C. W. fat diet	4	4478	551	5193	− 715
No. 53 J. C. W. carbohydrate diet	3	5132	587	5104	+ 28
No. 52 J. C. W. fat diet	3	5120	607	5309	− 189
Average of 4 experiments with carbohydrate diet	15	4532	558	5167	− 635
Average of 4 experiments with fat diet	15	4524	554	5236	− 712

These experiments show somewhat higher values for the carbohydrates than for the fats. It is questionable whether this is always true, for Atwater also published the results of some experiments in which this was not the case. That, as a matter of fact, the fats are to be considered as direct sources of muscular work, without requiring any preliminary conversion into carbohydrates, seems apparent; for if we assume that the fats are first transformed into carbohydrates, there will be a loss of energy during their oxidation. In such a transformation, 36 per cent of the potential energy of the fat would become free energy. Now 1 gram of animal fat produces 9.50 calories, and 1 gram of cane-sugar 3.96 calories. By the combustion, in the bomb calorimeter, 10.53 grams of fat and 25.25 grams sugar are required to produce 100 calories. The same number of calories would, of course, be liberated in the body during complete combustion. If the carbohydrates alone were the source of muscular energy, 36 of the 100 calories from the 10.53 grams of fat would not be utilized. These would be set free in the body during the transformation of the fats into carbohydrates and appear as heat. The ratio of the 10.53 grams fat to the 25.25 grams carbohydrate would be 64 : 100. This, as a matter of fact, is not the case, as the following table shows. In it the relative values of a carbohydrate and of a fat diet, as shown by the above experiments, are compared; in one case the amount of energy transformed per day is given in calories, and in the other the percentage of utilization of the fat diet is compared with that of the carbohydrate diet, and fat alone is compared with carbohydrate.

PERCENTAGE UTILIZATION OF ENERGY.

Experiment.	Energy from Fat Diet Compared with that of Carbohydrate Diet.	Energy in Fat Compared with that in Carbohydrate.
	Per cent.	Per cent.
Experiments No. 40 and 41	99.2	98.3
Experiments No. 43 and 44	96.8	92.8
Experiments No. 46 and 47	98.3	96.2
Experiments No. 52 and 53	97.7	94.8
Average	98.0	95.5

Instead of the theoretical ratio of 64 : 100 we find that the fat stands to the carbohydrate in the proportion of 95.5 : 98.0. Thus, it is evident, unless we choose a far more complicated explanation, that the energy which the body receives in the form of fat is a direct source of muscular energy, and that a preliminary transformation of fat into carbohydrate does not take place.

If we apply these relations to the metabolism of a diabetic, we will appreciate the great derangement of his energy economy. In the severe form of this disease, the organism loses not only the greater part of the energy of the carbohydrates, but also the energy required to transform fat or protein into sugar; and as a part of the sugar so formed is eliminated by the system, the loss becomes a double one. The fact that the diabetic, whose blood and tissues are saturated with sugar, and who is already greatly injured as regards the economy of energy by means of the loss he suffers because of his inability to consume sugar, even prepares more sugar from the other nutrients, only to eliminate it eventually as such, shows us that the assumption that diabetes is only a simple derangement of carbohydrate metabolism does not satisfactorily explain the disease. Up to the present time the most prominent symptom, that of glucosuria, has dominated the entire investigation of problems concerning diabetes, and it is very probable that this is the reason why the disease, as a whole, is so little understood.

We have intentionally gone somewhat into detail concerning these relations, because we are unable to follow any other function so exactly as that of muscular work. All the other organs are only indirectly accessible to our observation. We do not know whether they also are able to utilize all three of these materials, fat, carbohydrate, and protein, in like manner as sources of energy. O. Cohnheim [1] has recently performed an experiment in this direction. He tried to decide whether the digestive glands obtained their energy requirements mainly or exclusively from protein. As we shall see later on, it is possible to stimulate the digestive glands without introducing into the alimentary tract food which would then participate in the metabolism. Cohnheim made an œsophageal fistula in a dog according to the method of Pawlow, and after a period of fasting fed the animal. The food eaten by the animal fell out through the fistula tube at every swallow, so that no nourishment was actually received by the dog. Not only are the salivary glands stimulated by this "fictitious feeding," but the stomach also. On account of the acid gastric juice passing from the stomach into the duodenum, the pancreatic gland also begins to secrete its fluid. By estimating the amount of nitrogen present in the urine on days of fasting and those on which the fictitious feeding day took place, Cohnheim succeeded in showing that the activities of the digestive organs were without influence upon the transformation of albumin. No increased elimination of nitrogen took place. This does not by any means prove that the digestive glands do not work with albumin. It is possible that, while the digestive glands are decomposing albumin, an equivalent amount of albumin is being "spared" in some other part of the body. Such an assumption becomes all the more plausible when

[1] O. Cohnheim: Z. physiol. Chem. **46**, 9 (1905).

we remember that the animal was fasting, and that such an organism is as economical as possible with its subsistence. On the other hand, it is possible that all three nutrients, fat, carbohydrate, and albumin, are drawn upon as sources of the energy required for the work of the glands, and that the consumption, of albumin especially, was so small, that it did not change the nitrogen content of the urine enough to be shown by our present methods of analysis.

We have already stated that the carbohydrates are also to be looked upon as a source of heat. It is possible to cause glycogen to disappear by merely chilling an animal. Albumin may also act in this way as a source of heat.

At all events, the discovery that the fats act as direct sources of muscular force, proves that the nutrients stand in intimate relation to one another. They replace one another partly by being transformed and partly by reason of their calorific values. This, however, does not by any means include all of the relations existing between the individual nutrients. As we have already seen, it is possible to keep a dog alive on meat alone. In this case the organism must obtain all of its requirements from the albumin (aside from the small amounts of fat and carbohydrate contained in the meat). If the quantity of meat is insufficient, the animal must draw upon its stores of fat and carbohydrate. It is a simple matter to determine the amount of meat necessary to just satisfy the energy requirements. If we exceed to the slightest degree the quantity of lean meat which is necessary to keep the metabolism in equilibrium, there will be no accumulation of albumin, but, on the contrary, there is an increased decomposition of albumin as a consequence. An accumulation of albumin, i.e., an increased amount of albumin in the cell material, may indeed be brought about by a liberal and long-continued feeding of albuminous material.[1] It has been shown, however, that the animal organism constantly seeks to maintain its albumin content, and consequently the functional condition of its cells, at a constant level. As soon as the diet ceases to contain the excess of protein, the accumulation of albumin in the cells quickly disappears. The previous equilibrium in the economy of the individual cells will be reëstablished.

If we make an animal go hungry, the elimination of nitrogen continues, and this is also true when an abundance of fat or carbohydrate is fed to the animal. The albumin, therefore, is not entirely replaceable, although it is possible to reduce the destruction of albumin by means of fats. This fat may come from the food or from the body itself. It is possible by means of fat and albumin to establish a new nitrogen balance; i.e., to

[1] More recent experiments make it seem doubtful whether the nitrogen retention and deposition of albumin correspond to one another. Great caution should be used in the estimation of the nitrogen balance in this direction. Cf. E. Abderhalden and Bloch: Z. physiol. Chem. **53**, 464 (1907).

determine anew the quantity of albumin which is absolutely necessary to prevent injury to the albumin content of the organism. This amount of albumin will be much less than is required in a diet of albumin alone. If we feed an animal a definite quantity of fat and albumin, it will be possible to reduce the albumin requirement more and more by increasing the proportion of fat in mixture. We finally reach a minimum amount of protein, and if we attempt to replace it by fat, we cause the body albumin itself to be attacked. This limit varies with different animals and at different times with the same animal; in every organism it is dependent upon the condition of the body, and, above all, upon the fat in the body at the time of the experiment. A fat animal will permit more albumin to be replaced by fat than will a lean one, for the former can contribute from its own supply of fat. On the other hand, by feeding fat we have a means of causing an accumulation of albumin. We can *spare* albumin not only with fat, but also with carbohydrates. By their assistance also, provided sufficient albumin is supplied, the albumin content of the body may be increased. Voit,[1] who performed experiments in this direction, came to the conclusion that the fats and carbohydrates did not have an equivalent effect in causing an accumulation of albumin. Carbohydrates are more efficient as "albumin sparers" than are the fats, as is shown by the following table:

Food.				Meat.	Gain (+) Loss (−)
Meat.	Fat.	Carbohydrate. Starch.	Sugar.	Transformed into Body Albumin.	in Body Albumin.
500	250	...	...	558	− 58
500	...	...	300	466	+ 34
500	...	...	200	505	− 5
800	...	250	...	745	+ 55
800	200	...	...	773	+ 27
2000	...	200–300	...	1792	+208
2000	250	...	...	1883	+117

Atwater[2] has recently determined accurately the comparative values of carbohydrates and fats as sparers of albumin. The fact that proteins are distinguished from the other nutrients by the amount of nitrogen which they contain, makes it easy to carry out the experiment. By simply comparing the amount of nitrogen in the food with that of the urine, we can at once get an idea as to how much albumin has been decomposed. The nitrogen content of the fæces tells us approximately how much albumin has not been absorbed. We speak of a "nitrogen equilibrium" when the amount of nitrogen ingested in the form of food is equal to that contained in the urine and in the fæces. If the latter is greater than the

[1] Carl Voit: Hermann's Handbuch der Physiologie, **6**, 143 (1881). [2] *loc. cit.*

amount introduced, it shows that albumin from the body has been decomposed. Conversely, if the nitrogen eliminated is less than that ingested, then we are justified in concluding that albumin has been accumulated.

The following table will give a summary of some of Atwater's experiments:

Kind of Experiment.	Subject.	Number of the Experiment.	Duration in Days.	Available Energy in Food.	Heat Equivalent to Work Performed.	Nitrogen			
						in Food.	in Fæces.	in Urine.	Gain (+) Loss (−)
				Calories.		Grams.			
Work experiments:									
Carbohydrate	J.C.W	40	4	4180	518	17.1	2.2	17.1	−2.2
Fat	"	41	4	4150	522	16.9	1.5	20.3	−4.9
Carbohydrate	"	44	4	4602	571	17.8	2.6	17.3	−2.1
Fat	"	43	4	4496	548	17.1	2.0	19.1	−4.0
Carbohydrate	"	47	4	4366	562	17.4	2.7	16.3	−1.6
Fat	"	46	4	4478	551	17.0	1.8	16.1	−0.9
Carbohydrate	"	53	3	5132	587	17.9	2.3	15.4	+0.2
Fat	"	52	3	5120	607	17.7	1.6	16.4	−0.3
Average for the experiments with carbohydrates	...	...	15	4532	558	17.5	2.5	16.6	−1.5
Average for the experiments with fat	...	...	15	4524	554	17.1	1.7	18.1	−2.7

From these experiments we find, in agreement with those of Voit, that carbohydrates as well as fats act as sparers of albumin, under the given conditions. The fact that in the carbohydrate experiments there was invariably more nitrogen in the fæces than in the experiments with the fats, is explained by the nature of the food. Vegetables predominated in the former case, and meat in the latter. We shall see later, that the protein in vegetables is utilized to a less extent than that of meat. If we subtract the nitrogen in the fæces from the total nitrogen of the food, we shall obtain the quantity of nitrogen which the organism has evidently utilized. If we insert these values in the above table, we shall find that less nitrogen was available when carbohydrates were eaten, than when fats predominated in the food. Nevertheless, there was less nitrogen present in the urine in the former case than in the latter, although the difference was not great.

These experiments still leave one question unsolved: How do the fats and carbohydrates act as sparers of albumin, when they are eaten together? In the above experiments, the food at one time contained protein and fat, and at another time protein and carbohydrate. Under ordinary conditions all three kinds of nutrients, fat, carbohydrate, and protein, are available to the organism. Tallquist [1] has, therefore, studied

[1] F. W. Tallquist: Arch. Hyg. **41**, 177 (1902).

the problem from this standpoint. During one period, the food contained 16.08 grams nitrogen, 44 grams fat, and 466 grams carbohydrates; and in a parallel experiment it contained 16.08 grams nitrogen, 146 grams fat, and 250 grams carbohydrates; each gives the same number of calories (2867 and 2873 calories). In both experiments practically the same nitrogen balance was reached. It appears that under these conditions the carbohydrates are isodynamic with the fats in respect to acting as albumin sparers. Landergren[1] explains the greater sparing of albumin in an exclusively carbohydrate diet, as compared to a diet of fats alone, by the assumption that the animal organism constantly requires sugar; and inasmuch as he does not believe fat can be changed into sugar, a part of the albumin must be utilized, in the case of a fat diet, for the formation of sugar. This part of the albumin is spared, if the food contains carbohydrates, so that the organism then has more albumin at its disposal for its remaining functions. The total albumin may be utilized as such, when sufficient fat and carbohydrate has been added to the diet. This explanation is at present only an hypothesis.

The results as a whole, which have been obtained in studying the relations of the carbohydrates and fats to the transformation of albumin, show that we may conclude with a great degree of probability that the Law of Isodynamics holds for these nutrients among themselves. Both are able to spare albumin to about the same extent. The fact that the feeding of carbohydrate alone, or fat by itself, should have shown a difference in favor of the former, may be explained by the assumption that this Law of Isodynamics is not an absolute one; i.e., the chemical composition of the foods undoubtedly plays an important part.

We have already mentioned the most important relations existing between the nitrogen-free nutrients; i.e., the fats and carbohydrates. We have shown that with fats the muscles are able to perform their work just as well as with carbohydrates. On the other hand, we know of experiments which prove beyond question that carbohydrates can replace fats; in fact, according to the Law of Isodynamics. If all nourishment is withheld from an animal, it will draw on its own body, not only attacking its own protein, but especially the fat deposits. If we substitute for the fat which would be used up in this way during starvation the same quantity of fat in food, we find that a complete replacement follows; i.e., the animal does not touch its fat reserves. The same effect can be obtained by substituting for the fat an isodynamic amount of carbohydrate. This is shown by the following table, which gives a summary of some of Atwater's experiments.

The loss of body fat was at one time greater with a carbohydrate diet,

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[1] E. Landergren: Skand. Arch. Physiol. **14**, 112 (1903).

and at another time greater with a fat diet. On an average it was less with the carbohydrate diet. Apparently sugar is a better sparer of body fat than fat itself. The difference is, however, very slight, and we may conclude from the experiments that isodynamic quantities of fat and carbohydrates are equivalent in this respect.

Kind of Experiment.	Subject.	No.	Duration in Days.	Available in the Food.				Gain (+) or Loss (−)			
				Protein in Grams.	Heat of Combustion of Food.	Energy of Material Oxidized in the Body.	Energy of External Work.	Protein.	Energy of Protein in Calories.	Fat in Grams.	Energy of Fat in Calories.
				Calories.				Grams.			
Carbohydrate . . .	J.C.W.	40	4	17.1	4180	5251	518	−13.6	− 77	−104.2	−994
Fat	"	41	4	16.9	4150	5304	522	−30.6	−173	−102.8	−981
Carbohydrate . . .	"	44	4	17.8	4602	5125	571	−13.1	− 74	− 47.1	−449
Fat	"	43	4	17.1	4416	5155	548	−25.0	−141	− 54.3	−518
Carbohydrate . . .	"	47	4	17.4	4366	5173	562	−10.1	− 58	− 78.5	−749
Fat	"	46	4	17.0	4478	5193	551	− 5.6	− 32	−71.6	−683
Carbohydrate . . .	"	53	3	17.9	5132	5104	587	+ 1.3	+ 8	+ 2.1	+ 20
Fat	"	52	3	17.7	5120	5309	607	− 2.1	− 12	− 18.6	−177
Average for the experiments with carbohydrates . .	...	...	15	17.5	4532	5167	558	− 9.5	− 54	− 60.7	−581
Average for the experiments with fats	...	...	15	17.1	4524	5236	554	−16.7	− 95	− 64.7	−617

The question that next arises is with regard to the way in which carbohydrates and fats behave when they are fed simultaneously. It would seem possible that they would be decomposed equally, and the liberated energy utilized, sometimes for one purpose and sometimes for another. It is also conceivable that one substance may be used up more rapidly than the other. This is a very hard problem to decide. We may determine the amount of nitrogen and carbon eliminated in the urine and fæces, and deduct from the total amount of carbon that corresponding to the protein (as indicated by the nitrogen value). The carbohydrates and fats would be represented by the rest of the carbon. It would be possible to decide which nutrient gave rise to the greater part of the carbon dioxide formed if we knew the amount of oxygen which was consumed at the same time. If carbohydrates were exclusively oxidized, the ratio of the volume of oxygen taken up, to that of carbon dioxide produced, would be equal to 1. The ratio of $CO_2 : O_2$ is called the " respiratory quotient." It is only 0.71 in the case of the fats. Experiments which have been made in this direction

indicate that the carbohydrates are attacked immediately after absorption from the intestine, thus sparing the body fat. We must not forget, with regard to such experiments, that the conclusions drawn are for the most part indirect. They are not conclusive. A comparison of the general metabolism on the basis of income and outgo alone, must necessarily lead to a one-sided decision. The results of physiological-chemical investigations must not be left out of consideration, and the finer details of the work must not be forgotten in studying the coarser outlines of metabolism as a whole. We have seen that the metabolism of carbohydrates in all of its phases is an extremely delicately regulated process. Sugar reappears, after absorption, in the liver, deposited in the form of glycogen. This does not vanish so easily. It is extremely difficult to obtain an animal which is free from glycogen. At all events, our knowledge regarding the transformation of the carbohydrates in the tissues is altogether opposed to the assumption that they are rapidly burned up after absorption, even during work. We know it is true that the muscles evidently prefer to utilize the energy from carbohydrates in their performance of work, but this very fact prevents us from believing that the organism consumes this valuable material in order to save the fats.

Studies on metabolism have shown us that there is a difference in the behavior of the protein in food and that of fats and carbohydrates, which are themselves very similar in their behavior. We have already stated that by increasing the amount of protein in food, there is an increased metabolism. This, however, is not the case, or at least not to the same extent, if we increase the quantities of fat or carbohydrate, as the following experiments, carried out by M. Rubner, show:

Day.	Income.				Total Metabolism in Calories.	
	Nitrogen in Grams.	Fat in Grams.	Carbohydrate in Grams.	In Calories.	Absolute.	Per Kilogram of Body Weight.
2	...	...	...	...	969	40.2
3	56.8	...	...	1513	1072	44.8
4	...	...	...	...	947	39.9
5	...	167	...	1536	963	40.9
6	...	...	...	...	922	39.6
7	...	...	411	1446	982	42.3
8	...	...	...	...	977	42.1

2, 4, 6, and 8 are fasting experiments. In them 40.4 calories per kilogram body weight is the average metabolism. The addition of albumin causes an increase in the number of calories amounting to 11.9 per cent; the addition of fat, 1.2 per cent; and that of carbohydrates, 4.2 per cent.

The figures show that the number of calories in each case were about the same in the three experiments. If such experiments are carried out unaccompanied by muscular work, the difference between the effect of albumin, on the one hand, and that of the carbohydrates and fats, on the other, towards the entire metabolism, becomes more marked.

We have now finished all that we care to say with regard to the mutual relations existing among the three most important organic nutrients. We have discussed two ways in which they may replace one another. On the one hand, it is possible by means of a chemical transformation for one nutrient to be converted into another, and, again, the replacement may be merely one of calorific values, without any such transformation being necessary. The latter method of one food replacing another is undoubtedly of the greatest importance in the whole economy of metabolism. It represents the greatest possible utilization of the available energy, and guarantees the satisfactory maintenance of the entire metabolism, even when one of the nutrients is not momentarily available. Albumin is an exception. It is only in part replaceable. If the organism is starving, it tries to preserve its albumin by consuming first the fats and carbohydrates available, thus protecting its tissues against severe injury. The former condition of the body is therefore quickly regained as soon as food is eaten again. It is only when the organism draws upon its own protein for the main supply of the required energy, that the end is near. It is of great interest to note, that the reserve materials held in store by the organism, and drawn upon during starvation, likewise enter into metabolism strictly in accordance with their calorific values. As soon as the body substances are called upon to act as combustible material, they also follow the Law of Isodynamics.

LECTURE XVI.

INORGANIC FOODS.

I.

Importance of Inorganic Substances as Building Material of the Cells and Tissue. — Water, Salts.

All of the food-stuffs which we have studied up to the present, are those by means of which the animal may obtain chemical energy. The conception of a food is, in fact, closely related to this property. We recognize, however, a group of compounds indispensable to the organism which it always receives with its food, but from which it can obtain no chemical energy. We refer to water and inorganic salts. Thus far we have considered the foods solely with regard to their value as sources of kinetic energy. We must not forget, however, that the organism is constantly wearing out its cell-material; in fact, individual cells may even be entirely thrown off, only to be regenerated and built up anew. Such processes are particularly noticeable in the case of growing organisms. In such cases the new cells formed in place of the old ones are often of larger dimensions. Yet, it must not be thought that the fully developed organism retains its cellular condition unchanged. At present we are not in a position to explain fully the metabolism within the individual cells. We have no means of knowing how long a single cell may live; we do not know how long it can exercise its function with the same material. All that we can say is that there are certain processes visible to the eye which give indication of a continuous breaking down and building up of cells. We know that hair, feathers, scales, etc., undergo constant changes, processes which take place in some species of animals very slowly, but continuously; while in other cases, as with the shedding of feathers in the case of birds, and the changing of skins with reptiles and amphibia, such processes take place within a relatively short time. Again, we know of the constant change in the cells of the epidermis, and in the cells of the mucous membrane. Similarly we know that there is a continual loss of material involved in the exercise of the function of numerous glands. In this connection we need merely mention the glands of the skin, — the sebaceous and sweat glands, — the salivary glands, and the numerous little mucous glands of the respiratory and digestive membranes. The same

is true of all the glands taking part in the digestive processes, beginning with those of the stomach and the mucous membrane of the alimentary canal, on to the large digestive glands of the pancreas and liver. In all such cases the organism suffers a constant loss of material. Again, the organism constantly requires the presence of salts, and water to flush out the waste material through the kidneys. Further, we have to consider the specific tasks of the single cells and cell-groups by means of which definite products are developed which play an important part in the metabolism of the organs, whether it be a ferment, or some other product such as adrenalin, which is formed in the suprarenal gland.

Furthermore, the fact that there is evidently a lively breaking down and building up even in tissues which we would scarcely expect to participate in active metabolism, is shown to us by a histologic study of the bones, which show evidence of a continual exchange of their building material. From the field of pathology, we find that the building up of the nerves and their restitution under some conditions may assume considerable dimensions. This is shown, for example, in the case of hypertrophic activity, which appears as soon as there is an additional requirement placed upon a certain organ, whether because of the fact that it must act as a substitute for another, or whether because its work becomes increased in some other abnormal way, as, for example, in the case of the heart in certain kinds of heart trouble. On the other hand, in convalescence after certain fevers, particularly typhoid, we find a sudden rejuvenation of the sunken cell-energy. Each cell takes up the building material from the circulating nourishment, and this is particularly true of albumin, which in a certain sense determines the functional activity of the cell. In a short time the organism is renewed. The loss of albumin which the body has experienced during the disease is quickly compensated. The old equilibrium in the economy of the cells is again established. Again, a sudden increased production of cell-material takes place after some local irritation. Thus we find that the organism concentrates a great number of leucocytes at an infected point, and finally perhaps large masses of pus are formed, all at the cost of the nourishment and the material composing the organs. On the other hand, sometimes we find the organism throwing off considerable quantities of exudate, as, for example, in pneumonia, which again uses up large amounts of material. On the other hand, if we consider the continual variations in the number of red and white corpuscles, and the variations in the lymphocytes, we obtain the impression that the cell-material of the fully developed organism is never at rest. We know practically nothing concerning the quantitative relations involved in all such changes. We do not know whether the material in the old cells is used to some extent in building up new ones, or whether the new cells are entirely formed from new material. We do

not know whether the individual organs can effect an exchange of materials, or whether the cells of one group can utilize the waste material of another.

The interesting studies of Miescher [1] on salmon give us some information in this direction. Previous to spawning, these fish migrate from the sea into fresh water, for example, into the Rhine. From the time that these fish reach the river up to the time that the eggs are laid, they take no nourishment. This fact was known to Barfurth [2] and to His.[3] Meischer estimated that the majority of the salmon remained in the Rhine for from six to nine and one-half months, a smaller number stayed up to twelve months, while some were there as long as fifteen months. During all of the time that the fish remains in fresh water, nothing is eaten. The intestines are always found empty; and, indeed, Miescher established the fact that the digestive glands during this period do not yield any active juices. A series of marked changes take place in the appearance of the fish during this period. When the salmon first reaches fresh water its organs of regeneration are quite undeveloped. Being provided with a powerful dorsal musculature, it is able to stem the most rapid currents in the Rhine. On comparing such a fish with one that is taken just before the spawning time, it seems scarcely possible that they are the same kind of fish. The large dorsal muscle has become shrunken; the sexual organs, on the other hand, have become enormously developed. There is a parallelism between the two changes. Miescher observed, for example, that the weight of the ovary increased from 9.4 grams to 15 grams, while simultaneously there was a diminution in the dry substance and in the albumin content of the dorsal muscles as shown by the following average values:

	Length in Millimeters.	Weight in Grams.	Weight of Ovary in Per cent of Body Weight.	Composition of Dorsal Muscle.	
				Per cent Albumin.	Per cent Dry Substance.
March	872	9305	0.061	18.45	33.6
July and August	886	8953	4.78	17.5	26.8
November and December	879	7428	...	13.2	18.5

The albumin lost by the muscles is evidently utilized in building up the sexual glands — in one case the eggs, and in the other the sperm cells.

[1] Die histochemischen und physiologischen Arbeiten von Friedrich Miescher, vol. ii. Leipsic, 1897. Pp. 116 *et. seq.*

[2] Troschel's Arch. Naturgeschichte, Jg. xli, I, 122 (1875).

[3] Untersuchungen über das Ei und die Eientwicklung bei Knochenfischen. Leipsic, 1873.

Direct observation confirms this. Microscopic examination of the ovaries and of the testes shows that most active processes of growth and transformation are taking place. On the other hand, the large dorsal muscle exhibits all the signs of a far-reaching release of its stored material and of even the contents of its cells, the muscular fibers. Everything is not given up, but as much of the material as can be spared is transformed, leaving enough behind so that subsequently when the salmon returns to the sea the muscles may be regenerated. It is the large dorsal muscle which entirely provides the material for the changes taking place in the body of the starving fish, whereas the remaining muscles undergo no change that indicates in any way a migration of material. It has never been satisfactorily explained just how this migration takes place. Miescher describes the appearance of small drops of fat between the muscle fibrils. The amount of these drops may become so great that the whole muscular fiber becomes opaque. It is obvious that in this way preparation is being made for a migration of fat. Besides albumin and fat, the muscle must give up phosphates which in the formation of eggs evidently become a part of the lecithin. The other salts and substances, such as cholesterol and the nuclein substances required to form the nuclei of the eggs, must likewise be obtained from the dorsal muscle. It is evident, therefore, that the migration of substance here attains large dimensions. Undoubtedly a study of this interesting biological phenomenon by modern methods would give us considerable insight into the extent of the syntheses capable of being carried out in the organism. Every supply from the outside is cut off. The entire sexual products are formed from material taken from the dorsal muscle. It is clear that a comparison of the amount of lecithin, nucleic acids, etc., contained in this muscle with those of the sexual products, would give us a good idea of the metabolism and of the chemical processes involved.

These observations permit us to draw certain conclusions concerning metabolism during starvation.[1] If every source of food supply is cut off from an animal, then the organism turns to its own body for nourishment. Carbon dioxide is constantly being eliminated and oxygen absorbed, and likewise the chemical composition of the urine gives unmistakable proof that combustion is continually taking place, which in warm-blooded animals suffices not only for the performance of mechanical work, but also for maintaining the temperature of the body. If we compare the relative proportions by weight of the separate organs of a starving animal with those of one that is well nourished, it is at once apparent that the different organs do not participate equally in the metabolism. Thus the nervous system and the heart show but slight, if any, changes in their composition

[1] S. M. Lukjanow: Z. physiol. Chem. **13**, 339 (1889). C. Voit: Z. Biol. **31**, 510 (1894). A. Hermann: Pflüger's Arch. **43**, 239 (1888).

as regards both organic and inorganic matter. Evidently these organs which are so indispensable to life are maintained at the expense of the less vital tissues. In such cases there is a continuous transportation of material from one tissue to another. The fact that one organ, which during starvation will normally lose material to a considerable extent, may under other conditions, i.e., when its function is of especial importance to the whole organism, be kept in full activity at the expense of other organs, is shown by an observation made by Pflüger [1] that the liver of dogs after extirpation of the pancreas, in spite of the resulting glucosuria, did not diminish in weight, whereas all the other organs — excepting the heart and nervous system — were greatly impaired. Now we know what an important part the liver plays in the metabolism of carbohydrates, so that we can easily understand that under the above conditions the function of the organ is of especial importance to the animal.

How economical the animal organism is with its materials that it has once built up, is shown by another of E. Pflüger's [2] observations. The larva of the nurse-frog (*Alytes obstetricans*) is fully grown at the end of May. It has then attained a length of about 8.1 centimeters, of which about 3 centimeters belong to the real body, and the remainder to its over-sized tail. After the larva has reached this stage, it no longer takes any nourishment. At the same time the tail begins to shrivel up. Its cell-material is liquefied and migrates to the true body; and as the tail disappears, the front and hind legs shoot out. Just imagine what important transformations must take place in this process of developing the limbs of the animal from what was the tail! As soon as the tail has all been absorbed, nourishment is again taken up from the outside.[3]

This suffices to give us some idea as to the mutual relations in the metabolism of the different organs. It is not likely that the above observations represent exceptional cases. It is far more probable that such changes are of common occurrence, and in a way this is quite similar to the relation known to exist between the glycogen in the muscles and that of the liver.

Even although the greater part of the nourishment absorbed is employed for the production of energy, a certain portion of it is taken, as required, — whether albumin, carbohydrate, lecithin, cholesterol or nuclein substances, — and utilized for the building up and extension of the cells. We certainly cannot limit our conception of the term "food" to those substances which we know to be sources of energy. The function of serving the

[1] E. Pflüger: Pflüger's Arch. **108**, 115 (1905).

[2] Pflüger's Arch. **29**, 78 (1882); **54**, 333 and 403 (1893).

[3] The phenomenon is not peculiar to the larvæ of the nurse-frog, but is common to larvæ of amphibia which pass through this stage. The larvæ of *Rana fusca* and of *Rana temporaria* at least show a similar behavior, although here the animal apparently eats up the tail. The material of the organ is utilized, at all events.

cells must also be included. As we have said, water and inorganic salts, neither of which imparts to the organism any chemical energy, must necessarily be considered as foods, for they exercise this function of being of use to the cells. Now we know that the animal organism takes up a considerable amount of salts with its food, while on the other hand it is equally well known that the organism constantly eliminates salt in the urine and in the sweat. These losses must naturally be replaced. For the salts it would seem, *a priori*, as if there were not much need for such replacement. We could easily imagine that the salts set free in the breaking down of cell-material could be used anew in the formation of new cells. With water it is quite another matter, because the organism needs water for a number of different processes. Its importance is shown by the fact that two-thirds of the animal organism consists of water. Every cell must contain water. It forms one of the prime conditions for a definite physical consistency of the cell. Water is absolutely necessary as a solvent for numerous compounds. It brings into play numerous chemical reactions, and takes part in the building up and breaking down of substances without number. It is a carrier of nourishment to the body, whether through the blood, the lymph, or the finest fissures between the cell; and, conversely, it provides the means for carrying away the waste products. When we remember in addition that the body must give up water to the air in the process of respiration, and that in water the animal organism possesses its most important means of regulating the temperature of the body, by virtue of its evaporation on the surface, it soon becomes apparent why water plays such an all-important part in the life process not only for animals but naturally for plants as well. In the combustion of the food, naturally some water is formed in the body, but this amount is so small that it by no means suffices to satisfy all the requirements. The animal organism must have a supply of water from without.

Now, are the inorganic salts also indispensable for the nourishment of the fully developed organism? An attempt has been made to answer this question by providing animals with food which is as free from ash as possible. The first to perform such experiments was Forster.[1] As food he made use of the meat residue obtained in the preparation of Liebig's Extract of Beef. After having been repeatedly boiled with water, such meat contains only 0.8 gram of ash, for each 100 grams of dry substance. This, together with fat, sugar, and starch, he fed to two dogs. Both of the animals experimented upon died very soon; in fact, much more quickly than if they had not been fed at all. The same result was obtained by feeding three pigeons with starch and casein. Against the conclusion that death was caused by lack of salt, G. von Bunge[2] very properly raised

[1] Z. Biol. **9**, 297 and 369 (1873).

[2] *Ibid.* **10**, 111 and 130 (1874).

the question that possibly the lack of salt may have had an indirect action. In the catabolism of that part of albumin containing sulphur, the cystine, there is formed, as we have seen, a considerable amount of sulphuric acid. This under normal conditions will unite with the basic salts contained in the nourishment and be eliminated as a salt of the acid. If now the nourishment contains none of these basic salts, then the sulphuric acid will constantly withdraw alkali from the cell components so that the system will not only fail to have salts, but the whole structure of the cells will be injured by taking away a part of the building material. Now if such a hypothesis be correct, then the addition to the nourishment, which is otherwise practically free from ash, of sufficient alkali to unite with this sulphuric acid should enable the animal to live longer. Lunin [1] showed by experiments that this was actually the case. He fed mice with casein, fat, and cane-sugar. The amount of ash contained in this mixture was only one-tenth of that in the mixture used by Forster. With this food and distilled water five mice lived respectively, 11, 13, 14, 15, and 21 days. Other mice were not given any food at all; two died in three, and two in four days.

Next Lunin fed six mice with the same mixture, to which some sodium carbonate had been added. These animals lived 16, 23, 24, 27, and 30 days, or nearly twice as long as the mice did in the previous experiment. Now the objection may be raised to this last experiment that here the sodium carbonate may not act, as Bunge reasoned *a priori*, as an alkali, but rather as a salt. In order to meet this objection, Lunin fed seven mice with the same mixture, except that the sodium carbonate was replaced by an equivalent amount of sodium chloride (common salt). These animals died at the end of 6, 10, 11, 15, 17, and 20 days; i.e., they did not live any longer than the mice in the first experiment. The experiments were repeated with potassium carbonate and potassium chloride, but with the same result.

Now although the addition of sodium and potassium carbonate to such a diet was sufficient to prolong the life of the animals, it was not able to maintain their existence for any length of time.[2] Note, however, that these animals had received only one salt in the nourishment. It might be thought that better results would be obtained with a mixture of salts. Lunin, therefore, compared the lengths of life of mice fed upon cow's milk, with that of mice fed with the above mixture of casein, fat, and cane-sugar, plus the same salts that are contained in milk. Care was taken to

[1] Ueber die Bedeutung d. anorganischen Salze f. d. Ernährung d. Tieres. Dissert. Dorpat, 1880, and Z. physiol. Chem. **5**, 31 (1881).

[2] Cf. C. A. Socin, Z. physiol. Chem. **15**, 93 (1891). Abderhalden u. Rona, *ibid.* **42**, 528 (1904). Henriques and Hansen, *ibid.* **43**, 417 (1905). Falta and Noeggerath, Hofmeister's Beiträge, **7**, 313 (1905).

maintain the same proportion of salts to organic material as in milk. Fed with such a mixture, six mice lived 20, 23, 29, 30, and 31 days, or about the same length of time as the mice fed with the former mixture containing alkali carbonate. Two mice, fed entirely upon cow's milk for a period of $2\frac{1}{2}$ months, remained in good health at the end of the experiment.

These experiments apparently prove that it is not possible to keep mice alive without feeding them salts, and, moreover, that an artificial mixture of salts fails to sustain the lives of mice for more than a short time. This result may be accounted for in a number of different ways. It has not shown that the early death of the mice in the first experiment was actually due to a lack of salts in the diet. It is equally conceivable that certain necessary organic constituents were wanting. Milk always contains, besides casein, a certain amount of albumin. It is possible that the albumin is of great importance for certain functions. Again, perhaps lecithin and cholesterol are essential. Possibly milk contains organic compounds of a nature unknown to us. Above all we must remember that the exceedingly important Law of the Minimum [1] holds for the nourishment of animals as well as for that of plants. In any diet the amount of each constituent required by the organism must be regulated in accordance with this principle. Perhaps some inorganic element, such as fluorine was lacking, and for this reason the other inorganic constituents were not sufficiently utilized. Naturally the same law holds with regard to the organic constituents.

Until recently but little was known concerning the physiological importance of salts in the plant and animal organism. It was known that they took part in the anabolism of the cells. In fact, potassium, sodium, calcium, magnesium, iron, phosphoric acid, fluorine, and chlorine are invariably found in every cell. In some cases, manganese, silicic acid, iodine, and arsenic are found in the animal cells. The plants receive their nourishment from the ground, and under certain conditions may contain other elements; for example, copper, zinc, and aluminium. Silicic acid in many cases of plant life is an important source of rigidity. The plant cell requires inorganic material. There is no doubt that the physiological importance of the salts is the same in both the animal and vegetable kingdoms, and the assumption that they form merely a passive building material for the cells is not justifiable. This is evidenced by the fact that the distribution of the inorganic material is not uniform throughout the organism. Thus we find in certain cells more potassium and less sodium, while in the liquids of the body, for example the serum, the relative amounts of the two are reversed. In the cells there is more phosphoric acid, but less chlorine. It is evident from this that the taking up of salts

[1] J. Liebig: Die Chemie in ihrer Anwendung auf Agrikultur und Physiologie, p. 332 (1876).

by the cells is an active process. Thus the cell withdraws from the serum, which is rich in sodium salts and contains less potassium, the potassium. It is possible that the researches of Bokorny [1] concerning the behavior of lower organisms to solutions of certain dyestuffs may throw some light on these processes.

He showed, namely, that the protoplasm of certain cells would take up definite compounds even from very dilute solutions, so that, for example, a colored solution would eventually become colorless. Bokorny assumed that chemical combination took place between the protoplasm and the substances in question. He showed, for example, that the *Spirogyra* and *Cladophora* would absorb silver from a solution containing only one part in 100,000,000. In a solution of 1 : 10,000,000 enough silver was taken up so that when treated with hydrochloric acid and hydrogen sulphide the algæ turned black. Likewise from very dilute solutions of copper and mercury salts the cells would remove the inorganic material. Toward other compounds, e.g. gold salts, the behavior was quite different. From gold solutions containing one part in 100,000 the gold is not removed by the above-mentioned algæ, nor by yeast cells. There is a specific action between these cells and the heavy metals which we cannot explain at present. The absorption of certain salts by the cells of the animal organism must take place in conjunction with certain definite and specific processes by means of which a definite selection in definite proportions is made possible. Quite recently we have become able to explain in a measure the part played by salts in the life of the cells. The function of the salts is undoubtedly chiefly an osmotic one. It is the task of the inorganic salts to regulate the osmotic pressure of the cell-fluid itself and of the intercellular fluid. It is clear that any change taking place in this pressure, whether by the taking up or giving off of water, causing a swelling or a shrinking of the cell, or, on the other hand, any changing in the concentration or dilution of the reacting mixture contained in the protoplasma, will immediately lead to most serious disturbances in the progress of certain reactions. Above all, the velocity of the reactions will be changed.

The part played by the inorganic material is not restricted, however, to maintaining this constant osmotic pressure between the liquids within and without the cell. This is evident from the fact that the cell requires certain definite salts. It is not possible, for instance, to replace successfully the potassium in the cell by an equivalent amount of sodium. The individual salts must exert for themselves a specific action, although it is not yet clear as to just what this may be. We are acquainted with quite a number of isolated facts in this connection, but with our present knowledge it is not possible to group them together and view them from one

[1] Chem.-Ztg. **29**, 1201 (1905).

standpoint so that definite conclusions may be drawn. It may be well to cite one or two examples showing that, in fact, there is a specific action of the inorganic salts. Overton [1] has shown that the muscles of a frog will retain their normal volume in a 0.7 per cent solution of common salt, and remain excitable at the end of 40 to 48 hours. In concentrated salt solutions their volume is diminished, whereas in dilute solutions it increases. Solutions of grape-sugar, cane-sugar, milk-sugar, mannitol, alanine, asparagine, etc., having the same osmotic pressure as the 0.7 per cent common salt solution, are equally indifferent as regards the osmosis. In such solutions, however, the excitability of the muscle is soon lost. On adding a little common salt to one of these solutions, however, it is again possible to excite the muscle. In fact, 0.068 to 0.074 per cent of salt suffices to render this effect. The next question is whether a change of the anion (Cl) keeping the cation (Na) constant will have any effect. It was found the chloride could be replaced successfully by equivalent amounts of the bromide, nitrate, sulphate, bicarbonate, chlorate, acetate, and secondary phosphate of sodium, showing that the anions had no influence here. In a series of further experiments the cation was changed, and it was found that the sodium could be replaced by lithium alone, while potassium, calcium, magnesium, strontium, and barium salts were unable to preserve the excitability of the muscle. It is perfectly obvious, therefore, that the sodium ions, besides serving to maintain a definite osmotic pressure, also exert a quite specific action upon the contractility of the muscle.

Jacques Loeb [2] succeeded in performing a very pretty experiment. If the medusa *Gonionemus* be placed in a solution of cane-sugar or of glycerol, the osmotic pressure of which corresponds to that of the ocean, its rhythmic pulsation ceases immediately. This is not the case, however, if a solution of sodium chloride or bromide is used in the above experiment.

Loeb showed, moreover, that the presence of the sodium ions alone was not sufficient to maintain the contractility of the muscle. In a 0.7 per cent solution of common salt the muscles of a frog after about an hour exhibit rhythmic contractions which last for over 24 hours. It appears as if the sodium ions irritate in some way the muscular fibers. It has even been stated that they have a poisonous effect. It is exceedingly interesting that it is possible to combat this irritation of the sodium ions (also obtained with rubidium and caesium ions) by the addition of the bivalent calcium, strontium, magnesium and manganese ions. This effect is not, however, due merely to the valence of the ion, for the bivalent barium, zinc, cadmium, and lead ions do not act in the same way. On the other hand the monovalent potassium ion has an effect opposite to that of the monovalent sodium ion. It is particularly interesting that

[1] Pflüger's Arch. **92**, 115 and 346 (1902).

[2] Am. J. Physiol. **3**, 383 (1900).

such closely related chemical elements as sodium and potassium should act so differently physiologically.

A quite similar observation was made with the *Fundulus heteroclitus*.[1] This little fish is not at all sensitive to variations in osmotic pressure. It exists in salt water as well as in distilled water, while on the contrary, when placed in a solution of pure sodium chloride of the same concentration as the ocean, it soon dies. Its eggs behave similarly. If to the solution of pure sodium chloride the ions of calcium, barium, strontium, magnesium, lead, cobalt, ferrous iron, zinc, manganese, chromium or aluminium, are added, the injurious effect of the sodium chloride is combated successfully. On the other hand, the ions of mercury, copper, cadmium, nickel, and ferric iron are without influence.

The dependence of the cell-function upon the nature of the salts present, and the antagonistic action of different salts, are shown very well by the following experiment: A salt solution of the concentration corresponding to sea-water is poisonous to the eggs of the fundulus. Calcium and magnesium exert no recognizable effect upon the eggs. If a fundulus egg be placed in a pure aqueous solution of either of the two last-mentioned salts, it does not develop. Development takes place, however, immediately on adding a sodium salt to the solution.

At this place, we will recall the experiments of Martin H. Fischer.[2] By the injection of a $\frac{1}{6}$ molecular solution of common salt into the veins, he was able to produce a glucosuria which in its entire behavior corresponded to that produced by the diabetic puncture. Such a glucosuria can, in the case of rabbits, be prevented by adding a little calcium chloride to the solution which is injected (975 cubic centimeters of $\frac{1}{6}$ molecular NaCl solution + 25 cubic centimeters of $\frac{3}{8}$ molecular $CaCl_2$ solution). After the elimination of sugar has ceased, it can be renewed by the injection of pure sodium chloride solution, and again checked by means of the calcium chloride solution.

This reciprocal effect is interesting, and its study opens up new fields of investigation. Each cell has evidently particular salts in specific apportionment. A disturbance of this relation by the preponderance of this salt at one time, and that salt at another time, may cause considerable trouble in the economy of the cell. For the present it is necessary for us to study the action of the individual ions separately and in artificial mixtures. As regards the proportions of the separate ions in the cells, our present methods of investigation can throw no light. It is true that by analyzing the ash, we can get some idea as to the inorganic constituents of an organ. Aside from the fact that such an analysis can never give us

[1] Jacques Loeb: Pflüger's Arch. **80,** 229 (1900). Cf. W. A. Osborne: J. Physiol. [Proc. Physiol. Soc. **33,** 10 (1905)].

[2] Pflüger's Arch. **106,** 80 (1904), and **109,** 1 (1905).

a conception of the way the different elements are combined in the cell, we need only to refer to the extremely delicate mechanism in the action of the individual ions to make one realize how far we are from understanding fully the mechanism of the cell itself.

Jacques Loeb by means of his comprehensive studies deserves great credit for having added so much to our knowledge concerning the action of salts and their reciprocal action, and especially by his work on artificial parthenogenesis.[1] At this place we can hardly take up in detail these numerous and highly interesting investigations. We shall come back to them again. A great number of such experiments have been made. Unfertilized eggs of the *Annelida* may be developed by placing them in sea-water to which a small amount of potassium salt has been added (e.g., one or two cubic centimeters of $2\frac{1}{2}$ N.KCl or KNO_3 solution to 100 cubic centimeters of salt water.) Such eggs develop apparently normal larvæ. Such a solution has no action upon the unfertilized eggs of the sea-urchin.

These experiments have been cited to show that the presence of inorganic ions is indispensable for the life-process. The manner in which they act is still unknown to us. It is possible that new light may be thrown upon this question by a study of the other components of the cell, and especially of their behavior towards the ions. Now the cells contain *colloids*, and in fact the life process itself is intimately related to their presence. There is no doubt that the peculiar physical condition of the cell-contents is of great importance to all of the different processes which take place within the cell. Indeed, this alone makes it possible for so many different reactions to take place side by side. Colloidal solutions diffuse but very slowly — in fact, scarcely at all — into one another. Increased viscosity of a medium, however, does not affect the rate of diffusions of crystalloids and electrolytes, nor the mobility of the ions, nor does it affect the degree of dissociation of electrolytes. A monomolecular reaction (e.g., the catalysis of methyl acetate by dilute hydrochloric acid) takes place in a jelly just as rapidly as in water. On the other hand, the colloid, on account of its internal friction, often prevents the formation of precipitates. This is effected, not by preventing the reaction from taking place, but rather by keeping the newly formed molecules in such an extremely minute state of subdivision that they do not come together to form visible complexes. The colloids cause an enormous increase in surface tension.

The entire conception of colloids is in a stage of rapid development. There is no way of defining precisely the part that they take in the life of the cell. Many isolated facts indicate that the future investigation of

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[1] Cf. Abderhalden: Arch. Rassen-und Gesellschaftsbiologie, Jg. I, p. 656 (1905).

colloids will undoubtedly greatly increase our knowledge concerning the work of the cell.[1] Here we are especially interested in the behavior of colloids towards ions. Hardy[2] has shown that ions exert a particular influence upon the condition of the colloid. Negatively charged colloids are precipitated by the electropositive cations, and positively charged colloids by anions. In these relations we find a new proof of how extremely delicate the whole mechanism of the cell is to enable it to maintain between the individual ions on the one hand, and the colloids on the other in such a relation that all of its functions can take place unhindered. It is perfectly clear without further explanation, that owing to the various reactions taking place within the cells, at one time the action of one ion is most prominent, while at another time it is that of a different one. The cell must always be able to neutralize at a given moment the action of any one ion. Undoubtedly physical chemistry has here pointed out new paths for further investigation, and there is no question but that it will enable us eventually to draw new conclusions along lines that have already been studied. Here again in the operations of the delicate mechanism of the cell, it is not right to attempt to distinguish between the physical chemistry and physiological chemistry of the cells. Here and there it is advisable to separate the two fields and allow them to develop individually, but again and again they must come back to a common basis and unite to form a broader field, which develops by different methods as a whole.

It is obvious from a study of the experiments already cited that salts and water are fully as important as regards the life of the cell as its organic nutriment. Just as the part played by the latter is quite a varied one, so in the same way the inorganic substances participate in a number of different processes. Although they do not furnish the body with energy, nevertheless they do come into play during the expenditure of muscular effort, whether by changes in the concentration of the solutions, or by variations in the osmotic pressure, or of the surface tension, etc.

Inorganic salts are usually present in the different foods to an extent entirely sufficient for all our demands. An exception to this general rule is the fact that man and certain animals require an additional supply of sodium chloride. Sodium chloride is the only inorganic substance which it is necessary to add to our diet. This is rather remarkable, because both animal and vegetable food already contains considerable sodium and chlorine. The explanation of this exceptional requirement in the case of

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[1] Cf. Hans Aron: Biochem. Zentr. **3**, 15, 16, 17, pp. 461 and 501 (1905). R. Zsigmondy: Zur Erkenntnis der Kolloide, Jena, 1905. H. J. Hamburger: Osmotischer Druck u. Ionenlehre in den medizinischen Wissenschaften, Wiesbaden, 1904. R. Höber: Physikalische Chemie d. Zelle u. d. Gewebe, Leipsic, 1902.

[2] Z. physikal. Chem. **33**, 385 (1900).

common salt we owe to G. von Bunge.[1] Bunge pointed out in the first place that among animals only the true herbivora, and never the carnivora, crave salt. This fact is familiar to hunters. They know that wild herbivora — ruminants and solidungulates — frequent the salt licks. Now the amount of sodium chloride which these animals obtain in their food is about the same per unit of the animal's weight as that obtained by the carnivora in its diet. It is hardly to be said, therefore, that there is a lack of sodium chloride unless we assume that this salt plays a particular part in the organism of the herbivora, an assumption which, in the light of recent investigations concerning the action of the individual ions, no longer seems so improbable. Bunge has shown, however, that vegetable food differs from animal food by the amount of potash which the former contains. The herbivora obtain three or four times as much potassium salt in the food as the carnivora do. All vegetables, especially potatoes, clover, and meadow hay, contain large amounts of potash. We know of but very few land plants which, like the varieties of *Chenopodium* and *Atriplex*, contain more sodium chloride than potassium salt. It is easy to account for the high potash content of plants by the distribution of the elements sodium and potassium on the earth's surface. By the weathering of silicate rocks, sodium carbonate is formed, which dissolves readily in rain water and trickles down into the earth. The potassium, on the other hand, remains with the other bases combined with silica and aluminium as an insoluble double salt. The latter remains near the earth's surface, while the sodium is flushed out by springs, brooks, and rivers, and carried on to the ocean. This accounts for the fact that potassium salts predominate on the surface of the earth, while the ocean is rich in sodium salts, especially the chloride.

Now why should an increased amount of potassium salts create a demand for a greater supply of sodium chloride? Bunge suggests the following ingenious explanation: If a potassium salt, for example the carbonate, comes in contact with sodium chloride, a partial decomposition takes place, a little potassium chloride and sodium carbonate being formed. Now, as regards the inorganic salts of the blood-serum, sodium chloride ranks foremost. It is found there to a considerable extent, and, as far as we know, the amount is kept fairly constant. Now, on bringing the serum in contact with the abundance of absorbed potassium salts obtained from the vegetable nourishment, this double decomposition between the sodium salt in the blood and the absorbed potassium salt will take place to some extent. In this way potassium chloride is formed, and a part of the sodium of the blood combines with the acid which was previously united with the potassium. By this process the composition of the blood is changed.

[1] Z. Biol. **9**, 104 (1873); **10**, 111, 295 and 323 (1874); Lehrbuch der Physiologie der Menschen, Bd. ii, p. 103 (1901).

The serum now contains a substance which did not occur in it previously, or at any rate not to the same extent as now; namely, the newly formed sodium salt. It is the duty of the kidneys, as we shall see later, to keep guard over the serum and to regulate its chemical composition. They eliminate every constituent which under normal conditions is foreign to it, and take away any excess of compounds which belong there. In the above case the kidneys eliminate the newly formed sodium salt together with the potassium salt. This process, therefore, results in the serum being deprived of sodium chloride.

Bunge succeeded in testing this theory experimentally. He himself took 18 grams of K_2O as phosphate and citrate in three doses during the course of the day, and showed that as a result his body lost 6 grams of sodium chloride. This does not constitute an abnormal amount of potash salts. A man fed largely on potatoes will easily take 40 grams of K_2O into his system during the day. The loss of sodium chloride is by no means restricted to the blood. There is a constant exchange of material between it and the cells. After what we have seen with regard to the effect of the ions, we can easily understand the possibility that a diminution in the amount of sodium contained in the cells, which is in no way replaceable by potassium ions, may lead to serious disturbances. The organism, at all events, will attempt as soon as possible to restore the disturbed equilibrium.

Bunge, to support his views, cited numerous facts. He showed, for example, that in France the country folk consume three times as much salt per capita as those who dwell in cities. Now it is a fact that in the country much larger quantities of vegetables are eaten than in the city, where the diet consists largely of meat. A further support of the assumption that the vegetables rich in potassium were the cause of the increased consumption of salt, was gained by a study of people who live almost entirely upon meat; e.g., certain races of hunters, fishermen, and nomads. To gain this knowledge, Bunge read through a large number of articles on travels, and also placed himself in correspondence with travelers. In this way he established the fact that at all times and in all countries where the people subsist solely upon animal nourishment, either they have no knowledge of salt, or do not care for it, whereas in countries in which the inhabitants subsist mainly on vegetables there is always such an unmistakable craving for salt, that it has become considered as one of the necessities of life. This is the case in both the north and south polar regions. Again, in countries in which the inhabitants live on meat and rice, there is no craving for salt. Rice contains but one-sixth as much potash as wheat, rye, barley, or Indian corn, one-twentieth as much as the legumes, and only from one-twentieth to one-thirtieth as much as potatoes.

Now what happens in the case of a people subsisting chiefly on vegeta-

bles, and yet living where there is no salt supply? Such people prepare a salt of their own. Thus, Bunge [1] was able to procure a salt obtained by ignition of a plant which was used by the negroes in the southern part of Khartum, Africa, as a seasoning for their vegetable food. The analysis of this salt showed it to contain 19.27 per cent Na_2O and 4.92 per cent K_2O, or nearly six equivalents of soda to one of potash. It is interesting here to find that the natives have selected a plant (Salsola, or salt-wort) which is especially characterized by its high soda content. The natural instinct, however, does not always assert itself so well in this direction, for there are other races which use for salt the ash of a plant rich in potash. Lapicque [2] examined such a salt. The inhabitants of the Angoni district in British Central Africa use a substance prepared by burning goat manure and wood. Analysis showed that it contained 21.98 per cent KCl and 0.47 per cent NaCl.[3] It is interesting to learn, however, that after Lapicque had shown the natives how to obtain common salt, they gave up the preparation of their own native condiment. Salt, for the inhabitants of Angoni, is an extremely expensive article of commerce. The natives toil for salt upon the plantations.

The assumption that the high potash content of vegetable foods causes losses in the sodium content of the blood and indirectly of the tissues, is not in agreement with certain observations. Thus, Landsteiner [4] fed a number of young rabbits exclusively upon meadow hay for 3½ months. At the same time another lot of similar animals was fed entirely with cow's milk, which contains for one equivalent of soda only 0.7 to 3.7 equivalents of potash. Now although these two series of animals were fed with nourishment containing quite different relative amounts of alkali, nevertheless, at the end of the experiment the soda and potash content of the blood was the same in each case. We know, furthermore, that in spite of the fact that rabbits and hares live on fodder rich in potash, they do not show the slightest craving after salt, and under normal conditions do not obtain any in addition to what their food contains. It is possible that the organism of these animals may be different in some way, so that the loss of sodium is avoided. On the other hand, it is a well-known fact that purely herbivorous animals, such as cows and sheep, can subsist upon fodder rich in potash for a long time without any extra salt, and there is no recognizable disturbance in the development of these animals. It is indeed possible, and even probable, that the potassium salts contained in the fodder do not have such a marked effect as pure potassium chloride, when taken by itself into the system at one time and absorbed as such.

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[1] Z. Biol. **41,** 484 (1901).
[2] L'Anthropologie (1896).
[3] Abderhalden: Pflüger's Arch. **97,** 103 (1903).
[4] Z. physiol. Chem. **16,** 13 (1892).

We perhaps do not yet know all the different ways in which potash can be eliminated. We shall see that for the heavy metals the intestines are an important vehicle for their elimination. It is even possible that the liver regulates the amount of potassium salts, holding a part back so that the blood does not at any one time come in contact with large amounts of them. At all events, the influence of potassium salts contained in the food upon the elimination of sodium salts by the urine must be tested with some food, such as potatoes, which is rich in potassium salts. The experiment should extend over a considerable period in order to determine whether any loss of sodium chloride is permanent or only temporary.

The organism must in every case have ways and means for keeping the soda and potash content of the blood constant in spite of variations in the food supply. It is worthy of mention that the serum of all species of animals [1] which have been investigated up to the present time always contains the same amounts of these two elements; the serum of the carnivora, as well as that of the herbivora, contains about 0.43 per cent of soda, and 0.026 per cent of potash. Perhaps the fact that the red corpuscles of the ruminants, in contrast to those of the horse, cow, rabbit, etc., contain considerable amounts of soda, may shed some light upon the fact that the former crave salt, while the latter do not. To be sure, the red corpuscles of the carnivora also contain larger amounts of soda. It is very interesting that in the milk of carnivora the two alkalies are present in approximately equivalent amounts, whereas in the milk of the herbivora and in human milk the potash predominates. The organism of the herbivora and of the carnivora, corresponding to their later nourishment, thus early becomes accustomed to a definite relation between the amounts of potassium and sodium. The beasts of prey, which live upon the entire animal, obtain sodium and potassium in almost equivalent amounts. On the other hand, the herbivora and the human race receive in many foods the two bases in the same relative amounts as in milk; some kinds of hay contain three equivalents of potash to one in soda, while in milk there are from one to six equivalents of potash to one of soda. We may, indeed, assume that the organism is adjusted to the general preponderance of potash over soda, and that disturbances take place only when the customary relation is changed greatly at the expense of the sodium, as would, for example, be the case if the food consisted entirely of potatoes. Rye, peas, and beans likewise contain very considerable amounts of potassium, as the following table prepared by Bunge shows:

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[1] Abderhalden: Z. physiol. Chem. **25**, 65 (1898).

1 Equivalent Na_2O Corresponds to:

	Equivalents K_2O.		Equivalents K_2O.
Beef-blood	0.07	Oats	15 to 21
White of hens' eggs	0.7	Rice	24
Yolk of hens' eggs	1.0	Rye	9 to 57
Organism of mammals	0.7 to 1.3	Hay	3 to 57
Milk of carnivora	0.8 to 1.6	Potatoes	31 to 42
Human milk	1 to 4	Peas	44 to 50
Milk of herbivora	0.8 to 6	Strawberries	71
Beef	4	Clover	99
Wheat	12 to 23	Apples	100
Barley	14 to 21	Beans	110

Bunge's conception that common salt widens the circle of our food supply, may well be a correct one. It makes it possible for us to enjoy potatoes and many other foods which are rich in potash.

As stated before, our ordinary food contains sufficient quantities of the inorganic salts, and it is, in general, not to be feared that too little of one or another salt will be taken into the system.

Our diet is ordinarily a mixed one. If one article of food or another contains too little of any salt, the deficiency is made up by something else that is eaten. The fact that an exclusive diet of substances lacking in this or that salt may lead to disturbances will be shown later. Now, although it be granted that we need take no thought concerning the supply of inorganic material required by adults, the question arises whether the customary food of growing individuals likewise satisfies the requirements of the organism. This is a justifiable question, particularly in the case of mammals, and especially human children; for right in the midst of their growing period a change takes place in their food which compels us to compare the composition of their first food, the milk, with that which is eaten subsequently. The amount of inorganic salts contained in milk must give us some idea as to the requirements of the young. The following table gives a summary of the contents of the ash from human milk and that of certain animals:[1]

Species.	100 Parts by Weight of Milk Contain in Grams:						
	K_2O	Na_2O	Cl	Fe_2O_3	CaO	MgO	P_2O_5
Man	0.0795	0.0253	0.0468	0.0008	0.0489	0.0065	0.0585
Dog	0.1382	0.0779	0.1656	0.0020	0.4545	0.0195	0.5078
Pig	0.0945	0.0776	0.0756	0.0040	0.2489	0.0157	0.3078
Sheep	0.0967	0.0864	0.1297	0.0041	0.2453	0.0148	0.2928
Goat	0.1302	0.0617	0.1019	0.0036	0.1974	0.0154	0.2840
Cow	0.1776	0.0972	0.1368	0.0021	0.1671	0.0231	0.1911
Horse	0.105	0.014	0.031	0.002	0.124	0.013	0.131
Guinea pig	0.0754	0.0700	0.0999	0.0013	0.2417	0.0241	0.2880
Rabbit	0.2516	0.1980	0.1355	0.0020	0.8914	0.0552	0.9966

[1] Emil Abderhalden: Z. physiol. Chem. **26**, 487 and 498 (1899); **27**, 408 and 356 (1899).

A glance at the above table shows that the composition of milk varies with different animals. The values also vary in the case of different animals of the same species, but during the suckling period the variation is only within narrow limits. We shall find that the amount of ash and also of the inorganic material bears a certain relation to the rapidity with which the tissue is formed as shown by the body weight.

It is interesting now to trace the relations between the composition of the milk (especially that of its ash) and that of the suckling. It is of course obvious that such a comparison will serve to give us merely a rough idea of these relations, for our present methods of analysis are not sufficiently delicate for us to attempt to explain the way in which the different elements are combined. The analysis of the ash, for example, merely shows us what inorganic material is present, and gives us absolutely no conception of the manner in which these elements are combined in the body. We do not know from this whether the phosphoric acid that we find was present entirely as calcium phosphate or other phosphates, or whether it represents in part lecithin. At the same time the knowledge of the constituents of the ash forms a basis for further investigation, and with the above-mentioned limitations gives us some means for comparison.

Now it is a striking fact, as the figures below will show, that milk has such a different composition from the elements out of which it is formed; namely, the blood, and especially the blood-serum. The cells of the milk-glands must possess the power of selection. Now, what determines the composition of the milk? Bunge,[1] with reference to this question, compared the composition of milk-ash with that of the suckling itself, and found in the case of dogs the following relations:

	100 Parts by Weight of Ash Contain in Grams:			
	Dog a Few Hours Old.	Dog's Milk.	Dog's Blood.	Dog's Serum.
K_2O	11.14	15.0	3.1	2.4
Na_2O	10.6	8.8	45.6	52.1
CaO	29.5	27.2	0.9	2.1
MgO	1.8	1.5	0.4	0.5
Fe_2O_3	0.72	0.12	9.4	0
P_2O_5	39.4	34.2	13.3	5.9
Cl	8.4	16.9	35.6	47.6

[1] Z. physiol. Chem. **13,** 399 (1889) and Arch. Anat. Physiol. **1886,** 539.

With rabbits the following values were obtained:[1]

	100 Parts by Weight of Ash Contain in Grams:			
	Rabbit 14 Days Old.[2]	Rabbit's Milk.	Rabbit's Blood.[3]	Rabbit's Serum.[3]
K_2O	10.84	10.06	23.75	3.19
Na_2O	5.96	7.92	31.38	54.72
CaO	35.02	35.65	0.81	1.42
MgO	2.19	2.20	0.64	0.56
Fe_2O_3	0.23	0.08	6.93	0
P_2O_5	41.94	39.86	11.11	2.98
Cl	4.94	5.42	32.66	47.83

In the case of the guinea pig we find:[4]

	100 Parts by Weight of Ash Contain:	
	New-Born Guinea Pig.	Guinea Pig's Milk.
K_2O	8.22	9.69
Na_2O	6.94	8.99
CaO	32.21	31.1
MgO	3.09	3.1
Fe_2O_3	0.23	0.17
P_2O_5	42.25	37.02
Cl	9.1	12.84

Camerer and Söldner[5] have made a similar comparison between human milk and the ash of infants:

	100 Parts by Weight Contain:				
	Infant.	Milk.		Infant.	Milk.
K_2O	7.8	31.4	Fe_2O_3	0.8	0.6
Na_2O	9.1	11.9	P_2O_5	38.9	13.5
CaO	36.1	16.4	Cl	7.7	20.0
MgO	0.8	2.6			

[1] Abderhalden: Z. physiol. Chem. **26,** 498 (1899).
[2] G. v. Bunge: Z. Biol. **10,** 323 (1874).
[3] Abderhalden: Z. physiol. Chem. **25,** 65 (1898).
[4] Abderhalden: *ibid.* **27,** 356 (1899).
[5] Z. Biol. **40,** 526 (1900); **41,** 37 (1900); **44,** 61 (1903).

L. Hugonnenq[1] obtained quite similiar values:

	100 Parts by Weight Contain:				
	Human Fœtus.	Human Milk.		Human Fœtus.	Human Milk.
K_2O	6.20	35.15	Fe_2O_3	0.39	0.18
Na_2O	8.12	10.43	P_2O_5	35.28	21.30
CaO	40.48	14.79	Cl	4.26	10.75
MgO	1.51	2.87			

Except as regards the composition of the human infant and human milk, we find by a comparison of the corresponding values that there is a striking agreement between the ash of the young animal and that of the milk. In the case of human beings, however, we do not find any such agreement. Bunge explains this fact by the assumption that the ash content of milk has not only the task of building up tissue, but also serves in the preparation of the excreta, especially the urine. The more rapid the growth of the suckling, the less apparent will be the influence of the latter function. It is, therefore, in general not to be expected that the percentage composition of the milk and that of the infant will agree so closely in the case of human beings as with animals, such as dogs, rabbits, and guinea pigs, which require the mother's milk for but a short time after birth, but are soon placed upon a diet of green fodder. It is easy to see that a milk corresponding closely to the chemical composition of the young as regards the inorganic constituents will be more suitable for animals which develop very rapidly, whereas with species which develop more slowly, the building up of the separate tissues does not take place so uniformly and there are not so many changes taking place at the time when the growing organism changes to another source of nourishment.

We now come back to our first question: Does the suckling when it abandons the mother's milk and passes to other food receive a sufficient supply of inorganic salts? The following table gives a summary of the amounts of inorganic substances contained in the more important foods:[2]

	100 Parts by Weight of Dry Substance Contain:						
	K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	Cl
Honey	**0.80**	0.0	0.007	0.04	0.002	0.09	0.05
Beef	1.66	0.32	0.029	0.15	0.024	1.83	0.28
Rye	0.61	0.01	0.062	0.22	0.007	1.03	0.03
Wheat	0.62	0.06	0.065	0.24	0.008	0.94	...
Potatoes	2.28	0.11	0.100	0.19	0.009	0.64	0.13
White of egg	1.45	1.45	0.130	0.13	0.000	0.20	1.32
Pear	1.13	0.03	0.137	0.22	0.009	0.99	...
Human milk	**0.58**	**0.17**	**0.243**	**0.05**	**0.004**	**0.35**	**0.32**
Yolk of eggs	0.27	0.17	0.380	0.06	0.024	1.90	0.35
Cow's milk	1.67	1.05	1.511	0.20	0.003	1.86	1.60

[1] Compt. rend. **128**, 1419 (1899). Cf. Cornelia de Lange: Z. Biol. **40**, 526 (1900).

[2] Bunge: Z. Biol. **45**, 532 (1902).

It is evident from these values that most of the foods are deficient in lime alone, as compared with milk. Now while we are not justified in assuming that the lime-content of milk is to be considered as the normal amount required for man's later development, still, on the other hand, we must not forget that probably the "law of the minimum" holds for animal organisms as well as for plants. Now lime plays a quite particular part in the development of the organism, especially for certain tissues, the bones and teeth. It is clear, therefore, that the system must have an adequate supply of the lime at its disposal. Of course the fully developed organism requires lime as well, for even then, as we have already seen, there is a constant building up and wearing down of tissue, and especially of bony tissue. The following table gives the amount of lime present in many of our foods. At the same time the amounts of iron they contain are also given. The values refer to 100 grams of substance dried at 120° C. They are arranged with increasing lime-content.

	In Milligrams:			In Milligrams:	
	CaO	Fe		CaO	Fe
Sugar	0	0	White of egg	130	0
Honey	7	1.2	Red cherries	136	1.2
Beef	29	16.9	Peas	137	6.4
Pig's blood	33	225.7	French plums	154	1.8
White bread	46	1.5	Plums	166	2.8
Malaga grapes	60	5.6	Huckleberries	196	6.4
Wheat	65	5.5	Human milk	243	2.3 to 3.1
Rye	62 to 71	3.7 to 4.9	Yolk of egg	380	10 to 24
Apples	66	1.9	Figs	400	4.0
Graham bread	77	5.6	Wild raspberries	404	3.7
Pears	95	2.0	Oranges	575	1.5
Potatoes	100	6.4	Cabbages (light green leaves)	717	5.6
Rice	103	1.0 to 2.0	Wild strawberries	873	8.1 to 9.3
Dates	108	2.1	Cow's milk	1510	2.3
Black cherries	123	1.9			
Cocoa beans	126	2.5			

These values show that it is by no means immaterial what food the infant receives at the time it leaves the mother's breast. When the child is six months old, it takes about one liter of milk daily. This amount contains, in round numbers, about 0.5 gram of lime. We do not know exactly how much lime the nursing infant requires at the time it is weaned. We can safely assume, however, that in the later periods of its growth, it requires relatively less. For one thing, the development takes place more gradually than is the case shortly after birth; and, again, the absolute amount of lime taken into the system increases with the quantity of food eaten. The following figures will give some idea of the rapidity of the development shortly after birth. Rabbits double their weight at the

end of six or seven days.[1] It takes nine days for dogs to accomplish the same result; at the end of eighteen days the weight was three times that at birth. With cats it requires nine or ten days for the weight to become doubled; in one case the weight was three times as much at the end of 19½ days, and four times as much at the end of 29½ days. Pigs develop less rapidly. On an average, it requires 14 days for the original weight to be doubled; with sheep 15 days, goats 32 days, calves 47 days, and 60 days in the case of a colt. The slowest development is shown in the case of the human offspring, which does not double its weight until about six months have passed away. Observations upon the cat show that the rate of development decreases with age. It is particularly striking only at the time of birth. The case of guinea pigs is not without interest. According to their development, they scarcely belong in the ranks of the mammalia; by eating green food shortly after birth, they rapidly increase in weight. At birth they are already remarkably well developed. Even then they are able to eat the same food as that of the mother, and thrive on cabbage, etc. The female of this animal possesses only two mammary glands, situated in the groin, and milk plays but a subordinate part in the nourishment of the new-born guinea pig. The fact that the first development of these animals takes place quite as rapidly as in the case of the most closely related animals, leads us to the assumption that in early times these animals came into the world in a much more undeveloped condition, and were forced to depend upon milk for nourishment, like other mammalia.

It has often been suggested that rickets, a quite common children's disease, is caused by a lack of lime-salts in the nourishment. In fact, this disease appears most frequently when the mother's milk for some reason is replaced by some other form of nourishment. There is no doubt that in considering the value of a food for replacing the mother's milk, too much stress has been laid upon the amount of fat, proteid, and carbohydrate. Certainly a mistake is being made unless equal attention is paid to the amount of inorganic substances contained in the nourishment. According to general experience, it is not possible to replace the mother's milk satisfactorily by the milk of some other animal. It is necessary to add something to cow's milk in order that there may not be any substance present in less than the proper amount. Then again it is particularly erroneous to judge the value of a food used to replace the mother's milk by its calorific value. We must never forget that the suckling must build up its tissue first of all. It is, therefore, by no means a matter of indifference whether this or that organic substance is relegated to the background, whether fat or carbohydrate. For the growing suckling, carbohydrates and fat cannot be considered as equivalent in this sense. They are

[1] Abderhalden: Z. physiol. Chem. **26,** 487 (1899); **27,** 408 and 594 (1899).

isodynamic only as regards their combustion value. The law of isodynamics would undoubtedly hold in the case of sucklings in this direction were it not for the fact that its body substance is increased so largely that it is necessary to arrange its diet according to particular lines. We must, to be sure, admit that by the transformation of carbohydrates to fat, and perhaps also by the reverse process, one of these nutrients may replace another, even in the construction of the cells.

The assumption that rickets is caused by a lack of lime in the nourishment is contrary to numerous observations. Rickets appears sometimes with a food that is rich in lime, even when the child is being fed upon mother's milk, though much less frequently than with other food. It might be thought that there still may be a deficiency in lime, and perhaps on account of the fact that, owing to a disturbed process of absorption, an insufficient supply of lime becomes available to the tissues. This brings us to the question as to the state of the lime when it is absorbed and assimilated.

We meet here with one of the most remarkable circumstances in the entire subject of physiological nutrition. Whereas our knowledge concerning the occurrence of organic nutrients is considerable, we know very little concerning the way in which the inorganic substances are combined in the food. We do not know whether they are present as inorganic salts, — in the animal and vegetable tissue, — or whether complicated organic compounds are at hand which contain these inorganic elements in a state of more or less firm combination. It is conceivable that lime, for example, can take part in the construction of tissue only when it is present in a definite state of combination. Such an assumption was especially justifiable at the time when it was not recognized that it was possible for the human organism to accomplish syntheses. Now that we have seen, however, that the animal cells are capable of accomplishing most complicated syntheses, it becomes more and more probable that they are also able to make use of inorganic salts in the formation of their tissue.

Although we know very little concerning the way in which lime is contained in the ordinary foods, still, on the other hand, it is to be expected that an explanation of the way lime is present in milk will throw the most light upon this question. There are a number of possibilities to consider. It may be that the lime is in some way combined with the protein in milk, or that it may be dissolved in it in the form of an inorganic salt. At all events, the fact that the lime is not present in any firm state of combination is proved by the following experiment performed by Bunge.[1] On diluting cow's milk with water and precipitating the casein by careful addition of acetic acid in the cold, only a trace of lime is found in the albuminous

[1] Z. Biol. **45**, 532 (1901).

precipitate. By boiling and concentrating the filtrate, the globulin and albumin of milk are obtained. These proteins likewise contain but little lime. It is not impossible, but quite probable, that the small amounts of calcium contained in these precipitates are merely carried down mechanically, without there being any state of combination between the albumin or globulin and the calcium. The greater part of the lime is found in the filtrate from the two precipitations. After the removal of the casein and of the two other protein substances, the addition of oxalic acid at once causes the formation of a precipitate. The filtrate from this last precipitate of calcium oxalate was evaporated to dryness and ignited with sodium carbonate in order to remove the calcium from any organic substance which might not have been precipitable by oxalic acid. This ash, however, contained merely a trace of calcium. This shows that the calcium, if originally combined with protein, must be present in some loose salt-like combination such that even dilute acetic acid suffices to set it free. It has, however, not been established definitely whether some other organic substance in the milk may not serve to keep calcium in solution in the presence of phosphoric acid.[1]

From these observations of Bunge we may say that it is undoubtedly true that lime is absorbable and assimilable as such. Against this assumption the objection may be raised that it is possible that the lime in the intestine first of all enters into combination with some organic substance and is then ready for absorption, and that the latter process depends entirely upon the formation of such an organic compound. On the other hand, it may be said that even in such a case the manner of combination in the alimentary canal must be a weak one, for it is not to be assumed that any sort of firm combination is formed. Such a loose form of combination would have the effect of keeping the calcium in solution, but it is perfectly certain that they have no other effect upon the process of assimilation. We cannot be wrong in assuming that every calcium compound which can be converted into a soluble condition in the intestine is capable of being absorbed and assimilated.

We have up to now found no reason for believing that the disease of rickets is due to a diminished capacity for absorption on the part of the alimentary canal.[2] It is far more probable that the cause of the faulty calcification of the bones is due to a diminished assimilation of lime. It is highly probable that the cause of this is not to be sought in an unsuitable condition of the calcium salts that are at the disposal of the tissues. It is

[1] L. Vaudin, Ann. inst. Pasteur, **8**, 502 (1894), has observed that the amount of citric acid in milk is proportional to the lime content. It is altogether out of the question to believe that the lime is simply dissolved by this acid, but possibly citric acid in conjunction with other organic substances may serve to keep the lime in solution.

[2] T. G. Rey: Deut. med. Wochschr. **35**, 569 (1895).

much more likely that the function of those cells whose duty it is to assimilate the required calcium does not exert itself normally. All the facts known concerning the bones of children who have suffered from this disease agree best with this conception. Above all there is a striking over-production on the part of the osteoplastic tissue. This results in the formation of soft bones deficient in lime, the so-called " osteoid tissue." At the same time there is an abnormal resorption of the tissue already formed. We cannot be far wrong, if we attribute the disease of rickets to a metabolic disturbance on the part of a certain group of cells; and here again the cells themselves stand in the foreground as organs of assimilation. We may say in this connection that it is not the cells which play the chief part in the assimilation of lime. The other component, which we cannot yet sharply formulate according to our present knowledge, and therefore designate in general by the term "plasma," is equally important. We have said nothing concerning the condition of the lime in the cells; and, in fact, we are not able at present to depict the calcification process which takes place in the formation of bones. We do not know whether the osteoidal tissue formed in the disease of rickets is capable of taking up lime at all, or whether it is able to deposit calcium salts from their solutions. If we recall what was said concerning the mutual relations of the individual ions, we shall be tempted to attribute the over-production of osteoidal tissue to some such influence.[1] We have seen that calcium chloride, for example, acts antagonistically towards sodium chloride, and have drawn the conclusion from all such observations that only by means of the inorganic substances in the cell acting together do we have any guarantee for the normal exercise of the functions of the cell. If any one element is missing, a disturbance must necessarily follow. A certain amount of opposing force is lost, and thus the action of a certain ion or a group of ions exerting a similar effect may be felt. With these suggestions we will merely state that all our present knowledge concerning rickets leads us to the conclusion that the disease cannot be attributed to a lack of lime in the diet of the child, and to-day we have no reason for assuming that the lime is present in the cells of those who are suffering from this disease in a form which the cells cannot assimilate. The cause of rickets is doubtless much more deeply seated, and is to be traced to the organization and metabolism of the cells and tissues concerned in the process of bone production. The whole course of this disease, which in fact is usually " outgrown," is in accordance with this view. It is not to be assumed

[1] Cf. Clowes and Frisbe, Am. J. Physiol. **14**, 173 (1905), who have found that a rapidly developing *adeno carcinoma* in mice contains considerable potash and less lime. Slow development of the tumor shows the reverse proportions. It is not possible to draw definite conclusions at present as to which process is primary and which secondary in nature, but studies in this direction certainly warrant attention.

that more lime is received in the later years, for, as a matter of fact, milk, which is invariably the basis of infant diet, contains more lime than almost any other article of food, so that the infant receives relatively more lime than at any subsequent time.

It is to be expected that the formation of the bones will be seriously affected if the lime in the food is intentionally made inadequate. Thus when Forster [1] and likewise Voit [2] fed young dogs with meat, fat, and water, free from calcium, a faulty bone-formation was soon apparent. Chossat,[3] and later on Voit,[4] observed that fully developed pigeons, which were made to subsist for a year exclusively upon washed wheat grains and distilled water, showed a deficient skeleton. The bones were very fragile, the skull and breast-bone being very thin, and penetrated with sieve-like perforations. These experiments merely prove that the bones require lime for their development. Such experiments have no connection at all with the disease of rickets. Particularly, the observations made with the pigeons remind one very much of osteoporosis, a morbid absorption of bone which often takes place in elderly people, especially in the region of the skull, and is obviously due to inadequate nourishment of the bony tissue.

Up to this point we have only traced the metabolism of the animal organism in two periods, namely, during growth and after the organism is fully developed. An especial observation with regard to the content of the food in inorganic salts is furnished by the organism of the mother during pregnancy and during lactation. At this time the organism of the child develops exclusively at the expense of the mother. If the material received by the mother in the food during this period is not sufficient, then the stores of her organism are attacked, and finally her own tissue is subject to resorption. The fœtus develops continually even when the mother is starving. Remarkable migrations of substance must take place during this entire process. Our knowledge concerning these relations is still very incomplete. The following table prepared by Hugonnenq [5] gives an idea of the amount of inorganic material taken up by the fœtus during its development.

We see from these values that towards the end of pregnancy there is suddenly a marked increase in the amount of inorganic material taken up by the fœtus. During the last three months it takes up almost twice as much inorganic material as during the first six months of its development. Altogether it withdraws about 100 grams of ash constituents from the

[1] Z. Biol. **9**, 369 (1873); **12**, 464 (1876).
[2] *Ibid.* **16**, 85 (1880).
[3] Compt. rend. **14**, 451 (1842).
[4] Ber. Vers. Deut. Naturforscher, München, **1877**, 243.
[5] Compt. rend. **128**, 1054 (1899).

mother. Of iron it takes up 0.294 gram (0.42 gram Fe_2O_3). Here again the greater part is taken up during the latter part of the period of pregnancy. This gives one the impression that the fœtus is probably provided with reserves in order to fit it for all contingencies which may arise with regard to its nourishment after the birth.

Age of the Fœtus.	Sex.	Weight		Fe_2O_3	
		of the Foetus in kilograms.	of the Ash in grams.	of the Entire Organism.	Calculated to 100grams Ash.
4½ months	♀	0.52	14.00	0.06	0.43
5 months	♀	0.57	18.72	0.06	0.33
5 months	♀	0.80	18.36	0.07	0.40
5 to 5½ months . . .	♀	0.12	28.07	0.11	0.38
5½ months	♀	1.29	32.98	0.13	0.38
6 months	♀	1.17	30.77	0.12	0.39
Towards end of pregnancy	♂	2.72	96.76	0.38	0.40
Towards end of pregnancy	♂	3.30	106.16	0.42	0.40

After its birth the child still receives nourishment at the expense of the mother. Milk now affords the vehicle for the transference of material. An idea of the amounts of material which the mother has to furnish the child is shown by the following figures: A male nursling takes about one liter of milk per day at the age of six months. This contains the following amounts of separate constituents:[1] Water 875.8 grams, casein 8.0 grams, albumin 12.1 grams, fat 37.4 grams, milk-sugar 63.7 grams, ash 3.0 grams. The ash is composed of 1.08 grams potash, 0.28 gram soda, 0.50 gram lime, 0.07 gram magnesia, 0.007 gram ferric oxide, 0.66 gram phosphoric acid, 0.53 gram chlorine.

This increased output is naturally felt by the organism of the mother, and it must be taken into account in the choice of her food. Here again it would be altogether wrong, as regards the nourishing of the nursling, to regulate the diet of the mother with regard to the calorific value of the food. It is the chemical composition of the food which is of utmost importance, for the milk must provide the infant not only with combustible matter, but above all with building material for its cells. Although the organism of the child can probably utilize carbohydrates for the production of fat, and can perhaps form sugar from albumin, it is impossible to effect such transformations in the case of inorganic salts. Even the assumption that the place of one salt may be taken by another one which

[1] Averages from 173 analyses. See J. Koenig: Die menschlicher Nahrungs-und Genussmittel, Berlin, **1904.**

is closely related to it, is not justifiable, for we have seen that the ions have a quite specific action. For this reason it seems as if more attention should be paid to the inorganic nature of the ash of milk, or of any milk-substitute, in considering its value as food for the child.

It is not right, however, to lay particular stress upon any one inorganic salt. At present we are not able to judge the relative merits of the different salts. As we have seen, almost all of the inorganic substances, with the single exception of lime, are present in sufficient quantities in the ordinary articles of diet.

One hundred grams of dry substance contain the following amounts of lime in milligrams: [1]

Beef	29	Plums	166
White bread	46	Human milk	243
Graham bread	77	Yolk of eggs	380
Potatoes	100	Strawberries	483
Peas	137	Cow's milk	1510

This little summary shows that a diet consisting chiefly of meat is not suitable for the nursing mother. Rich in lime are the yolk of eggs, and especially cow's milk. We can well imagine, *a priori*, that the organism of the mother during the entire period when the child receives its nourishment at her expense will suffer materially if there is lack of lime in her food, and in such cases she will be obliged to draw upon her own supplies of lime to furnish the child with the amount that it requires.

Osteomalacia, a disease in which the bones gradually lose their solid constituents and finally become thin as parchment, soft and flexible, occasionally occurs during pregnancy. It would seem probable that this disease bears some relation to the increased requirement of lime-salts on the part of the organism of the mother. The child develops at the expense of the mother's tissue. All that we know concerning the disease, however, is contrary to this assumption. It occurs more frequently in certain localities.[2] Its appearance is not restricted to the period of pregnancy. It is at such a time, however, that the symptoms are most pronounced, and usually the disease then progresses more rapidly. The histological study of osteomalacial bones shows that it is not lime alone that the bones have lost. It is true that one of the most noticeable changes is the decalcification of the individual lamellæ; but at the same time there takes place, with varying intensity, a new formation of osteoidal tissue. The assumption that this decalcification of the bones probably does not stand in any direct relation to the development of the fœtus is supported

[1] G. von Bunge: Z. Biol. **41**, 155 (1900).

[2] Cf. L. Gelpke: Die Osteomalakie im Ergolztale, Basel, 1891.

by observations of the metabolism which takes place during the disease. Thus Goldthwait, Painter, Osgood, and McCrudden[1] found that when the nourishment contained 4.56 grams of lime there was an elimination of 3.859 grams by the urine and of 1.80 grams by the fæces. Thus the organism lost 1.10 grams of lime in the process. Hand in hand with the decalcification, a formation of an organic substance takes place. This substance is characterized by a high sulphur and a low phosphorus content. Curiously enough, while the calcium is being carried away from the organism, magnesium is being held back. Considering all these facts together, we are led to the conclusion that the disease is not caused by a giving up of lime to the organism of the child, but that evidently there is a severe metabolic disturbance of the bony tissue which naturally must be influenced indirectly by the important transformations which are taking place in the entire metabolism of the organism of the mother due to the presence of the new being. But just as in the disease of richitis the lime plays a more or less passive part, it is indeed highly probable that here again the absence of lime is not directly responsible for the trouble, but that the loss of lime takes place secondarily as a result of the disease. The lime is loosened from its state of combination in the bones, and is eliminated as refuse out of the system. The primary trouble is a disturbance in the economy of the bony tissue. It has been frequently suggested that the cause of the decalcification is due to the appearance of acids. This was deduced from the fact that lactic acid is found in the urine of those suffering from osteomalacia. The appearance of the lactic acid, however, does not prove anything in this direction. In fact, cases of the disease are known in which no lactic acid could be detected in the urine; and we know, furthermore, that the acid may appear in the urine for quite a number of different reasons without the lime-content of the bones being affected at all. The appearance of lactic acid in the urine does not indicate where the acid originates. There is absolutely no foundation for the assumption that the acid in osteomalacia is formed in the bony tissues and serves to dissolve out the lime.[2]

Fehling's[3] observation, that removal of the ovaries serves to check the disease, has shed a peculiar light upon the nature of the disease. After this operation, lime is once more retained by the system, and the newly formed osteoidal tissue calcifies. At present we can merely assume that the loss of the ovaries brings back the metabolism to normal paths. We

[1] Am. J. Physiol. **14,** 389 (1905).

[2] Cf. C. Schmidt, Ann. **61,** 329 (1847). Moers and Muck, Deut. Arch. klin. Med. **5,** 485 (1869). Nencki and Sieber, J. pr. Chem. **26,** 43 (1882). M. Levy, Z. physiol. Chem. **19,** 239 (1894).

[3] Arch. Gynäk. **39,** 171 (1891); **48,** 472 (1895). Cf. also Winckel: Sammlung klinischer Vorträge N. F. No. 71.

may suspect that the ovaries have previously produced something which has caused the metabolic disturbance. Such an hypothesis, however, has not up to the present time been established experimentally. We must for the present be content with a knowledge of the observed facts, and await a fuller explanation of the peculiar mutual action between the ovaries and the bony tissue as a result of further investigation.

LECTURE XVII.

INORGANIC FOODS.

II.

We have started with milk as a standard for determining the requirements of the animal organism as regards inorganic material. This is justifiable inasmuch as it is certain that milk contains in proper proportions all the inorganic salts which are necessary for the development of the growing individual. Under ordinary conditions there is little fear of an insufficient supply of these elements in the case of adults, except, of course, during periods of pregnancy and of lactation. Our ordinary mixed diet contains a sufficient amount of all the inorganic substances, even when on account of social reasons the nourishment is obtained from material which is not of full value. We shall come back to this point.

In comparing the composition of milk with that of the other foods, we left one important fact unmentioned; namely, the relatively low iron content. There are, in fact, but few articles of food which contain less iron than milk, as is shown by the following table arranged with increasing iron content:[1]

	Milligrams of Iron per 100 Grams Dry Substance.		Milligrams of Iron per 100 Grams Dry Substance.
Sugar	0.0	Plums	2.8
Egg albumin	0.0	Raspberries	3.7–3.9
Honey	1.2	Figs	3.7–4.0
Red cherries	1.2	Shelled hazel nuts	4.3
Rice	1.0–2.5	Rye	3.7–4.9
Scotch barley	1.4–1.5	Cabbage (etiolated leaves)	4.5
Oranges	1.5	Barley	4.5
White bread	1.5	Shelled almonds	4.9
Wheat flour	1.6	Wheat	5.5
Greengage	1.8	Malaga grapes	5.6
Black cherries	1.9	Cabbage (light-green leaves)	5.6
Apples	1.9	Huckleberries	5.7–6.4
Pears	2.0	Potatoes	6.4
Dates	2.1	Peas	6.2–6.6
Cow's milk	2.3	Beans (white)	8.3
Human milk	2.3–3.1		
Cocoa beans	2.5		

[1] G. von Bunge: Z. Biol. **45**, 532 (1901). Cf. Häusermann: Z. Physiol. Chem. **23**, 555 (1897).

	Milligrams of Iron per 100 Grams Dry Substance.		Milligrams of Iron per 100 Grams Dry Substance.
Carrots	8.6	Asparagus	20.0
Wild strawberries	8.1–9.3	Yolk of eggs	10–24
Wheat-bran	8.8	Cabbage (outer dark-green leaves of the head)	17–38
Lentils	9.5	Spinach	33–39
Almonds (brown skins)	9.5	Pig's blood	226
Hazel nuts (brown skins)	12.7	Hematogen	290
Dandelion greens	14.3	Hemoglobin	340
Beef	16.9		

The remarkably small amount in milk of an inorganic element to which we are accustomed to ascribe so great significance is very striking. Iron, we know, forms an important constituent of the hemoglobin. Now in comparing the composition of the ash of the suckling with that of the milk, we found that the former contained considerably more iron. This obliges us to draw the conclusion that the new-born animal is already provided with a store of iron. The organism of the female can supply its young with nourishment in two ways, — at first through the placenta, and later by way of the mammary glands. Evidently for some reason or other in the case of iron the former method is preferred. In corroboration of this Bunge[1] showed that the amount of iron contained in a new-born rabbit is greatest at the time of birth, and decreases from day to day until it reaches a minimum at the end of the period of lactation, increasing immediately as soon as the animal changes to a food richer in iron. The following table shows the results obtained by Bunge:

Age of Rabbit.	Milligrams of Iron per 100 Grams of Body Weight.	Age of Rabbit.	Milligrams of Iron per 100 Grams of Body Weight.
Embryo arranged according to weight.	6.4 8.5 9.0	13 days after birth	4.5
1 hour after birth	18.2	17 days after birth	4.3
1 day after birth	13.9	22 days after birth	4.3
4 days after birth	9.9	24 days after birth	3.2
5 days after birth	7.8	27 days after birth	3.4
6 days after birth	8.5	35 days after birth	4.5
7 days after birth	6.0	41 days after birth	4.2
11 days after birth	4.3	46 days after birth	4.1
		74 days after birth	4.6

Rabbits are fed on the milk of the mother for about three weeks. These values show that the lowest iron content coincides with the end of the

[1] Z. physiol. Chem. **16**, 173 (1892); **17**, 63 (1893).

period of lactation. If the assumption be correct that the organism of the mother ordinarily provides her young with sufficient iron before the birth, and that the percentage of iron diminishes during lactation, it would seem probable that in the case of guinea pigs there would be no such maximum iron content at the time of birth, for there is no need of such a supply, inasmuch as the animal immediately after birth begins to feed on green fodder, which is rich in iron. In fact, as Bunge has shown, this assumption is verified by the facts:

Age of the Animal.	Milligrams of Iron per 100 Grams Body Weight.	Age of the Animal.	Milligrams of Iron per 100 Grams Body Weight.
Embryo	4.6	5 days after birth	5.7
	4.4	9 days after birth	4.4
	5.6	15 days after birth	4.4
	5.3	22 days after birth	4.4
6 hours after birth	5.0	25 days after birth	4.5
1½ days after birth	6.0	53 days after birth	5.2
3 days after birth	5.4		

Here, as we expected, there is no maximum of the iron content at birth as compared with later periods; there is likewise no minimum.

Why does the animal organism prefer, as a rule, to supply the new being with such a store of iron at birth as will permit it to be satisfied with a low iron content in the milk? Bunge's idea is that the assimilation of iron is a difficult process. The organism of the mother is, therefore, as economical as possible with its stores of iron, and prefers that the young should receive it through the more certain path of the placenta than through the alimentary canal of the offspring.

The importance of the iron stored in the organism may, however, be in another direction. First of all it is to be decided in what form the iron is deposited in the new-born animal. We can, indeed, imagine that to some extent at least it is present as an antecedent of hemoglobin, so that it can be quickly changed into the latter as occasion demands. On the other hand, it is also possible that the offspring is provided with a large amount of hemoglobin itself. It is true that the naked, helpless being which develops so rapidly after birth requires a large amount of oxygen to effect the various oxidation processes which take place within it, and in order to make use of all this oxygen it requires hemoglobin. The following summary shows that, as a matter of fact, the amount of hemoglobin present per kilogram of the body weight is greatest at the time of birth.[1]

[1] Emil Abderhalden: Z. physiol. Chem. **34**, 500 (1902).

RABBITS, SERIES I.

Age in Days.	Absolute Weight of the Hemoglobin in the Entire Animal Minus the Intestine.	Hemoglobin per 1000 Grams Body Weight.
1	0.675	12.61
3	0.699	10.91
6	0.760	6.61
10	0.773	4.87
14	0.981	3.81
18	1.096	3.21
22	1.122	2.41

RABBITS, SERIES II.

Age in Days.	Absolute Weight of the Hemoglobin in the Entire Animal Minus the Intestine.	Hemoglobin per 1000 Grams Body Weight.
1	0.836	13.37
3	0.868	11.27
8	0.999	6.44
12	1.083	5.25
18	1.175	4.08
22	**1.384**	**3.01**
28	2.830	5.47

RATS.[1]

Age in Days.	Absolute Weight of the Hemoglobin in the Entire Animal Minus the Intestine.	Hemoglobin per 1000 Grams Body Weight.
1	0.026	12.96
6	0.048	6.42
11	0.064	4.88
22	**0.105**	**4.64**
28	0.221	6.70
32	0.296	7.39

These values which in the case of the rabbits were obtained with animals of one and the same litter, whereas in the case of the rats a whole litter was analyzed at the end of each period, show that the absolute amount of hemoglobin increases slowly after birth. At the end of the lactation period there was twice as much hemoglobin in the rabbits as at birth. In the case of the rats, an examination of the contents of the stomach and

[1] The values for each day were those of a whole litter.

intestines showed that they began to take nourishment, other than the milk of the mother, at the end of the twenty-second day. During this time the absolute amount of hemoglobin had increased more than threefold. It was perfectly possible for this increase to arise from the amount of iron contained in the milk. Now if we compare the amounts of hemoglobin present per kilogram of the animal's weight, it is evident that at birth the relative amount of hemoglobin present was remarkably high; this value diminishes little by little as the animal gains in weight, reaching its lowest value toward the end of lactation. This minimum becomes all the more remarkable when we remember that with full-grown rabbits there is from 7 to 10 grams of hemoglobin per kilogram of the animal's weight. As soon as the animal's nourishment changes from milk to green fodder rich in iron, both the absolute and relative amounts of hemoglobin increase quite materially.

It is interesting to know how much iron is present in the new-born rabbits in some other form than hemoglobin. In the following table this is computed on the assumption that the hemoglobin of rabbits contains 0.336 per cent of iron.[1] The value thus obtained is then deducted from the amount of iron that Bunge[2] found per kilogram of the animal's weight.[3]

RABBITS, SERIES I.

Age in Days.	Absolute Weight of the Hemoglobin in the Entire Animal Minus the Intestine.	Hemoglobin per 1000 Grams Body Weight.	Milligrams Iron as Hemoglobin per 1000 Grams Body Weight.	Milligrams Total Iron per 1000 Grams Body Weight. (After Bunge).	Milligrams Iron not Present as Hemoglobin per 100 Grams Body Weight.
1	0.675	12.61	42	139	97
3	0.699	10.91	37	99	62
4	...	...	...		
6	0.760	6.61	22	85	63
10	0.773	4.87	16	43	27
11	...	...	...		
13	...	...	...	45	32
14	0.981	3.81	13		
17	...	...	...	43	32
18	1.096	3.21	11		
22	1.122	2.41	8	43	35

[1] O. Zinoffsky: Z. physiol. Chem. **10**, 32 (1885).

[2] *Loc. cit.*

[3] This computation is naturally not accurate, but gives relative values.

RABBITS, SERIES II.

Age in Days.	Absolute Weight of the Hemoglobin in the Entire Animal Minus the Intestine.	Hemoglobin per 1000 Grams Body Weight.	Milligrams Iron as Hemoglobin per 1000 Grams Body Weight.	Milligrams Total Iron per 1000 Grams Body Weight.	Milligrams Iron not Present as Hemoglobin per 1000 Grams Body Weight.
1	0.836	13.37	45	139	94
3	0.868	11.27	38	99	61
4	...	...	...		
7	...	...	...	60	38
8	0.999	6.44	22		
11	...	...	...	43	25
12	1.083	5.25	18		
17	...	...	...	43	29
18	1.175	4.08	14		
22	**1.384**	**3.01**	**10**	**43**	**33**
27	...	...	...	34	16
28	2.830	5.47	18		

A glance at these tables shows distinctly that shortly after birth a considerable amount of the iron is present in some other form than hemoglobin. This amount of iron diminishes rapidly during the first few days after birth, and is evidently transformed into hemoglobin. After about the sixth day from birth, the amount of iron not present as hemoglobin, per 1000 grams of the animal's weight, remains fairly constant. Inasmuch as the animal is constantly gaining in weight during this period, it is evident that iron must be continuously deposited in the tissues. This iron comes from the milk. On the other hand, the absolute amount of hemoglobin likewise increases. At all events, the above values show the minimum amount of iron which is in the tissues of the rabbit and which is held there most tenaciously. From this point of view it is not difficult to understand why the new-born animal is provided with so great an amount of stored-up iron. During the first few days considerable hemoglobin is formed, and, if there were not this supply of iron, it would be necessary for the milk to contain much more iron than usual in order to satisfy the requirement. A study of the cells of the milk-glands, however, shows that on the whole they are nearly fitted to furnish a quite definite secretion. The task of the cells in satisfying the demands of the young organism is, therefore, lightened by the fact that the offspring already has this extra supply of iron available. As Hugonnenq [1] has shown in the case of the human fœtus, this storing up of iron takes place chiefly during the last three months before birth. There is nothing whatever to indicate that the iron is stored up merely because it is a substance difficult for the young organism to absorb and assimilate.

[1] Compt. rend. **128**, 1054 (1899).

The above tables are of interest in another respect. They show that the iron stores of the suckling plus the amount of iron in the milk are, towards the end of the period of lactation, no longer sufficient for the formation of the required amount of hemoglobin. The organism is evidently in a state of iron starvation. As soon as fodder rich in iron is taken into the system together with the milk, the hemoglobin values increase rapidly. This shows how undesirable it is that the human offspring should be restricted to a milk diet much longer than the ordinary period of lactation (7 to 9 months). The child then requires more iron in its food.

Iron has, from days of antiquity, always been considered as playing an especially important part in the nourishment of the human organism, and chiefly on account of the pathological condition known as chlorosis, or green sickness. This occurs particularly in young women at, or near, the age of puberty. The name is derived from the peculiar, characteristic, greenish-yellow colored skin of such patients. It was early recognized that this most prominent symptom — together with the paleness of the mucous membrane — was to be traced to anæmia, an impoverishment of the blood. The disease has always been combated by prescribing iron. On examining the blood of those suffering from this disease, it is found that there is a deficiency in the amount of hemoglobin per unit of volume. It is not, as in forms of anæmia caused by loss of blood, or otherwise, due to the fact that the total number of blood corpuscles is diminished and consequently the hemoglobin content, but rather that the amount of hemoglobin contained in the individual corpuscles is too small. While in many cases the actual number of red corpuscles remains normal, still it often sinks considerably. The only interest that this disease has for us at this place is to the extent that the study of it, and particularly of the therapeutic action of iron, has cleared up the question concerning the absorption and assimilation of this metal and of the other inorganic elements which are required by the organism. The fact that inorganic iron salts have a favorable action upon chlorosis has been known for a long time. How the iron acted was not known, — it was merely taken for granted that it was absorbed and assimilated; i.e., utilized for the formation of hemoglobin. This view was perfectly plausible as long as nothing definite was known concerning the manner in which the iron is held combined in the hemoglobin molecule. If the hemoglobin merely contains the iron in a loose, salt-like state of combination, it would be perfectly plausible to think of an assimilation of inorganic iron salts. It is now known, however, that the iron contained in hematin, a compound very closely related to hemoglobin, is in a very firm state of combination. It resists the action of boiling, concentrated potassium hydroxide and boiling hydrochloric acid. By dissolving it in concentrated sulphuric

acid the iron is split off and the hematin is changed into hematoporphyrin. The assimilation of the iron, therefore, cannot be a very simple process, and consequently at the time when it was denied that the animal cells possessed the power of effecting syntheses, it is inconceivable why the assumption should, nevertheless, have been made that inorganic iron as such took part in the formation of hemoglobin. This standpoint was emphasized by G. von Bunge,[1] who raised the question as to the form in which the iron is contained in our food supply. Is it present in the form of simple inorganic iron salts, or as complicated organic compounds? He prepared first of all from the yolk of eggs, which must of course contain the iron necessary for the formation of hemoglobin in the blood of the chick, a compound which by its entire chemical behavior was shown to contain iron in a very firm state of combination.[2] On extracting the yolk of a hen's egg with alcohol and ether, none of the iron goes into the extract. All of it remains in the residue, which consists chiefly of albumin and nucleins. From this residue the iron cannot be extracted by means of alcohol and hydrochloric acid. Since all salt-like compounds containing iron combined with inorganic or organic acids can be removed by the above reagents, it is to be assumed that the iron is held in a closer form of combination than is the case with its ordinary salts. Bunge next isolated the compound containing iron which was formed by the action of the juices of the stomach during the digestion of protein. The part containing iron does not go into solution. It is indigestible, and corresponds in its entire behavior to that of a nuclein substance. The iron cannot be extracted by alcohol containing hydrochloric acid, but is dissolved out by aqueous hydrochloric acid, the rate of solution increasing with the concentration of the acid. Although the iron contained in hematin does not react with the ordinary reagents used for detecting the presence of iron, namely, ammonium sulphide, and acid solutions of potassium ferro- and ferricyanides, the iron contained in the nuclein substance does give these tests. By dissolving the nuclein in ammonia, and then adding potassium ferrocyanide and acidifying with hydrochloric acid, a white precipitate is produced which turns blue on standing. In the case of potassium ferricyanide the precipitate remains white. On adding ammonium sulphide to the ammoniacal solution of the nuclein a green coloration is formed, gradually turning black in the course of twelve hours. Recently this compound, to which Bunge gave the name hematogen, has been prepared by Hugonnenq and Morel,[3] and purified from albumin as much as possible. On analyzing it they obtained

[1] Verhandl. des 13. Kongresses für innere Medizin, p. 133 (1895).

[2] Z. physiol. Chem. **9**, 49 (1884).

[3] Compt. rend. **140**, 1065 (1905).

the following values: 43.5 per cent C; 6.9 per cent H; 12.6 per cent N; 8.7 per cent P; traces of S; 0.455 per cent Fe; 0.352 per cent Ca; and 0.126 per cent Mg.

It appears that we have here a compound representing a preliminary stage in the formation of hemoglobin. The method of proving this, however, is not entirely satisfactory. The conclusion rests largely upon an elementary analysis. It need hardly be mentioned that this does not mean much in the investigation of such a highly complicated organic substance. A quite similar compound has been prepared by G. Walter,[1] from the eggs of the carp. Walter found in his preparation 48.0 per cent C, 7.2 per cent H, 14.7 per cent N, 0.30 per cent S, and 2.4 per cent P; in a second product he obtained the values 47.8 per cent C, 7.2 per cent H, 12.7 per cent N, 2.9 per cent P, and 0.25 per cent Fe.

There is no doubt that the plants also, to some extent at least, contain iron in the form of complicated organic compounds; and, in fact, it seems evident that nuclein substances containing iron are present. Plants rich in iron, especially spinach, are well suited for the preparation of such products. By a similar treatment to that described for the preparation of hematogen from egg-yolk, a product relatively rich in iron is obtained, which, like hematin, is not acted upon by the juices of the stomach, gives a similar elementary analysis, and shows corresponding chemical reactions. These compounds are always obtained in an amorphous state, so that it is difficult to judge of their purity. To attempt to draw any conclusions as to their identity, or as to the relation of these compounds to one another, would be extremely hazardous.

We do not know the form in which the iron is contained in milk. The amount present is so small that up to the present time it has not been possible to isolate any compound containing iron.

Starting with the hypothesis that the iron in our food is present, at least to some extent, in a state of firm combination with organic material, Bunge next raised the question as to whether the animal organism is so constituted that it is able to absorb inorganic iron salts. It was not an easy question to answer. It was customary to recognize the absorption of a substance only when it, or one of its related compounds, was found in the urine. This is not the case when inorganic iron salts are taken into the system.[2] Only after subcutaneous introduction is there any considerable amount of iron to be found in the urine. The fact that when iron is incorporated into the system in this way it has a poisonous effect upon the organism, gives further support to the assumption that inorganic iron salts cannot pass through the intact intestine,[3] for there has never

[1] Z. physiol. Chem. **15**, 477, 489 (1891).

[2] R. Kobert: Arch. exper. Path. Pharm. **16**, 361 (1883).

[3] H. Mayer u. Francis Williams: Arch. exper. Path. Pharm. **13**, 70 (1881).

been any poisoning observed from taking iron salts, provided the doses and the manner in which they are introduced are so chosen that large amounts of iron do not get into the circulation by erosion of the alimentary canal. Little by little the view became accepted that iron and the heavy metals find their principal place of elimination not in the kidneys, but in the intestines. It is true that a part of the iron leaves the body through the urine, for the latter invariably contains small amounts of iron. The amount, however, is very slight, and is not materially increased when iron is taken as medicine. Long ago it was suggested that probably a large part of the iron was eliminated through the intestines, and was, therefore, to be found in the fæces. The fact that after taking iron into the system, the most of it did actually appear in the fæces, led to the erroneous conception that no absorption took place. It has been shown in the first place that subcutaneous and intravenous injection of iron salts always result in a part of the iron being eliminated through the intestines.[1] On the other hand, it may be said that this does not represent a normal condition. It was indeed conceivable that the organism seeks to get rid of the iron salts poisonous to it as quickly as possible by all the means available. The credit of having done the most towards explaining the absorption and elimination of iron salts belongs chiefly to Kunkel, Quincke, and Hochhaus.[2] These investigators made use of ammonium sulphide as a reagent for tracing the course of iron in the tissues;[3] in some cases, potassium ferrocyanide was used as well. If, for example, mice are fed for a long time upon milk which, as we have seen, contains but little iron, then, on placing the alimentary canal of these animals in ammonia and ammonium sulphide, the iron test does not appear, or at most there is only a slight green coloration.[4] The same is true of the other organs of mice, after they have subsisted upon milk alone for a considerable period. The best tests for iron are given by the

[1] Hamburger: Z. physiol. Chem. **2**, 191 (1878–79); Lapicque: Arch. Physiol. normale et pathol. **1895**.

[2] Kunkel: Pflüger's Arch. **50**, 1 (1891); **61**, 595 (1895). Filippi: Arch. exp. Path. Pharm. **34**, 462 (1895). Hochhaus u. Quincke: *ibid.* **37**, 159 (1896). Quincke: *ibid.* **37**, 183 (1896). W. F. C. Woltering: Z. physiol. Chem. **21**, 190 (1895–96), and Over de resorptie van ijrerzonten in het spysverteringskanaal, Utrecht, 1895. W. S. Hall: Arch. Anat. Physiol. **1896**, 49; **1894**, 455. A. Macallum: J. Physiol. **1894**, 186. Abderhalden: Z. Biol. **39**, 113 (1899). A. Hofmann: Virchow's Arch. **151**, 484 (1900). G. Swirski: Pflüger's Arch. **17**, 466 (1899). Tartakowsky: *ibid.* **100**, 586 (1903). Hubert Sattler: Ueber Eisenresorption u. Ausscheidung im Darmkanal bei Hunden u. Katzen Inaug. Diss. Kiel, 1904, and Arch. exper. Path. Pharm. **52**, 326 (1905). Franz Müller: Deut. med. Wochschr. No. 51 (1900) and Deut. Med.-Ztg. No. 30 (1901). Virchow's Arch. path. Anat. klin. Med. **164**, 436 (1901).

[3] Cf. R. Gottlieb: Z. physiol. Chem. **15**, 371 (1891).

[4] A. Mayer was the first to apply this method (Dorpat, 1850). Later, Perls (Virchow's Arch. **39**, 42 (1867)) made use of $K_4Fe(CN)_6$.

liver and spleen.[1] The facts are quite different in the case of animals which have been given iron with the milk. In the stomach there is obtained but little, if any, reaction for iron, while in the duodenum there is a marked green coloration. Often the reaction is localized to definite zones. It is frequently found, for example, that merely the top of the intestinal villi are colored green. If the tissue of the intestine is examined under the microscope, numerous little kernels containing iron are to be found embedded in the protoplasm of the intestinal epithelium, and for the most part these are directly beneath the cuticular borders of the cells. Now and then tiny leucocytes may be seen laden with innumerable little particles of iron. These are noticed in the stroma of the villi. In the submucosa also, cells containing iron may be noticed once in a while. In the jejunum, however, it is quite different. Here, as a rule, the iron reaction is shown only in the solitary follicles and in the *Peyer's patches*. In the ileum, the iron reaction is not, as a rule, very pronounced, while the cæcum and large intestine again give a strong test. Coming from the intestinal canal, especially the duodenum, lymphatics filled with cells containing iron may often be seen leading to the mesenteric glands, which likewise show a pronounced iron reaction. The liver and spleen now give a very strong test, and evidently these organs serve as storage places for iron salts. The muscles and bone-marrow, etc., likewise show a green coloration when tested with ammonium sulphide.

Before going farther, we wish to emphasize the fact that the observed conditions which were obtained with mice, rats, rabbits, guinea pigs, dogs, and cats, are not to be attributed to an irritating effect of the iron. It is perfectly clear that if the epithelium of the intestines is injured in any way, the absorption relations which normally take place in the intestines will be considerably affected. Such irritation has indeed often been observed in experiments where large doses of iron salts were fed to animals, but in the experiments now in question the amounts of iron were very small. Thus, rats were given but 0.4 to 0.5 milligram iron in the course of a day, rabbits 4 milligrams, guinea pigs 2 to 3 milligrams, dogs 3.5 to 4 milligrams, and cats 4 milligrams per day in the form of ferric chloride added to the milk. This small amount of iron was taken up gradually in the course of the day.

Now what is the significance of the above discoveries? The simplest assumption is that iron begins to be absorbed in the duodenum, and is then, to some extent at least, carried first of all to the lymphatic ducts. Observations made by Gaule[2] make it seem probable that part of the iron is carried away to the thoracic duct, and thus reaches the circulation. It is

[1] Abderhalden: *loc. cit.*

[2] Deut. med. Wochschr. **1896,** Nos. 19 and 24.

certain that a part of the absorbed iron is also conducted to the portal vein of the liver. The latter organ forms one of the chief storage places of iron salts. The spleen also absorbs considerable iron. It is probable that the lymph carries some of the iron back to the intestines, and is eliminated through the cæcum and large intestine. Evidently the leucocytes play an important part in the processes of taking up and transporting the little particles of iron, for they are often observed laden with iron particles, both in the paths of absorption and in those of elimination. There is no doubt that a large portion of the iron introduced into the organism is again eliminated through the intestines. It is at present uncertain as to what parts of the intestines share in this elimination. This is particularly true of the small intestine, which perhaps participates in both the absorption and elimination of iron. We must not forget, above all, that microchemical reactions have a limited value. They serve merely to give us a qualitative picture of the activity of definite compounds, but never give us much idea as to the amounts of substances entering reactions. Moreover, the method used in the above experiments for detecting the presence of iron qualitatively was not entirely satisfactory. We have already mentioned, for example, that the iron in hematin does not react with the ordinary reagents used in testing for the presence of iron, and that hematogen only gives a noticeable reaction after the solution has stood for some time. It is easy to conceive that the animal organism may contain its iron firmly bound in other compounds besides hematin, so that in any case the mere fact that we do not detect the presence of iron by a qualitative test does not prove that the tissue tested does not contain this element. On the other hand, the fact that we do not get these iron tests in cases where other methods (examination of the ash) show that it is actually present, does not give any reliable information concerning the way that the iron is held in combination. We have already seen that colloids play an important part in the animal organism, and that they are apparently capable of preventing certain reactions from taking place. Thus we found that the formation of precipitates was often prevented, even in cases where the reaction had actually taken place. The precipitate is not seen, merely because the internal friction between the colloid particles prevents the tiny parts of insoluble substance formed from uniting to cause a visible precipitate. This point is all too frequently lost sight of in scientific work. In many cases the statement that iron is present in a firm state of "organic" combination is not justifiable.

It is, we regret to say, at present impossible to follow with sufficient accuracy the question of the absorption of iron and the distribution of the absorbed iron among the separate organs. The amounts of iron absorbed are so small that with our present methods it is entirely out of

the question for us to attempt to picture the course of the iron through the organism, and to decide how much inorganic iron is absorbed, and for how long.

The conditions which we meet with in the case of iron as regards its absorption and elimination are similar with other elements. Apparently other inorganic elements are taken up in about the same way and are similarly eliminated. Thus we know that if we add lime [1] to the food, a part of it is eliminated in the urine, whereas a considerable portion is deposited in the large intestine. Inorganic elements which are not usually found in the organism follow the same course. Thus, Steinfeld [2] found that subcutaneous injection of bismuth into birds caused the vermiform process and large intestine to be colored black. The relations of elimination are similar with mammals. Thus Steinfeld found on repeating this experiment with dogs and cats that the lymph vessels of the intestines were filled with a black substance.

The fact that when iron was administered, a part of the dose was absorbed in the duodenum, and then again eliminated at the end of the intestine, does not tell us what happens to the iron that is contained in our ordinary food. This had to be determined by a direct experiment. It was found [3] that animals fed exclusively upon meat or exclusively upon vegetables gave exactly the same reactions for iron in the intestines and in the tissues as those which were dosed with iron salts. The behavior of the hemoglobin and hematin was particularly interesting. Both of these compounds contain iron in a form which renders it impossible to detect it by the ordinary chemical reactions. If now a rat was fed with milk containing either hematin or hemoglobin, while another rat was fed with milk alone, then the alimentary canal of the latter would not give any iron reaction, whereas if the intestinal tract of the first rat was placed in a solution of ammonia and ammonium sulphide, an intense green coloration appeared at once. Thus the iron has been loosened from its state of combination in hematin; it has evidently become ionized. This shows it is extremely probable that unchanged hematin is not absorbed as such. The formation of hemoglobin in the animal organism is in all cases the result of a synthesis or a change in the way in which the iron is combined. This assumption cannot at present be verified definitely, because, as we have mentioned, it is possible that besides the compounds in which we are able to detect iron by the ordinary reagents, there may be others present

[1] R. W. Raudnitz: Arch. exper. Path. Pharm. **31,** 343 (1893); J. G. Rey: Deut. med. Wochschr. No. 35, 569 (1895). G. Rüdel: Arch. exper. Path. Pharm. **33,** 79 (1894). J. G. Rey: *ibid.* **35,** 295 (1895).

[2] Dissertation, Dorpat, 1884. Meyer and Steinfeld: Arch. exper. Path. Pharm. **20,** 40 (1886).

[3] Emil Abderhalden: *loc. cit.*

which are capable of absorption in which the iron is too firmly bound to react with such chemicals and thus escapes microchemical detection. It is at least decidedly interesting to know that even under normal conditions of nourishment the tissues of the animal organism always contain iron in compounds which permit its detection by means of the ordinary reagents.

The mere fact that inorganic iron salts artificially added to the nourishment are capable of absorption and deposition in the tissues does not show by any means that this iron is actually assimilated. It would be insufficient to consider the assimilation of iron from the standpoint of blood formation alone. There is no doubt that iron forms an integral part of all cells and tissues of the body. Certainly, this iron in the tissues is just as essential for the normal functions of the separate organs as is the case with the iron in hemoglobin. Some idea of these relations may be obtained from the values cited for the iron in the tissues of rabbits during the period of suckling. It is evident from these values that the tissues hold with great tenacity to a definite minimum amount of iron, even when the hemoglobin content of the entire organism is so far diminished that strong anæmia results. At present we have no definite information as to the part played by this iron in the tissues. We do not even know what amounts are present in the separate organs. It is likewise difficult to decide what portion of the total iron has been introduced and what portion is about to be eliminated. Thus it is easy to realize how the question concerning the assimilation of iron has been covered by that of the formation of the hemoglobin.

At the present time it has never been definitely decided whether the iron introduced into the system in the form of inorganic salts takes part in the formation of hemoglobin or of hematin. We find ourselves here, as is the case with many other biological experiments, confronted with the condition that one and the same discovery may be used, to support entirely opposing views. The old experience of physicians, that iron pills combat chlorosis, can be explained by the assumption that the iron in the pills goes to form the hemoglobin. On the other hand, judging from analogy with other observations, it is perfectly plausible to assume that the iron in the pills merely has an indirect effect in that it excites those organs which take part in the formation of blood into increased activity. First of all, it is necessary to determine whether chlorosis is actually caused by a deficiency of iron.

Let us see how much iron there is in the human organism. A mouse contains 100 milligrams iron per kilogram of its weight, a guinea pig contains but 52 milligrams per kilogram, and a rabbit about 46 milligrams. If we assume that the last value is about right for a human being, then we would have in a person weighing 70 kilograms about 3.2 grams of iron. The question is How much iron does the human organism eliminate? Star-

vation experiments on man show that there is 7 to 8 milligrams of iron eliminated daily from the intestines.[1] Now a liter of milk contains about 2.3 milligrams of iron. With milk alone as food, therefore, it would be necessary to drink at least four liters per day, for during starvation the organism is extremely economical with its stores, and the amount of iron then eliminated represents a minimum. Certain of our foods, such as white bread, rice, cherries, apples, etc., are very deficient in iron.[2] It is, therefore, easy to believe that people subsisting exclusively upon such nourishment will have impoverished blood; and, as a matter of fact, many cases of chlorosis may be accounted for in this way. The practising physician, however, knows of many cases of chlorosis in which unquestionably enough iron has been taken into the system to satisfy the ordinary requirements. In such cases we are forced to assume that the function of absorbing iron has in some way become disturbed, or else that the iron compounds taken into the system for some reason are not utilized by the organs which serve to form the blood. As a matter of fact, disturbances in the function of the intestinal tract have been observed frequently in chlorosis. Even in such cases, however, which are, by the way, exceptional, it is not known whether these intestinal disturbances are of a primary nature, or whether they are not rather a result of the impoverished blood and the disturbances of nutritional relations which result therefrom.

The attempt has been made to decide by means of experiments upon animals what the relations are between iron introduced into the organism in an inorganic form and the formation of the blood. Two different methods have been tried. We have seen that at the end of the lactation period the suckling possesses a low hemoglobin content, and that this increases rapidly as the animal begins to partake of food richer in iron. If, on the other hand, the animal is kept for a prolonged period upon a milk diet, a marked anæmia results. That this is, as a matter of fact, the case,[3] is shown by comparing the amounts of hemoglobin in animals which have from birth been fed only upon milk, with that of animals which, at the end of the suckling period, have passed over to a diet richer in iron (vegetables). Now the addition of inorganic iron salts to the milk has no effect upon the absolute amount of hemoglobin contained in the animals, nor upon the amount per kilogram of the animal's weight. The added iron, however, is found to have a remarkable effect in accelerating the growth of the animal.[4]

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[1] Lehmann, Müller, Munk, Senator, and Zuntz: Virchow's Arch. **131,** Suppl. 1, pp. 18 and 67 (1893).

[2] Cf. p. 380.

[3] Emil Abderhalden: Z. Biol. **39,** 193 (1899).

[4] Emil Abderhalden: Z. Biol. **39,** 483 (1899).

A second method[1] for deciding the question concerning the assimilation of iron is to withdraw equal quantities of blood from two animals of the same age and, as far as possible, of the same condition of nourishment, and then to compare the regeneration of blood in one case where the animal is fed entirely upon milk, and in another where iron is added to the milk. Here all authors agree that the animal to which the inorganic iron has been fed is able to replace the lost blood much more quickly than the other animal to which only the iron contained in milk is available. The disagreement in this result with that obtained in above experiments may perhaps be due to the different conditions of the animals in the two types of experiments. In the first instance, animals were chosen whose iron stores at the beginning of the experiment were in a quite exhausted state. All of the iron in the different tissues was brought down to the lowest limit. The animals in the second cases were in an entirely different condition. These possessed a considerable supply of stored-up iron, and would have been able to supply the loss in hemoglobin iron from these stores alone. That the addition of inorganic iron exerts a favorable action might be in fact explained on the assumption that it excites the action of the blood-forming organs. We must admit, however, that such an explanation does not seem well founded in this case, for it would seem probable the large loss of blood would of itself serve to incite the organs which take part in the formation of blood into increased activity. On the other hand, it is conceivable that the inorganic iron replaces the iron in the tissues, so that the latter is left free to take part in the formation of hemoglobin. No one has ever been able to prove this directly, but it seems to be a likely explanation, as we shall see from what follows.

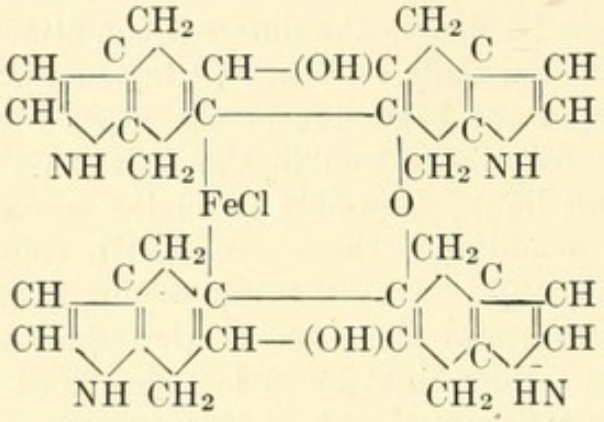
Franz Müller[2] hoped to settle this question by an examination of bone-marrow, one of the principal places where the red blood-corpuscles are formed. Müller fed dogs, taken immediately upon the completion of the lactation period, on the one hand exclusively with milk, and on the other with milk and iron. After subsequently killing the animals, he found that the bone-marrow of the animals fed with iron contained considerably more nucleated, red blood-corpuscles than the animals fed with milk alone. Again, this discovery may be interpreted in two different ways. We may assume that the inorganic iron takes part directly in the formation of hemoglobin, or, on the contrary, that it merely exerts an indirect action.

When we take into consideration all of the different experiments that have been performed in this connection, we must unhesitatingly admit that it has never been satisfactorily proved whether the iron taken into

[1] Kunkel: Pflüger's Arch. **61,** 596 (1895). Eger: Z. klin. Med. **22,** 335 (1897).

[2] *Loc. cit.* Cf. also A. Hofmann: *loc. cit.*

the system in an inorganic form can take part in the formation of hemoglobin. The question then naturally arises, Have the proper means been found for solving this problem? Up to this point, in discussing the formation of hemoglobin, we have considered but one component of hematin; namely, the iron. It seems hardly justifiable to limit the entire discussion to this one component. We know that the chemical composition of hematin is very complicated.[1] M. Nencki and J. Zaleski [2] have suggested the following structural formula for hemin, the hydrochloric acid ester of hematin:



We introduce this formula here merely to show how complicated its synthesis must be if the animal cells are to build it from inorganic iron. The formula also shows that obviously the iron itself does not play such an all-important part in the synthesis; that is to say, it is probably not so very difficult to introduce the iron into the molecule. It is infinitely more important to know whether the organism has other organic material available for the formation of the hematin.

In the above formula we see that two hematoporphyrin molecules (the iron-free cleavage-products of hematin) are held together by an iron atom. The formation of hematin, therefore, depends just as much upon the presence of material from which hematoporphyrin can be made as upon the presence of iron. Now we know, as will be explained subsequently in detail, that hematophorphyrin is closely related to chlorophyll,[3] for similar decomposition products are formed from each of these two compounds. This suggests the thought that chlorophyll can perhaps be brought into relation with hematin, and thus with the formation of hemoglobin. There is absolutely no doubt that these compounds are closely related to one another. The fact that both, as we shall see later on, possess similar biological functions is sufficient to explain this relationship, although, as a matter of fact, the task of chlorophyll is quite different, as far as our knowledge goes, from that of hemoglobin. Now, inasmuch

[1] See Lecture XXIV.

[2] Ber. **34**, 997 (1901).

[3] L. Marchlewski: Die Chemie des Chlorophylls (1895).

as the original source of all substances in the animal organism can be traced back eventually to the vegetable kingdom, the thought suggests itself that chlorophyll serves as the building material in the formation of hemoglobin. The herbivora devour this compound in considerable quantities, and, on the other hand, the carnivora receive considerable amounts of hemoglobin with their nourishment. Unfortunately, we know nothing definite concerning the transformations which take place with these two products in the alimentary canal. Of hemoglobin we now know that in its absorption it is so changed that the iron in it becomes detectable with ordinary chemical reagents. According to our experiences as regards the significance of digestion, it is perfectly possible that chlorophyll and hemoglobin are broken down into similar products, and thus that the organs which form the blood have practically the same kind of building material presented to them in each case. The actual amount of chlorophyll and hematin available does not need to be very large. Unfortunately, we do not know how much hemoglobin is formed daily, or how much is decomposed, so that we have no data to judge as to the normal extent of hemoglobin formation.

We arrive at this line of thought because evidently the synthesis of hematin is a very complicated one, and we are aware of no material from which it could be formed apparently so readily as from chlorophyll or its decomposition products.[1] At all events, these views do not by any means imply that inorganic iron cannot take part in the formation of hemoglobin. Even if we grant that the nucleoalbumins, or nucleoproteids, serve as the building material of hemoglobin, it does not seem to us that this makes the direct assimilation of iron impossible, for these substances contain iron in a relatively loose state of combination. The formation of hematin from albumin compounds containing iron must necessitate quite considerable molecular rearrangements in order to get the iron in the state of union known to exist in hematin. Now the question arises as to whether milk possesses the material, other than iron, in sufficient quantity for the formation of hematin. We have a perfect right to doubt this. It would be perfectly useless to provide the organism with a sufficient amount of hematoporphyrin, the principal component of hematin, when an adequate supply of iron, the minor constituent, is not available. It is far more probable that milk contains these two constituents in about the same relative amounts as in hematin. It is consequently perfectly

[1] Perhaps the cleavage products of protein come into consideration as building stones. Both proline and glutamic acid could furnish material for the pyrrole ring. Plant albumins contain large amounts of glutamic acid. The reserve albumins are rich in the above-mentioned amino acids. Perhaps this has something to do with the formation of chlorophyll, and it is perfectly possible that the animal organism chooses the same way for preparing its hematin.

possible that in the above-mentioned experiments in which iron was added to the milk, there was not much effect on the amount of hemoglobin formed, because there was an insufficient supply of the other building material out of which hemoglobin is formed. Again, we have up to this point left out of consideration the fact that besides hematin, there is another component of hemoglobin, namely globin, which is an albumin substance of highly complicated structure. The formation of the hemoglobin molecule is complete only after the hematin has united with the globin molecule.

Enough has been said to show that the formation of hemoglobin does not solve the question as to the part that iron plays in its formation. The kernel of the whole question has not yet been attacked. We cannot hope for a solution of the problem until we understand clearly the formation of hematin. The mere fact that the addition of iron to nutriment poor in iron does not have any distinct influence upon the formation of hemoglobin, in no way speaks against the participation of inorganic iron in the synthesis of hemoglobin in the case of normal nutrition, but it indicates that the other building material is wanting as well as the iron. Furthermore, the fact that when the animal passes over to a form of nourishment richer in iron, there is a rapid increase in the extent of the hemoglobin formation, is explained not only by the increased amount of iron in the food, but as well by the fact that the other material required for the production of this substance is likewise available to a much greater extent.

Let us now return to chlorosis. We must first of all emphasize the fact that the anæmia produced by an exclusively milk diet, or by loss of blood, has nothing whatever to do with the disease in which there is an impoverishment of the blood. In the case of typical chlorosis, the composition of the blood is abnormal in spite of the fact that there has been available a sufficient supply of substances which take part in the formation of blood. It is characteristic of the disease that it occurs in the full-bloodedness of the female organism's development, in the years of puberty, and it gradually disappears without any change in the nourishment sufficient to account for the correction of the disorder. One gets the impression that demands are suddenly made upon the blood-forming organs which it is not able to satisfy. It would be easy to conceive that the blood losses brought about by menstruation are the cause of the increased demands upon the hematopoietic system. It has been shown, however, that the amount of iron lost in the flow of blood during menstruation [1] is very slight, and need hardly be taken into consideration. Bunge, from his observations that the suckling was born with a store of iron, made the suggestion that the organism, in order to be able to give up this supply of iron, must begin to store up iron before the time of conception, so that it

[1] Hoppe-Seyler, Brodersen and Rudolph: Z. physiol. Chem. **42**, 545 (1904).

would have, aside from its food, a sufficient supply of iron during pregnancy. In order to test this hypothesis Bunge [1] made a number of experiments, and determined the amount of iron in the chief storage place, the liver, in both male and female cats and dogs, but the results obtained were not altogether harmonious.

Although we are not in a position to assign a cause for chlorosis, still it is perhaps possible to explain the curative effect of the iron preparations. If we, however, study closely the whole process of the hemoglobin formation, it seems to us less probable that the iron preparations added to the nourishment actually take part directly in the making of blood. It would be easy to understand such an action, if chlorosis were actually caused by a deficient supply of iron. We are perfectly certain, however, that in the majority of cases this is not true. We have every reason to presume that our nourishment in general, besides containing sufficient iron, likewise contains enough of the other substances required for the formation of hemoglobin. Just as the tissues of the bones in richitis are not capable of assimilating lime, so, evidently, the tissues of the hematopoietic organs are not able to utilize the material which forms the building stones of hemoglobin. It has been suggested that the iron added in the form of inorganic salts exerts an irritating effect upon these organs, and urges them into renewed activity. The unsatisfactory character of such an explanation is evident. It assumes that the iron fed to the body in an inorganic condition, behaves otherwise than that contained in "organic" compounds. We have, however, at present no insight into the ways and means by which absorbed iron reaches the circulation, nor as regards the form in which it is present in the different fluids and tissues. We only know that the iron in the organs can be detected by means of the ordinary chemical reagents, irrespective of whether the animal has been fed with milk and inorganic iron, or milk and, say, hemoglobin. Even while these observations do not justify the assumption that the different iron compounds all behave alike after reaching the intestine,—i.e., that they may be changed into the same state of combination,—still, on the other hand, we have no justification for the assumption that the animal organism distinguishes between iron that is fed in an inorganic condition from "organic" iron. According to all our general conceptions of the process of digestion, it appears to us as extremely probable that even the formation of hematin is preceded by a deep-seated decomposition. In this case the animal organism unquestionably breaks down and again builds up. If we look at the formula of hemin given above, it seems to us as highly improbable that in general "organic" iron is necessary for the synthesis of hematin. Yes, in fact, we can even imagine that the disease of chlorosis actually depends upon the fact that the cells of the body are no longer

[1] Z. physiol. Chem. **17**, 78 (1893).

capable of making use of iron which is supplied in the form of highly complicated organic compounds, or at least are unable to convert them into such a state that they can be utilized for the formation of hematin. Perhaps the preparatory decomposition in the alimentary canal has not taken place, and thus the iron may circulate to some extent in the tissues, but in a form from which the blood-forming organs are not able to set it free. From this point of view, it is easy to understand the favorable action of inorganic iron salts. Here we intentionally break away from the older idea that the animal organism is dependent upon the *nature* of the food that it receives. It is far more important that it receives all the materials that it requires. The way these elements are originally combined in the food is more or less a matter of indifference, provided they are susceptible of decomposition. It is indeed this far-reaching decomposition and the renewed construction which makes the animal organism to a considerable extent independent of the kind of nourishment it receives. To be sure, we must admit that apparently the animal cells are not capable of utilizing certain kinds of combinations. Thus, it is improbable, for example, that it is capable of constructing cholesterol, and perhaps not hematoporphyrin from its simplest components, although it is precisely here that we meet with the possibility that certain decomposition products of the albumins may be utilized for the syntheses. At all events, we are not justified in believing that hematogen and similar substances are necessarily direct preliminary stages in the formation of hemoglobin. It is indeed possible that these compounds contain all the necessary material for the formation of the red pigment of the blood, although this has never been proved directly.

The theory we have thus developed concerning the action of inorganic iron salts is not necessarily correct. By the addition of iron preparations to the food, we increase the iron supply for the entire organism. It is conceivable that this results in an indirect action upon the organs which produce the blood. We know that widely different organs are intimately related to one another in their metabolism and in the exercise of their functions. In this connection, we need refer only to the regulation of the sugar-content of the blood by means of the liver. Similarly the amount of hemoglobin in the blood is a very constant quantity. Evidently we have here another case of regulation. On the other hand, we have seen also that the different ions exert a quite specific effect, and that it is, indeed, possible for the predominance of one ion to cause a certain specific action. It is conceivable that the heaping up of iron in the cells of the body, and perhaps specially in the cells of certain organs, may give a new impulse to the organs which participate in the formation of blood.

The action of the " inorganic " iron was formerly attributed to a protective effect. Thus the sulphur in alkaline sulphides, instead of com-

bining with the more complicated organic compounds containing iron, was believed to unite preferably with the iron of inorganic compounds. The iron in the nutriment would thereby be protected, and remain in the system instead of being eliminated as sulphide of iron. Since it has been shown, however, that even inorganic iron salts are absorbed, and it has been proved, moreover, that alkaline sulphides are not present in the stomach and small intestine, this protective theory may well be discarded.[1]

From the experiments that have been cited, it is perfectly clear that in reality we know very little concerning the cause of chlorosis, and especially concerning the action of iron preparations. On account of the complexity of the processes involved, we can hardly expect at present to understand perfectly the relations between the iron and the pathology of the blood formation. Above all, we need to know more about the process of blood formation, and especially as regards the formation of hemoglobin itself. At the same time the solution of the problem of the formation of hemoglobin does not by any means necessarily solve the whole problem of the formation of the blood. We then have to consider the formation of the blood corpuscles. The stroma of the blood corpuscles must also be built up, and, in such a way that it can take up the hemoglobin. A long chain of processes leads from the separate building materials of hemoglobin, the iron and the organic compounds, to the finished blood corpuscles capable of exerting their important functions. The chain may be broken at many places, and thereby the whole process of blood formation disturbed. This so infinitely complicated problem has been attacked only from one side, that of the iron. Undoubtedly iron is indispensable in the formation of blood, but equally indispensable are all the remaining and building materials which are far more complicated, — the hematin, hemoglobin, and even the corpuscles themselves.

This is not the place to discuss whether iron preparations actually do have any effect upon chlorosis. It is indeed conceivable that it is the dietetic and hygienic measures that are taken that are alone effective in iron therapeutics. We have followed chlorosis and iron therapeutics thus far only in the hope that we would be able thereby to get some idea of the relation of iron to the formation of blood. On the contrary, the above conclusions apparently lead us to the opinion that chlorosis itself is not difficult to understand, — we can account for its appearance if we assume that the function of the organs producing blood are in any way disturbed, — but on the other hand, the fact that inorganic iron preparations [2] are successful in combating the disease rather stands

[1] Cf. Kletzinsky: Z. Ges. Aerzte Wien X, II, 281 (1854). Hannon: Presse médical (1851). Woltering: *loc. cit.* G. von Bunge: Lehrbuch d. phys. chemie, p. 94 (1894).

[2] Nearly all of the " organic " iron preparations on the market belong to this class of iron salts, for they contain the iron in a loose state of combination.

in the way of the general conception which prevails concerning its cause. According to the theory, however, that it is not the function of synthesizing the material which is disturbed, but rather the function of decomposing the material containing the iron, it is easy to understand the favorable action, of iron salts, for in them the organism receives material which for some reason it cannot itself obtain. At all events, in cases of iron therapeutics it should be borne in mind that hemoglobin cannot be formed from iron alone, so that care must be taken to supply the remaining material necessary, in the form of meat, eggs, and green vegetables.

The importance of iron for the tissues has been in the past almost forgotten in the discussion of the relations of iron to the formation of blood. Here again iron plays an important part, but unfortunately we now know but little in regard to the way it is combined in the tissues and cells. It evidently occurs in different forms. Thus, iron compounds have been prepared from the liver, spleen, and muscles of man by P. Marfori [1] and O. Schmiedeberg.[2] According to these investigators, the iron is present in much the same state of combination as in hematin. The substances thus prepared were apparently absorbed by dogs after they had been starved, or fed upon a diet free from iron. The exact nature of these iron compounds has by no means been fully explained.

Copper plays, in the case of invertebrates, a similar part to that taken by iron in the vertebrates.[3] It is found combined with albumin. The compound which corresponds to hemaglobin is called *hemocyanin*. It is found especially in many mollusks and crustaceans. The blood of the cephalopoda has been examined chiefly in this connection. The arterial blood of these animals is blue, the venous blood colorless. In certain other mollusks (*Pinna squamosa, Doris, Patella, Chiton*) manganese [4] replaces the copper.

We have, up to this point, failed to mention certain inorganic salts which occur in milk, and unquestionably are indispensable foods. Thus, milk always contains some magnesium. This element forms an integral part of plant and animal cells and also of the animal fluids, blood and lymph. The amount of magnesium in milk is in general relatively small. Its function in the animal economy is not yet definitely known.[5] Appar-

[1] Arch. exper. Path. Pharm. **29,** 212 (1891), and Arch. ital. biol. **21,** 1 (1894).

[2] Arch. exper. Path. Pharm. **33,** 102 (1894); cf. Filippi: *ibid.* **34,** 462 (1895).

[3] Cf. Harless: Müller's Arch. **1846,** 148. Schlossberger: Ann. **102,** 86 (1857). Frédéricq: Arch. Zoöl. expér. **7,** 535 (1878); Compt. rend. **87,** 996 (1878). Dhéré: Compt. rend. soc. biol. **52,** 458 (1900). Griffiths: Proc. Roy. Soc. Edinburgh, **18,** 288 (1890–91); **19,** 127 (1892); Compt. rend. **114,** 496 (1892). Cf. Otto v. Fürth: Vergleichende chemische Physiologie der niederen Tiere, p. 74, Jena, **1903.**

[4] Griffiths: Compt. rend. **114,** 840 (1892); **115,** 259 and 474 (1892); **116,** 1206 (1893), and Respiratory Proteids, London, **1897.**

[5] Cf. Lecture XVI, p. 354 et. seq.

ently it bears about the same relation to calcium as potassium to sodium. J. Malcolm [1] has shown that the introduction of soluble magnesium salts into adult animals causes a loss of calcium. In growing animals it tends to prevent the taking up of calcium. Soluble calcium salts apparently have no effect upon the elimination of magnesium salts. In osteomalacia we have also come to recognize a certain antagonism between these two kinds of salts.[2]

Fluorine[3] likewise occurs in small amounts in milk, and forms a regular constituent of bones and teeth, besides being found in the blood.[4] In spite of the small amount present it cannot be disregarded. For this element, as well as for all others, the Law of the Minimum holds.

Phosphorus is of much greater importance both for the growing and adult organism. We find phosphorus in the cells in the form of very important compounds, namely lecithin, the nucleins and nucleoalbumins. We know, furthermore, that phosphorus combined with the alkaline earths forms one of the most important constituents of the human skeleton, and is also present in the same form in other tissues. Phosphorus is present in milk, partly in organic combination, as in casein which belongs to the group of nucleoalbumins, and partly as inorganic salt. Milk also contains some lecithin. At present it is not known exactly how the phosphorus is distributed between these different compounds in the different kinds of milk. Apparently the amount of lecithin present is not very large.

There is no reasonable doubt that the living organism can utilize phosphoric acid directly in the formation of lecithin. It is similarly possible that it forms a part of its nucleins from the latter substance. The fact that the animal organism can form lecithin from phosphates without difficulty is apparent from the experiments already cited of Miescher upon salmon.[5]

Phosphorus is especially important in the construction of nervous tissue. The brain of a new-born infant weighs about 400 grams. This weight is doubled during the period of lactation. According to Schlossmann's computations,[6] the nursling assimilates during this period for the building up of its central nervous system alone about 0.75 gram of phosphorus. The skeleton requires much more of this element. In fact, if we estimate the total amount of phosphorus required by the infant during the first year of its life, we shall find that it amounts to from 50 to

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[1] J. Physiol. **32,** 183 (1905).

[2] Cf. Lecture XVI, p. 377.

[3] G. Tammann: Z. physiol. Chem. **12,** 325 (1888). S. Gabriel: *ibid.* **18,** 281 (1894).

[4] J. Nicklès: Compt. rend. **43,** 885 (1886); Tammann: *loc. cit.*

[5] Cf. Lecture XVI, p. 351.

[6] Med. Klinik. No. 11 (1905); Arch. Kinderheilk. **40,** 1.

60 grams. The amount of phosphorus required in the food is naturally even far greater, because in the above estimate it was not taken into consideration that phosphorus is constantly being eliminated in the form of phosphates. In one liter of human milk there is present 0.19 gram phosphorus, ass's milk contains 0.76 gram, cow's milk 0.79 gram, and goat's milk 0.96 gram. Human milk, therefore, is deficient in phosphorus; it contains less than any of the other kinds of milk which have been analyzed. This is a remarkable fact, for we know that the human offspring is able to construct, while still nursing, a nervous system which is but slightly developed. Compared to human milk, that of the above animals is extremely high in phosphorus. There must be some reason for this difference. Bunge, who noticed this fact in his analyses of different kinds of milk, compared the percentage composition of the ash with the rate of development of the species.[1] It is to be assumed *a priori* that an animal which develops rapidly will require more building material than one whose development is slower. If we compare the time required by the suckling to double its weight at birth with the amounts of albumin and ash — perhaps the most essential constituents for the formation of the tissues — contained in 100 parts of milk, it is evident at a glance that the amount of these increases in proportion as the development of the animal is rapid. This is shown by the following table:[2]

Species.	Days Required to Double Weight.	100 Parts by Weight of Milk Contain:			
		Albumin.	Ash.	Lime.	Phosphoric Acid.
Man	180	1.6	0.2	0.03	0.05
Horse	60	2.0	0.4	0.12	0.13
Cow	47	3.5	0.7	0.16	0.20
Goat[4]	22	3.7	0.78	0.20	0.28
Sheep[4]	15	4.9	0.84	0.25	0.29
Pig[4]	14	5.2	0.80	0.25	0.31
Cat[3]	9½	7.0	1.02	...	...
Dog[3, 4]	9	7.4	1.33	0.45	0.51
Rabbit[3]	6	14.4	2.50	0.89	0.99

The composition of the milk of a single species is by no means constant. The amount of albumin and ash diminishes with the age of the suckling. This likewise has an effect upon the rate of growth as shown by the following values:[5]

[1] Fr. Pröscher: Z. physiol. Chem. **24,** 285 (1897).
[2] Abderhalden: *ibid.* **27,** 594 (1899).
[3] Abderhalden: Z. physiol. Chem. **26,** 487 (1899).
[4] *Ibid.* **27,** 408 (1899).
[5] *Ibid.* **27,** 457 (1899).

One hundred parts by weight contain: (*a*) Before the animal has doubled its weight at birth:

Species.	Casein.	Albumin.	Total Protein.	Fat.	Sugar.	K_2O.
Pig	3.71	1.65	5.36	6.32	3.19	0.105
Sheep	4.08	0.80	4.88	9.29	5.04	0.097
Goat	2.91	0.76	3.67	4.33	3.61	0.130

Species.	Na_2O.	Cl.	Fe_2O_3.	CaO.	MgO.	P_2O_5.	Total Ash.
Pig	0.082	0.083	0.004	0.268	0.017	0.329	0.871
Sheep	0.086	0.129	0.004	0.245	0.015	0.293	0.841
Goat	0.062	0.102	0.004	0.197	0.015	0.284	0.771

(*b*) After the animal has doubled its weight:

Species.	Casein.	Albumin.	Total Protein.	Fat.	Sugar.	K_2O.
Pig	3.23	1.06	4.29	7.21	3.71	0.099
Sheep	4.07	0.52	4.59	9.44	5.22	0.096
Goat	2.56	0.58	3.14	2.93	3.92	0.133

Species.	Na_2O.	Cl.	Fe_2O_3.	CaO.	MgO.	P_2O_5.	Total Ash.
Pig	0.074	0.067	0.004	0.241	0.014	0.300	0.783
Sheep	0.085	0.121	0.004	0.235	0.015	0.281	0.809
Goat	0.062	0.111	0.004	0.199	0.016	0.285	0.784

During lactation the albumin content of milk diminishes gradually, while substances such as sugar and fat, which are less essential as building material, but are rather to be regarded as sources of energy and heat, tend to increase in amount.

The remarkably small amount of phosphoric acid contained in human milk is nevertheless sufficient for the development of the child, although this takes place much more slowly than is the case with most mammals. The above relations between the rate of growth and the composition of the milk make it perfectly apparent how difficult it must be to replace one kind of milk with that of another species. Evidently if the new milk contains any constituent in amount less than the required minimum,

there will be, necessarily, disadvantageous results. The value of any milk substitute should in no case be determined by the fact that it contains all of the elements required, nor by the fact that it contains in abundance something (e.g., albumin, lime or phosphorus) which we are accustomed to regard as especially essential. It is of chief importance that there is nothing present in quantity below the required minimum. Even though a milk substitute may be rich in phosphorus, it may be of but little value; for, in order that the cells may utilize this phosphorus, it is necessary that a sufficient amount of certain other substances should be present. This shows where the greatest emphasis is to be placed. There is no question that the unsuccessful results obtained in the artificial feeding of infants have been due chiefly to the non-observance of this principle. It is obvious that the mother's milk can never be replaced satisfactorily by some other milk, or milk-substitute. This accounts for the greater mortality among "bottle babies" than among those that are breast-fed. It is our duty to make it generally known that on the one hand there is no perfect substitute for the mother's milk, and on the other hand to show that when a replacement is unavoidable, the food should be adjusted in accordance with the requirements established as a result of biological investigation.

Chlorine is also an important constituent of milk. It occurs as chloride of sodium and of potassium, and is distributed throughout all the cells of the body. The alkali chlorides, especially the sodium compound, play an important part in the formation of the urine. We shall come back again to the relations of chlorides to the juices of the stomach.

Finally, milk contains another element, sulphur, which is present in a firm state of combination in the proteins casein, albumin, and globulin. In this connection the reader is referred to what was said concerning the decomposition products of protein.[1]

As far as we know, this comprises all the elements contained in milk. It is, to be sure, possible that other elements are present in small amounts. Thus, it has been suggested that milk may contain iodine. This assumption was made merely because iodine plays an important part in the economy of the cells. It is, however, perfectly possible that the newborn child either contains iodine already stored away, or else that it makes use of what it has only for later functions.

There has been a great deal of discussion as to whether the animal organism normally contains arsenic. It is certain, however, that if such be the case, the amount present is extremely small. The question concerning the arsenic content is an old one, and has been zealously discussed by toxicologists in medical jurisprudence.[2] The contradictory results concerning the normal occurrence of arsenic in the thyroid gland is prob-

[1] Cf. Lecture VIII, p. 157.

[2] M. Orfila: Traité de médecin légal, 4th edition, Vol. III, part I, p. 285 (Paris, 1848).

ably due to differences in the material examined. The amount of arsenic in animal tissues must depend upon the nature of the food. Gautier [1] and Bertrand [2] have found arsenic, while others, as, for example, Kunkel,[3] have not.

It should be mentioned finally that recently the claim has been made that lithium is also a normal constituent of the human organism. Erich Herrmann [4] found this element present in stages of development where the nourishment had been provided solely from the blood of the mother. The lungs were found to be particularly rich in lithium.

From what has been said, it is apparent that inorganic salts are of great importance as foods, both for the developing and adult organisms. The cells also require the presence of salts for the proper exercise of their functions. The cells are constantly being broken down and built up anew. The more recent investigations concerning the action of the separate salts make it seem most probable that our knowledge concerning the part that inorganic substances play in the metabolism of the cells will shortly be widened greatly, and that before long the inorganic substances in our food will be the subject of considerable more interest corresponding to their importance.

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[1] Compt. rend. **137,** 295 (1903), and Bull. soc. chim. Paris, **29,** 913 (1903). Cf. also Compt. rend. **137,** 158 and 232 (1903), and Bull. soc. chim. Paris, **29,** 639 (1903).

[2] Ann. inst. Pasteur, **16,** 553 (1902); **17,** 1 (1903); Ann. chim. phys. **28,** 242 (1903); Bull. soc. chim. Paris, **29,** 790 and 920 (1903), and Compt. rend. **137,** 266 (1903).

[3] Z. physiol. Chem. **44,** 511 (1905).

[4] Pflüger's Arch. **109,** 26 (1905).

LECTURE XVIII.

OXYGEN.[1]

All the foodstuffs which we have considered up to this point are introduced into the animal organism by way of the alimentary canal. There is one substance required to nourish the body which differs from all the other organic and inorganic foods, not only in the form in which it is taken up, but also in the manner of its introduction. We refer to oxygen, which is taken up as a gas into the animal organism through the lungs and carried by the blood to the tissues. With the oxygen, as such, there is no available energy introduced into the organism. It possesses no chemical kinetic energy, so that it belongs to the same class of substances in this respect as the salts and water. In every way, however, oxygen occupies an exceptional position. Plants set it free by the aid of chlorophyll and the influence of the sun's rays in the assimilation of carbon dioxide and water. Energy is required for this process and becomes stored up as chemical energy. Conversely, in the animal cells oxygen again combines with the substances formed in the plants, energy is set free, and we find as the final end-products, water and carbon dioxide, both of which can again take part in the cycle.[2]

The first one to clearly recognize the importance of oxygen for the life process was Lavoisier. He sharply outlined the important rôle which this substance plays in the combustion processes taking place within the animal organism. With this knowledge, there was laid one of the most important foundation stones for the entire physiology upon which, in the period following, stone after stone was piled in rapid succession until finally the structure was established, the particulars of which we are now studying. No discovery in the whole field of physiology was so decisive for further investigation as this. Lavoisier himself, it is true, believed that the lungs were the seat of all the oxidation processes taking place in the animal organism. The oxygen taken from the air was supposed to oxidize the substances brought to the lungs by means of the blood. Such an assumption was *a priori* hardly plausible, for in this combustion

[1] Cf. Christian Bohr: Handbuch der Physiologie der Menschen, Vol. I, p. 54, 1905.

[2] Fundamentally, there is no such sharp distinction between plant and animal cells. Plants also utilize chemical energy, but in them the reduction processes far exceed those of oxidation in the daytime, though in the absence of light (night-time) the latter processes are more prominent.

energy is set free which is required by the life-processes taking place in the tissues and cells. If all combustion took place in the lungs, then at a single place the greater part of the total energy would be set free. The tissues and cells could only secure for themselves a part of this energy by means of certain cleavage-processes. An examination of the gases in the blood would necessarily decide this question. If the oxygen actually combined with the combustible substances directly in the lungs, then it was certainly to be expected that the blood itself would contain but little if any oxygen. This idea appealed to Magnus,[1] who analyzed the gases in blood and showed that a certain amount of oxygen was present until the capillaries were reached and at this point a part of it began to disappear. This proved beyond question that all of the combustion processes could not take place in the lungs. It left undecided the question whether the oxidation processes took place exclusively in the blood, or whether oxygen passed through the walls of the blood-vessels into the tissues. It is conceivable that the tissues constantly give up these oxidizable substances to the blood. In fact, certain discoveries support this assumption. If the supply of oxygen be entirely cut off from an animal, it suffocates. Its blood then contains but traces of oxygen. On exposing such blood to oxygen, the latter disappears in a short time, and the amount of carbon dioxide in the blood increases. The blood of an animal which has not been suffocated shows the same phenomenon, but to a much less extent. Ludwig and Schmidt,[2] who carried out these experiments, explained this discovery on the assumption that oxidizable substances were constantly being given up to the blood which immediately underwent combustion provided the supply of oxygen were adequate. If this supply were cut off, these substances began to accumulate in the blood. Now we know that the blood contains cells, the white and red blood corpuscles, which themselves undergo metabolism, and thereby may easily consume oxygen and yield carbon dioxide. The above experiments, therefore, are not sufficient to prove satisfactorily that the combustion takes place chiefly in the blood. Afonassiew[3] then showed that, as a matter of fact, only the blood-corpuscles and not the serum of a suffocated animal could take up oxygen in this way. The assumption that the combustion takes place in the cells and tissues themselves was furthermore supported by the following experiment: Pflüger and Oertmann[4] removed the blood from a frog, washed out the last blood corpuscles with a 0.75 per cent solution of common salt, and finally

[1] Ann. Physik. **40,** 583 (1837); and **64,** 177 (1845).

[2] Ber. über die Verhandl. der Sächs. Ges. Wissen. Leipzig. Math.-physikal Klasse, **19,** 99 (1867).

[3] *Ibid.* **24,** 253 (1872).

[4] E. Oertmann: Pflüger's Arch. **15,** 382 (1877); E. Pflüger: *ibid.* **10,** 251 (1875).

replaced all the blood in the various vessels by this saline solution. This animal was then placed in an atmosphere of pure oxygen, and consumed as much of the gas and evolved as much carbonic acid gas as a normal frog.

To-day there is not the slightest possible doubt that oxygen diffuses into the tissues, and that the cells themselves obtain their energy by the combustion of their nutriment, which takes place in their immediate vicinity. We know a great many facts which are in harmony only with this assumption. One of the principal proofs is that the blood itself possesses no oxidizing properties.[1] If, for example, salts of lactic acid, acetic acid, etc., are placed in the blood they remain unchanged, whereas in their passage through the organism they are quickly and completely oxidized. This experiment becomes more convincing if carried out with surviving organs. If, for example, blood is conducted through the liver of a dead animal by the portal vein, it can be shown that ammonium formate introduced into the blood disappears and in its place urea is formed. This is never the case, however, if the formate is merely exposed to the action of the blood without coming in contact with the liver-cells; in such cases the ammonium formate remains unchanged. Evidently there is a mutual action between the blood and the cells of the liver which is necessary to cause this complicated reaction to take place. This is merely one example out of many.

The fact that oxygen actually passes through the walls of the blood-vessels is strikingly shown by the way the fœtus is provided with this element. It is well known that there is no direct connection between the vascular system of the mother and that of the child. The circulation of the fœtus is isolated. The umbilical arteries carry the blood rich in carbon dioxide and poor in oxygen from the fœtus through the umbilical cord to the placenta. Here these arteries break down into extremely fine branches. They change into the form of chorionic villi in the enlarged capillaries of the mucous membrane, in the intravillous spaces of the decidua. To this region the organism of the mother sends blood rich in oxygen. In order that this oxygen may enter the fœtal circulation — i.e., that it may enter into the umbilical veins, — it must first penetrate the epithelium and vascular walls of the chorionic villi, and conversely the venous fœtal blood of the umbilical arteries gives up its carbon dioxide in the same way.

Another proof that the oxygen passes through the walls of the blood capillaries lies in the fact that the saliva contains a constant amount of free oxygen. According to E. Pflüger,[2] it contains 0.5 per cent by volume

[1] Cf. E. Pflüger: Pflüger's Arch. **6**, 43 (1872); Hoppe-Seyler: *ibid.* **7**, 407 (1873).

[2] Pflüger's Arch. **1**, 686 (1868). See also R. Külz: Z. Biol. **23**, 321 (1887). J. L. Bancroft: Biochem. J. **1**, 1 (1906).

of this gas. This oxygen must come from the circulation, and is undoubtedly to be regarded as oxygen that has not been consumed by the oxidation processes taking place in the cells of the salivary glands.

Paul Ehrlich[1] has proposed a very pretty method for following the course of the oxidation processes taking place in the tissues. If a dyestuff which becomes decolorized on reduction and again resumes its color on oxidation is injected into the veins of an animal, it is easy to recognize the presence of oxidizable substances in the tissues. Methylene blue is especially suited for such experiments. If this has been injected into the veins, it will be found that a freshly-killed animal will be of normal color; but after being exposed to the air for some time, the color of methylene blue eventually appears, showing that the tissues have contained this dyestuff in a reduced form.

The assumption that the consumption of oxygen must actually take place in the tissues and cells has been based frequently upon numerous observations concerning the oxygen supply of lower organisms. Thus the observations of Kupffer[2] and of Max Schultze[3] regarding the direct supply of oxygen to the cells of the body are a good example. The former showed that insects which had no real vascular system conduct the atmospheric oxygen directly to the tissues by means of an infinitely-fine tracheal system. The finest little runners of the tracheæ send out branches to the individual cells, so that the latter by means of these tiny tubes take the oxygen directly from the air. Again, Schultze showed that in the organs of phosphorescence of *Lampyris splendidula*, the finest ends of the tracheæ lead to the individual cells, which cause the phosphorescence.

Although these observations undoubtedly indicate the ability of highly organized animals to take up and utilize directly the oxygen of the air, yet they do not prove conclusively that also in the highest organized animals such a direct introduction of the oxygen to the cells actually takes place. In the ascending series of animal species, with the division of labor and specialization of the separate cell groups becoming more and more complicated, it would not seem impossible that perhaps one particular cell group may be limited to quite restricted functions; and that, for example, the cells receive their energy, in the more highly developed organisms, from certain *cleavage* processes, while the energy produced by oxidation serves merely as the source of heat for the organism. We have already seen[4] that intestinal parasites, and even frogs, can live for

[1] Med. Zentrb. **1885**, 113. Das Sauerstoffbedürfnis des Organismus, Berlin, **1885**. Cf. C. A. Herter: Z. physiol. Chem. **42**, 493 (1904), and Am. J. Physiol. **12**, 128 (1904). Herter and Richards: *ibid.* **12**, 207 (1904). C. A. Herter: *ibid.* **12**, 457 (1905).

[2] Beiträge zur Anatomie und Physiologie (1875). Cf. E. Pflüger: Pflüger's Arch. **10**, 251 and 270 (1875).

[3] Arch. mikros. Anat. **1**, 124; **5**, 186.

[4] Lecture IV, p. 74.

a long time without oxygen, and produce carbon dioxide; and, on the other hand, we have cited the experiments of Fick and Wislicenus,[1] who showed that the energy set free in the cleavage processes was altogether insufficient to account for the work which these authors were capable of accomplishing. We came to the conclusion then, that under some circumstances the muscular cells, in order to satisfy the demands placed upon them, must utilize all the chemical energy available from the food materials it receives.

On the other hand, the assumption that the cells in general are satisfied to accomplish their work with the energy resulting from cleavage processes, is supported by the fact that there are unicellular organisms which not only do not require oxygen, but on which in fact this gas even acts as a poison. These are the anaërobic bacteria. All sorts of varieties of bacteria are known, ranging from those to which oxygen is indispensable to those which get along best without it. There are, in fact, bacteria which are temporarily anaërobic; i.e., they can get along without oxygen for a time. It is characteristic of all these bacteria that they eliminate carbon dioxide, no matter whether they take up oxygen directly from the air or not. The bacteria which are wholly anaërobic, and those which are temporarily so, must be able to obtain oxygen from the nutriment upon which they subsist. In the latter case, the assumption might be made that they store up compounds rich in oxygen, which they consume during the anaërobic period, just as the muscular cells are evidently capable of storing up oxygen, when they are in a state of rest, which they require when the muscles are being used.

We shall later on[2] go more into detail concerning the significance of this progressive breaking down by stages of the nutriment on the part of the cells in our body, and shall find that by means of this alternate simple decomposition and oxidation it is possible to obtain energy from the food as it is required. At all events, all our observations indicate that each individual cell in the body must have the possibility of obtaining oxygen for oxidation processes, and for the regulation of its internal economy. We shall soon become acquainted with facts which compel us to accept this assumption.

Let us now attempt to trace the course of the oxygen from the time it is taken up by the lungs till it is given up to the cells of the individual tissues. The blood plays an intermediate part in the process. It takes the oxygen from the lungs and gives it up to the tissues. The first gas-exchange is commonly spoken of as *external respiration*, and the latter as *internal respiration*. The question that interests us first of all is how does the oxygen circulate in the blood. There are two possibilities to be con-

[1] Lecture IV, p. 69.

[2] See Lecture XIX.

sidered. The oxygen may be simply absorbed by the blood, or it may be that there is some compound in the blood which unites with this oxygen. In the former case the absorption of oxygen must follow the gas laws,[1] of which we shall briefly sketch the most important particulars.

The absorption of a gas by a liquid, when there is no chemical reaction between the two, is dependent upon the nature, the temperature, and the pressure of the gas; and, in fact, the *weight* of gas which is absorbed by a definite liquid is proportional to the pressure under which the gas is placed. Now according to Boyle's law,[2] the *weight* of a definite volume of a gas is directly proportional to the pressure, so that evidently the *volume* of gas absorbed is independent of the pressure. Again, if instead of a single gas a mixture of gases stands above a liquid, then each individual gas will be absorbed independently of the others, and the amount absorbed is governed entirely by the pressure which this gas exerts (Dalton's law). This pressure is called the partial pressure. The partial pressure can be computed as soon as one knows the total pressure exerted by all of the gases present in the mixture, and the percentage composition of the mixture. The partial pressure is the same percentage of the total pressure that the gas in question is present in per cent *by volume* in the mixture.[3]

If a liquid is allowed to remain in contact with a definite gas mixture for some time, the liquid will become saturated with gas. When this has taken place, then the pressure exerted by each gas in the liquid is equal to the partial pressure of the same gas in the mixture above the liquid. There is a state of equilibrium between the gas in the atmosphere and that in the liquid. If this equilibrium is disturbed, for example, by diminishing the amount of gas in question in the gaseous mixture, then the liquid will give up this gas until once more the pressure in the mixture is in equilibrium with the pressure exerted by the gas in the liquid. This fact may be taken advantage of, if we wish to determine in a simple manner what pressure is exerted by a gas which is dissolved in a liquid. The liquid is placed in contact with a gas mixture of a definitely known composition and pressure (whereby, as stated above, the partial pressure

[1] Cf. Text-books on Physics. For comparative purposes the volumes of gases are reduced to 0° C. and 760 millimeters, barometric pressure. As regards the methods used for examining the gases in blood, see E. Pflüger's Untersuchungen aus dem physiologischen Laboratorium zu Bonn, Berlin, 1865. Alexander Schmidt: Verhandl. Sächsischen Gesellsch. Wissensch. **19,** 30 (1867). A. Kossel and A. Raps: Z. Physiol. Chem. **17,** 644 (1893). Neesen: *ibid.* **22,** 478 (1897). Müller: Pflüger's Arch. **103,** 541 (1904). J. Geppert: Die Gas-analyse und ihre physiologische Anwendung nach verbesserten Methoden, Berlin, 1886.

[2] The name Mariotte's law is often given to this principle (earlier discovered by Boyle), that at any given temperature the volume of a given weight of gas varies inversely as the pressure which it bears.

[3] The partial pressure of a gas in a mixture is the same pressure that the gas exerts when present by itself in the volume occupied by the mixture.

exerted by the gas in question may be accurately computed), and the liquid shaken with this gas mixture for some time. Now, according as the gas contained in the liquid exerts a less pressure, the same pressure, or a greater pressure, than is exerted by the same gas in the gas mixture, there will be either an increase, no change, or a diminution in the amount of the particular gas above the liquid.

Let us now come back to the question of the condition of the oxygen as it circulates in the blood. According to the above principles, it ought to be easy to decide whether there is any chemical combination between the oxygen and the blood. From the amount of oxygen in the air that is breathed into the lungs, or, what is the same thing, from the partial pressure of the oxygen in the alveolar air, taking into consideration the temperature of the body and the composition of the blood, we can compute how much oxygen the latter could take up by simple absorption.

There are a number of different methods which are derived from the above principles for determining the gas content of a liquid. Since with rise of temperature the amount of gas absorbed diminishes, it is evident that the gas can be expelled from a liquid by heating it. When the liquid begins to boil, i.e., when it is itself being converted into vapor, all of the absorbed gases have been expelled. The pressure of the gas in the liquid is now equal to zero, whether it is due to the fact that there is a complete vacuum above the liquid, or because the gas which was absorbed by the liquid has been replaced by some other gas (e.g., water vapor) in the atmosphere directly in contact with the liquid. According to the above description of the behavior of gases, the effect in the latter case, as far as the absorption of the gas goes, must be exactly the same as if all the gases had been removed, for then the partial pressure of the given gas in contact with the liquid would have become zero.

The air that is breathed into the lungs contains in round numbers 79 per cent by volume of nitrogen, 21 per cent oxygen, 0.03 per cent of carbon dioxide, and varying amounts of water vapor. If we compare the amount of oxygen taken up by the blood in the lungs, with that computed to be present from the partial pressure of the oxygen in the gas mixture that reaches them, it is found that far too much oxygen is removed by the blood, so that it is quite out of the question to consider the phenomenon as one of simple absorption.[1] Nitrogen and argon, which, as far as we know, take no part in the metabolism of the living animal organism, behave quite differently when in contact with the blood. They are, for the most part, merely absorbed mechanically. The absorption coefficient for nitrogen amounts at the body temperature to about 0.013 to 0.02. Oxygen, however, in its absorption by the blood, in no way follows the gas laws. The amounts of oxygen absorbed by the blood, when it is exposed to differ-

[1] Cf. Hüfner: Arch. Anat. Physiol. **1890**, 1; **1895**, 209.

ent partial pressures of this gas, show, within certain limits, but a slight variation. The blood-plasma is able to take up only 0.65 per cent by volume of oxygen.[1] As a matter of fact, the arterial blood contains more than 70 times as much oxygen. Again, if blood be placed under an air-pump, the oxygen is not removed from it at all in proportion to the change in gas pressure. The oxygen does not leave the blood to any extent until the pressure has been reduced to 358 millimeters Hg.

Since the blood-plasma contains, as stated above, 0.65 per cent by volume of absorbed oxygen, the rest of the oxygen contained in blood must be held in some sort of chemical combination; and, obviously, this union is effected with the blood corpuscles. In the arterial blood of a dog, Pflüger[2] found, on an average, 22 per cent of oxygen by volume. If now a solution of hemoglobin is employed, containing the same amount of hemoglobin as the blood, it will be found that this solution, within narrow limits, is capable of absorbing the same amount of oxygen as the blood. This shows that it must be the hemoglobin which combines with the greater part of the circulating oxygen in the blood. When hemoglobin absorbs oxygen, it is changed into oxyhemoglobin; one molecule of hemoglobin unites with one molecule of oxygen, or for one gram of hemoglobin there are required 1.56 cubic centimeters of gas (measured at 0° C. and 760 millimeters pressure). Dog's blood contains approximately 14.5 per cent of hemoglobin. From this it is evident that 22.6 per cent by volume (1.56×14.5) of oxygen can be absorbed by the blood of a dog. This agrees well with the amount found by actual experiment. It is, however, not permissible to apply the results obtained by working with hemoglobin solutions directly to the absorption of oxygen by the blood under different conditions. There are a number of facts known which show that there are certain differences as regards the behavior of the two liquids. This may be accounted for in different ways. It is conceivable that certain changes may have taken place in the preparation of the hemoglobin solution. On the other hand, the possibility exists that the hemoglobin in the blood is influenced by the way it is contained in the blood corpuscles. It is,

[1] Christian Bohr, Skand. Arch. Physiol. **17**, 104 (1905), has recently computed the absorption coefficients of the plasma and blood for different gases:

	Oxygen.		Nitrogen.		Carbon Dioxide.	
	15° C.	38° C.	15° C.	38° C.	15° C.	38° C.
Water	0.0342	0.0257	0.0179	0.0122	1.019	0.555
Plasma	0.033	0.023	0.017	0.012	0.994	0.541
Blood	0.031	0.022	0.016	0.011	0.937	0.511
Blood corpuscles	0.028	0.019	0.014	0.009	0.825	0.450

[2] E. Pflüger: Zentrb. med. Wiss. **1867**, 722, and Pflüger's Arch. **1**, 274, 288 (1868).

moreover, very questionable whether hemoglobin itself is a simple substance. The component containing iron is constant in its composition, but the relation between this and the globin (the protein constituent), or, in other words, the number of globin molecules which unite with the hemochromogen, varies in different cases. It is necessary to mention this fact in this connection, because it is unquestionably true that differences in the results obtained by investigators are due, to some extent at least, to the fact that the results obtained by working with hemoglobin solutions have been applied directly to the absorption by the blood.

Hemoglobin itself consists of a protein, globin, and another constituent, hemochromogen,[1] which contains iron; it is the hemochromogen alone that unites with oxygen. This is shown by the fact that hemochromogen absorbs as much oxygen from the air as an equivalent amount of hemoglobin. When hemochromogen is oxidized to hematin, the hemoglobin of the blood becomes oxyhemoglobin. Whereas the oxygen combined in hematin cannot be removed by means of an air-pump, this is not the case with oxyhemoglobin itself. This fact is of great importance for the understanding of the transportation of oxygen by the blood and its giving up of the same to the tissues. Oxyhemoglobin belongs to the class of compounds which are said to be dissociable. Before we go into further particulars concerning this transportation of oxygen by the blood and the subject of internal respiration, we must make perfectly clear what are the conditions in the animal organism upon which the dissociation of the oxyhemoglobin depends. We have already mentioned the fact that the blood-plasma contains absorbed oxygen. The amount present is, corresponding to the gas laws, relatively small. It is perfectly clear that it follows the laws of gas absorption. First of all, the amount of this gas must be in equilibrium with the alveolar air. On the other hand, this absorbed oxygen, in its transportation to the various tissues, must necessarily constantly seek to be in equilibrium with the pressure of the oxygen in these tissues, and, likewise, in accordance with the well-defined gas laws. Now, as we shall soon see, the tissues are constantly consuming oxygen and forming carbon dioxide therefrom. For this reason the pressure of oxygen in the tissues is kept lower than that of the absorbed (or dissolved) oxygen in the blood. Hence oxygen is constantly entering these tissues from the blood. There is no doubt at all that, in the first place, this absorbed oxygen is given up. Now in proportion as this absorbed oxygen is given up by the blood, oxyhemoglobin becomes dissociated, i.e., begins to give up its oxygen to the plasma. If this conception be correct, it must be possible to remove eventually all the oxygen from the blood by means of the air-pump, even at low temperatures, i.e., without the aid of heat, which itself tends

[1] Cf. Lecture VII, p. 141.

to dissociate the oxyhemoglobin. This has, in fact, been found to be the case.[1] For the cells themselves, therefore, at any given moment, only the uncombined oxygen is available. The oxygen contained in the oxyhemoglobin serves, as it were, as a reserve supply. It is only the merely mechanically absorbed oxygen which determines the pressure of the oxygen in the blood, and determines thereby the gas exchange. Naturally the extent of the giving up of oxygen to the tissues depends solely upon the magnitude of this pressure exerted by the absorbed oxygen in the blood. The fact that the oxygen in the blood is not merely absorbed, but for the most part held in a state of loose combination, is of great significance for the entire metabolism. The animal organism is, within fairly wide limits, independent of the partial pressure of the oxygen in the surrounding atmosphere. From rarefied air, the blood removes the oxygen and combines it with hemoglobin. We can imagine that the process takes place in somewhat the following manner: First of all the oxygen is absorbed by the blood-plasma from the alveolar air in accordance with the gas laws; but inasmuch as this dissolved oxygen constantly tends to combine with the hemoglobin, more and more oxygen is taken up from the air. In this way a considerable store of oxygen is laid away in the organism, by means of which it is able to satisfy, at any time, any unusual and unexpected demands for this important gas.

The question next arises as to how the amount of oxygen that is taken up by arterial blood corresponds to the quantity absorbed when a sample of blood is thoroughly shaken with air. Experiment has shown that normally the blood is nearly saturated with oxygen, for but little more is absorbed when it is shaken with air. The taking up of oxygen by the blood is dependent upon certain definite conditions. This is evidently true of a small amount that is mechanically absorbed by the plasma. The amount of oxygen which unites with the hemoglobin, on the other hand, is entirely independent of the laws which govern the absorption of gases in cases where no chemical combination takes place. Paul Bert [2] studied the influence of the temperature. At higher pressures he was not able to detect any definite influence, but with lower gas pressures there was less absorption at the temperature of the body than at, say, the room temperature. The fact that the absorption of the oxygen depends upon the pressure of the gas is clearly shown by the following table prepared by Krogh.[3] Krogh examined the blood from horses at 38° C., and determined the amount of chemically combined oxygen; i.e., from the total amount of

[1] Christian Bohr: Blutgase und respiratorischer Gaswechsel, Handbuch der Physiologie des Menschen. Vol. I, pp. 221, 222 (1905).

[2] La pression barométrique, Paris, 687 (1878). Cf. also A. Löwy: Zentr. Physiol. **13**, 449 (1899); Arch. Physiol. Anat. **1904**, 231 and 565.

[3] Skand. Arch. Physiol. **16**, 390 (1894).

oxygen absorbed, the small amount merely absorbed by the plasma was deducted. These values may be represented graphically by plotting the oxygen pressures as abscissæ, and the amount of oxygen absorbed as ordinates. Two such plotted curves representing the curves of oxygen tension, as Bohr called them, are shown below in which the dotted line represents the absorption by the plasma, the other that quantity which is chemically bound.

Pressure of Oxygen in Millimeters.	Horse Blood at 38° C.			
	In 100 c.c. Blood.		Oxygen Absorbed.	
	Combined Oxygen.	Oxygen Dissolved in the Plasma.	Per cent Chemically Combined.	Dissolved in 100 c.c. Plasma.
10	6.0	0.020	30.0	0.030
20	12.9	0.041	64.7	0.061
30	16.3	0.061	81.6	0.091
40	18.1	0.081	90.4	0.121
50	19.1	0.101	95.4	0.152
60	19.5	0.121	97.6	0.182
70	19.8	0.141	98.8	0.212
80	19.9	0.162	99.5	0.243
90	19.95	0.182	99.8	0.273
150	20.0	0.303	100	0.455

In the above table column 4 shows the per cent of chemically combined oxygen, placing arbitrarily that absorbed at 150 millimeters pressure at 100 per cent.

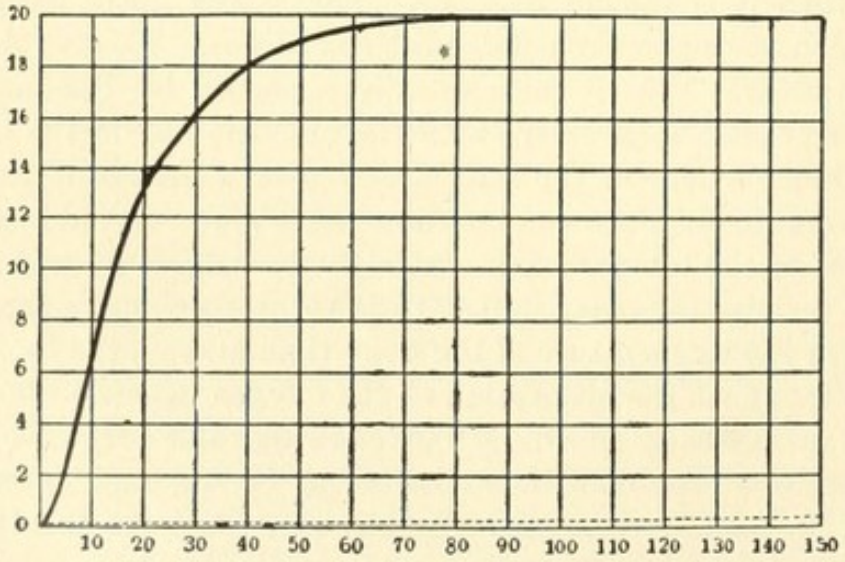


FIG. 3. Absorption of Oxygen at Various Pressures by Horse's Blood at 38°.

These two curves show very clearly the different behavior of the chemically combined oxygen, and that which is nearly absorbed. The amount of the latter increases regularly in proportion to the pressure as it should

in accordance with the gas laws. The chemically bound oxygen, on the other hand, is only affected materially by low pressures.

We must not fail to mention that recently Ch. Bohr[1] has made certain observations which tend to shatter the belief that the oxygen combination by hemoglobin is a constant quantity.[2] Bohr speaks of a specific oxygen capacity of the blood in different animals and different individuals. The proportion of hemoglobin, or of the iron contained in it, to the amount of oxygen consumed, varies. In order to explain this fact, Bohr makes the assumption that the pigment in the blood is not a simple substance, but is composed of different components, which separately combine with varying amounts of oxygen. It is conceivable that each individual blood corpuscle originally incloses a uniform pigment, the specific nature of which is gradually attained in various ways. This would account for the fact that iron and oxygen are often found in unequal atomic proportions. It must be emphasized, however, that Bohr's assumption is to be regarded merely as an hypothesis. It is by no means satisfactorily proved. We have mentioned these investigations of Bohr, partly because they open up again to experimental research one of the few fields which had apparently been investigated exhaustively. Once more our interest is aroused concerning all the questions regarding the transportation of oxygen, new inquiries are suggested, and a process which has been regarded as simple, is perhaps to be looked upon as of quite complicated mechanism.

In order to understand correctly the transportation of oxygen in the blood, the process by which it is taken up in the lungs and given up to the tissues, we must for the present stop attempting to trace the course of the oxygen, but concern ourselves with one of the end-products which results from the oxidation (combustion) of the organic substances in the tissues; namely, carbon dioxide. This is necessary, because, according to Bohr's investigations, it is evident that definite relations exist between the oxygen content of the red corpuscles and the carbon dioxide content of the blood. The carbon dioxide in the blood comes, as we have said, from the tissues. It is formed everywhere in the cells as one of the end-products in the metabolism of oxygen. There must, therefore, be developed in the separate tissues a certain carbon dioxide gas pressure, which is in equilibrium with the pressure exerted by this gas in the surrounding cell-complex, and also with that in the blood. When the arterial blood, freshly laden with oxygen, passes through these tissues, which are rich in carbon dioxide, then gas will diffuse into the blood, for the pressure exerted by the carbon dioxide in the blood is less than that of the tissues. The amount of carbon dioxide in arterial blood has been found to average 40 per cent by volume, but this varies greatly. In venous blood, i.e., that which is flowing away

[1] Handbuch der Physiol. *loc. cit.* p. 93.

[2] G. Hüfner: Arch. Anat. Physiol. **1894**, 130.

from the tissues, there is more of this gas. The following table will give some idea of the amounts of the separate gases in arterial and venous blood.[1]

	Oxygen.	Carbon Dioxide.	Nitrogen.
Arterial blood	20	43.6	1.2
Venous blood	12	50.0	1.2

The venous blood from different vascular regions shows an extremely varying content of the different gases. The carbon dioxide content of all the venous blood taken as a whole, must be represented by that of the right heart; for it is here that the venous blood coming from the whole vascular system is mixed. Schöffer,[2] working in Ludwig's laboratory in 1860, compared the composition of arterial blood with that of the right heart. The following table gives the results obtained by Bohr and Henriques.

	Oxygen.		Carbon Dioxide.		Nitrogen.	
	Artery.	Right Heart.	Artery.	Right Heart.	Artery.	Right Heart.
I. . .	25.6	17.3	44.0	51.5	1.23	1.31
II. . .	21.3	11.9	42.6	48.5	1.19	1.06
III. . .	20.3	14.4	45.9	50.3	1.18	1.40
Average	22.4	14.5	44.2	50.1	1.20	1.26

If we compare the amount of carbon dioxide present in arterial blood with that in venous blood, it is at once apparent that it cannot be a case of simple absorption. The quantity present is far too large. Like oxygen it must be chemically combined for the most part. The amount of carbon dioxide absorbed by the blood does in fact depend upon the pressure of the gas, with which it is in equilibrium; but the absorption is not proportional to this pressure, as it would be in a case of simple absorption. With what substance in the blood is this gas combined? The relations here are far more complicated than in the case of oxygen. With the latter there is but one kind of combination, — that with hemoglobin. Carbon dioxide, however, combines with different substances in the blood. A part of

[1] Christian Bohr: Handbuch, *loc. cit.* p. 83.

[2] Sitzungsberichte d. Wiener Akad. **41**, 613 (1860). C. Ludwig: Mediz. Jahrbücher, Wien, 1865. N. Zuntz and Hagemann: Ergänzungsband III zu der landwirtschaftl. Jahrb. **27** (1898).

this gas is merely dissolved both by the plasma and by the red corpuscles, following the laws of gas absorption. The average gas pressure of the carbon dioxide in the organism may be taken as 30 millimeters. Corresponding to this pressure, the amount of carbon dioxide physically dissolved in 100 cubic centimeters of blood amounts to 2.01 cubic centimeters. Now the total amount of carbon dioxide absorbed under these conditions is about 40 per cent by volume, so that approximately only 5 per cent of the total carbon dioxide absorbed is merely held in solution.

It is of interest to know how the carbon dioxide is distributed between the blood-corpuscles and the plasma. According to Setschenow,[1] about two-thirds of the carbon dioxide in dog's blood is held by the plasma, and one-third by the blood corpuscles. Kraus [2] found similar values with the blood of oxen. In blood from horses, however, Frédéricq [3] found only one-fourth with the corpuscles and three-fourths with the plasma.

A. Jaquet [4] studied the influence of the carbon dioxide gas pressure upon the absorption of the gas by the plasma at 37.5° C. The absolute values of this absorption vary greatly. They depend upon the alkalinity of the plasma. Although the values given in the following table are, therefore, only relatively true, still they show how the amount absorbed depends upon the pressure exerted by the gas.

CARBON DIOXIDE ABSORPTION BY THE SERUM.

Pressure of CO_2 in mm.	CO_2 chemically combined in 100 c.c.	Pressure of CO_2 in mm.	CO_2 chemically combined in 100 c.c.
14.8	45.8	26.6	61.7
16.5	57.4	42.7	63.7
17.0	58.5		

It is evident from the above figures that with low pressures there is a marked increase in the absorption with increasing pressure. With pressures above 20 millimeters, however, this increase is not so marked.

Let us now see what the nature of the chemical combination is between the carbon dioxide and the plasma. First to be considered are the salts of the alkalies, especially carbonates. The amount of these present in the plasma is quite large, the sodium salts predominating. Now we know that monocarbonates are changed to bicarbonates by the absorption of carbon dioxide, and conversely that loss of the same gas gives rise to the formation of monocarbonates again. It would thus be very easy to account for the transportation of the carbon dioxide by the blood. In reality,

[1] Mémoires de l'Acad. de St. Petersburg, **26,** 59 (1879).
[2] Festschrift Graz. p. 19 (1898).
[3] Compt. rend. **84,** 661 (1877); **85,** 48 (1878).
[4] Arch. exper. Path. Pharm. **30,** 311 (1892).

however, the relations are by no means so simple. The dissociation of the bicarbonate in solutions of the same concentration as the serum at 37° C., becomes noticeable only when the pressure of the gas upon the solution is less than a few millimeters. With a pressure of 0.2 millimeter about three-fifths of the total dissociable carbonic acid still remains chemically combined. According to the observations of Jaquet, cited above, the carbonic acid of the plasma behaves quite differently. Complete saturation is not effected with 15 millimeters of carbon dioxide pressure. The behavior of the carbonic acid loosely combined in the plasma, therefore, cannot be explained by its relations to bicarbonates and monocarbonates. The fact that by means of the air-pump more than half of this carbonic acid may be expelled from the plasma, speaks, more than anything else, against any such assumption. Inasmuch as we know of no other compounds in the plasma which would be capable of uniting with carbon dioxide to any considerable extent, we are forced to believe that other weak acids are present in the plasma which are constantly striving to unite with the alkali. Sertoli [1] long ago looked for such acids, and considered as such the protein substances of the plasma, especially the globulins. To-day there is no longer any doubt that these protein substances are actually present in the form of alkali salts in serum. They are driven out of these compounds if there is an excess of carbonic acid present. N. Zuntz and A. Löwy [2] have shown this assumption to be true in a very convincing manner. They found that the amount of diffusible alkali in the serum increased by conducting carbon dioxide into it. This is to be attributed to the fact that as the carbonic acid is forced into the serum, the alkali albuminates which are not diffusible are decomposed, and alkali carbonates which are capable of passing through the membrane are formed in their place.

The next point to be decided is whether the alkali contained in the serum is entirely combined with albumin when the partial pressure of the carbon dioxide gas is equal to 0, or whether an excess of alkali is present? If, other than alkali albuminates, there were no other alkali salts of weak acids present in the serum, then it would be expected that if the alkali were completely combined with the protein (i.e., when the partial pressure of the carbonic acid gas was zero), all of the carbon dioxide would have been driven out of the plasma. This is not the case, as E. Pflüger [3] has shown. In one experiment he found 4.9 per cent by volume, and in another 9.3 per cent of carbon dioxide which remained in the plasma, and

[1] Sertoli: Hoppe-Seyler, Medizin-chem. Untersuch. Berlin, 1868, p. 350. Cf. N. Zuntz: Hermann's Handbuch der Physiol. Bd. **4**, 64 (1882). Torup: Die Kohlensäurespannung des Blutes, Kopenhagen, p. 36 (1887). Kurt Brandenburg: Z. klin. Med. **45**, H. 3 and 4.

[2] Pflüger's Arch. **58**, 511 (1894).

[3] Die Kohlensäure des Blutes, p. 11, Bonn. 1864.

could not be removed by the air-pump. Pflüger proved this amount of carbonic acid to be present by adding acid to the plasma. In other words, Pflüger assisted the action of the protein, which was insufficient to take the place of all the carbonic acids in the blood, by the artificial addition of a stronger acid, which expelled the remainder of the carbonic acid that was combined with the alkali.

From these experimental results, we can describe the combination of the carbonic acid in the plasma, somewhat as follows: A part of the carbon dioxide is evidently present, even with low pressures of gas, as bicarbonate. With increasing carbon dioxide pressures, a part of the gas replaces the protein in its combinations with the alkali. In this way we are able to understand much better how the carbon dioxide gas exchange takes place, although it cannot yet be said that the entire process has been satisfactorily explained.

The reason that we do not at present understand clearly the exact way in which the carbon dioxide is combined in the plasma, and have no exact data concerning the dissociation of the separate compounds, is because the plasma itself is a complicated mixture of unlike substances, which mutually influence one another in a number of different ways. The study of the behavior of the bicarbonates alone, or, on the other hand, of the albuminates, does not lead to results which can be applied directly to the plasma, for it is at present impossible for us to imitate precisely the conditions prevailing in this fluid.

There is no doubt that alkali phosphates which are always present in the plasma, even although in small amounts, also have an effect upon the combination with carbonic acid. When exposed to the action of carbon dioxide, Na_2HPO_4 is attacked with the formation of NaH_2PO_4 and $NaHCO_3$.

According to the results obtained by Setschenow,[1] the removal of the alkali from alkali albuminates takes place only with carbon dioxide pressures which are greater than those ordinarily prevailing in the living organism. Thus we may have in these compounds a regulating mechanism which prevents the carbon dioxide pressures from exceeding a certain maximum. If the pressure of the gas exceeds this normal value, the alkali albuminates then serve to unite with the excess of the carbonic acid, and thus prevent any considerable pressure being exerted by the gas.

Carbonic acid is likewise contained in the *blood corpuscles*, partly free, and partly in a state of chemical combination. At 38° C., and 30 millimeters gas pressure, there is present in the blood corpuscles corresponding to 100 cubic centimeters of blood, about 0.6 cubic centimeter of the gas, which is simply physically dissolved. The greater part of the carbon dioxide absorbed by the red corpuscles does not follow the laws for gas

[1] Mémoires de l'Acad. de St. Petersburg, **26**, 60 (1879).

absorption. This part is chemically combined, and in fact, chiefly with the pigment, hemoglobin. This may effect the absorption of carbon dioxide in two ways. In the first place, the globin in it and the remaining proteins of the blood, may strive to combine with the alkali, as does the carbonic acid. On the other hand, the hemoglobin itself may unite directly with the carbon dioxide. The first way in which the hemoglobin influences the absorption of the carbon dioxide is perfectly analogous to what we have just been discussing with regard to the plasma. Here, also, the union between the hemoglobin and the alkali will not be dissolved until the pressure of the carbon dioxide has reached a certain value. Thus N. Zuntz [1] found the compound between the hemoglobin and alkali was not decomposed appreciably, until the pressure of the carbon dioxide was greater than 70 millimeters. This benefits the organism only in time of exceptional need.

We shall now consider the nature of the chemical union between the carbonic acid and the hemoglobin itself. We have seen that hemoglobin combines with oxygen, and that this property is peculiar to that part of the molecule which contains the iron, while the globin participates indirectly in the reaction only in as much as the union of the globin with the hematin brings forth relations which change the firm state of combination between oxygen and hematin into one which is more readily dissociable. It is conceivable that the carbon dioxide unites with the same part of the hemoglobin molecule that oxygen does. We do in fact know of gases of which this is true, as, for example, carbonic oxide (carbon monoxide). One volume of the latter gas replaces one volume of oxygen.[2] This carbonic oxide compound with hemoglobin is also dissociable. The carbon monoxide may be replaced by oxygen again. This takes place when the partial pressure exerted by the oxygen exceeds that of the carbonic oxide.[3] The fact that the carbonic oxide actually combines at the same place as the oxygen was shown to be very probable by Hoppe-Seyler,[4] who showed that it was combined in the iron-containing radicle of the molecule. Now it is well known that carbonic oxide has a poisonous effect; and apparently this is due to the fact that it replaces the oxygen in its combination with the hemoglobin, so that it seriously affects the supply of oxygen for the tissues. Such an action is unknown in the case of carbon dioxide. It is also *a priori* hardly probable that oxygen and carbonic acid should each strive for possession of the hemoglobin molecule. It has also been shown

[1] Zentr. med. Wiss. **5**, 529 (1867).

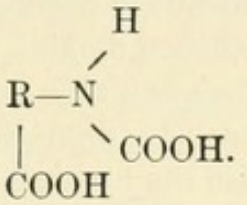
[2] G. Hüfner: Arch. Anat. Physiol. **1895**, 209. Hüfner and Külz: J. pr. Chem. **28**, 256 (1883); and Hüfner: *ibid.* **30**, 68 (1884).

[3] Recently Hüfner and Küster have undertaken experiments to determine the relations by weight in which hemochromogen combines with carbonic oxide. Arch. Anat. Physiol. **1904**, 387.

[4] Z. Physiol. Chem. **13**, 477 and 493 (1889).

that the carbon dioxide is taken up quite independently of the union of hemoglobin with oxygen.

This is made very clear by the experiments of Bohr.[1] He showed that the presence of oxygen had no apparent effect upon the amount of carbonic acid absorbed under different pressures. The hypothesis is also supported by the fact that the transformation of hemoglobin into methemoglobin[2] does not in any way affect its combination with carbon dioxide,[3] while that with oxygen is disturbed. It seems probable that the carbon dioxide is not combined with the hematin part of the molecule, but rather with the globin. This appears the more probable since M. Siegfried [4] has recently shown that carbonic acid is associated (i.e., chemically bound) by the action of amino acids and protein substances. From such compounds it is very easy to set the carbonic acid free again; i.e., dissociate it. From glycocoll, for example, a carbaminoacetic acid is formed. From the amphoteric amino acid a relatively strong dibasic acid results.[5] The carbamino acids correspond to the general type:



The union of carbonic acid with globin is also dependent upon the pressure of the gas, especially when this is low. This is shown by the following table. It gives the amount of carbonic acid chemically combined per gram of hemoglobin at different gas pressures of carbonic acid. The concentration of the hemoglobin solutions amounted to 2.69 per cent; the temperature was 38° C.

Tension of CO_2 in Millimeters.	CO_2 Absorption per Gram of Hemoglobin.	Tension of CO_2 in Millimeters.	CO_2 Absorption per Gram of Hemoglobin.
10	1.260	60	2.363
20	1.647	100	2.701
30	1.902	200	3.113
40	2.091	300	3.312
50	2.240	...	3.990

[1] Zentr. Physiol. **4**, 49 and 253 (1890); and Skand. Arch. Physiol. **3**, 47 and 64 (1892).

[2] See Lecture XXIV.

[3] Skand. Arch. Physiol. **8**, 363 (1898).

[4] Z. Physiol. Chem. **44**, 85 (1905); **46**, 490 (1905). See Lecture XI, p. 235.

[5] It is evident that such compounds may be formed in the tissues, and that in this way any momentary excess of carbonic acid can be combated. Thus, the cells can assist the oxidation processes. It is also possible that the carbonic acid assimilation on the part of plants, as Siegfried has suggested, may also take place through the formation of carbaminic acids, and that the latter, and not the carbonic acid, are reduced.

The concentration of hemoglobin in the blood amounts to 15 per cent on an average. Bohr [1] computed from the above values that with a carbon dioxide pressure of 30 millimeters, about 8.1 cubic centimeters would be held in combination in 100 cubic centimeters of blood. Allowing for the 0.6 cubic centimeter of the gas which is held in merely physical solution by the red corpuscles, then, as the total amount of carbon dioxide absorbed by the corpuscles at that pressure amounts to about 14 cubic centimeters, there remains unaccounted for somewhat over 5 cubic centimeters of carbon dioxide. This must be combined with other substances in the blood corpuscle. These other substances are evidently the alkalies present which can form bicarbonates.

We have now considered the absorption of carbon dioxide by the plasma and by the corpuscles, each acting independently, and it remains to decide whether such a mixture as is present in the blood has any reciprocal effect upon such absorption. N. Zuntz [2] has shown that if an equilibrium has been established, at a definite carbon dioxide pressure, in the exchange of the dissociable substances between the plasma and blood corpuscles, that a change in the pressure of the carbon dioxide will disturb this equilibrium. Thus an increased pressure of the gas makes the serum more alkaline, while the chlorine content simultaneously diminishes, as the following table shows. It demonstrates likewise the reversibility of the entire process.[3]

INFLUENCE OF CARBON DIOXIDE UPON THE COMPOSITION OF THE BLOOD IN CORPUSCLES AND SERUM.

	(*a*) Blood *a*, shaken with air.	(*b*) Blood *a*, exposed to action of CO_2 for 30 min.	(*c*) Blood *b*, after conducting air through it for two hours.
Specific gravity of serum	1.026	1.030	1.026
Total solids in 50 cc. serum	4.157	4.532	4.122
Volume of $\frac{N}{10}$ $AgNO_3$ solution corresponding to the chlorine in 100 cc. serum	99.4	90.7	102.4

This increased alkalinity of the serum is explained by assuming a migration of the alkali carbonates to take place from the corpuscles to the plasma. There is, however, no experimental evidence in support of this assumption. Gürber [4] has shown that the potash is not driven from the

[1] Handbuch f. Physiol. *loc. cit.* p. 115.

[2] Beiträge zur Physiol. des Blutes, Inaug. Diss. Bonn, 1868.

[3] H. J. Hamburger: Osmotischer Druck und Ionlehre in den mediz. Wissensch. Vol. I, p. 263, Weisbaden, 1902.

[4] Sitzb. physikal. med. Ges. Würzburg, **1895**, 28.

corpuscles by the increased pressure of the carbon dioxide gas. This author believes that the carbon dioxide expels the hydrochloric acid from sodium chloride by virtue of its mass-action. Sodium carbonate is formed, while the hydrochloric acid, set free, migrates to the blood corpuscles. The reciprocal relation of the plasma and blood corpuscles has not, however, been explained satisfactorily up to date.

Then, again, has the carbon dioxide tension of the blood any influence upon the absorption of oxygen? We have already seen that the converse is not true, because the carbonic acid and oxygen are combined at different places in the hemoglobin molecule. It has been believed for a long time that the pressure exerted by carbon dioxide in the blood does effect the absorption of oxygen. From the above facts, however, it would seem hardly probable *a priori*. Bohr, Hasselbach, and Krogh,[1] nevertheless, have shown that as a matter of fact the absorption of oxygen is affected by carbon dioxide pressures, which are not above the physiological values. With increasing carbon dioxide pressures the absorption of oxygen becomes less. A few figures will show how great this effect is:

Oxygen pressure in mm.	Oxygen absorbed at CO_2 pressures of —				
	5 mm.	10 mm.	20 mm.	40 mm.	80 mm.
5	11	7.5	5	3	1.5
10	28.5	20.5	14	9	4
15	51	36	27	18.5	8
20	67.5	54	41	29.5	14
40	89	84	77	66.5	49
60	95	93.5	19.5	86	73
80	98	97	96	94.5	87
100	99	98.5	98	97	95
150	100	100	100	99.8	99.5

Bohr explains this by assuming that the entrance of carbonic acid into the globin molecule changes the affinity of the globin for hematin, and thereby influences the absorption of oxygen by hematin at low oxygen pressures. The biological significance of these discoveries may be explained perhaps as follows: We have already seen that, as regards the gas-exchange with the tissues, it is not the total oxygen contained in the blood which is effective, but chiefly that which is contained dissolved in the plasma; it is this that causes the oxygen pressure of the blood. If now an increased amount of carbon dioxide be produced, then as the hemoglobin cannot hold as much oxygen in combination as before, there will be more oxygen in the dissolved state, so that the oxygen pressure of the,

[1] Zentr. Physiol. **17**, 661 (1904), and Skand. Arch. Physiol. **16**, 402 (1904).

blood becomes greater, and there is a more lively gas-exchange between the tissues and the blood.

We shall now attempt to trace the gas-exchange of the blood with the alveolar air on the one hand, and with the tissues on the other. In the lungs, two processes are continually taking place side by side. Blood laden with carbon dioxide constantly reaches these organs, there to discharge this gas and take up a fresh supply of oxygen. The dark-colored venous blood is hereby changed into bright-red arterial blood, and this reënters, through the veins of the lungs, the general circulation. In order to understand the entire gas-exchange in the lungs, we must remember that the blood-vessels of the lungs (the region where the pulmonary arteries end, and the veins begin) represents an infinitely-fine, capillary network, spun round the alveoli. In this way an enormous amount of surface is exposed, which enables us to comprehend how, in spite of the relatively quick passage of the blood through the lungs, a complete gas-exchange takes place. The size of the respiratory surface has been variously estimated. Aeby[1] found that the lung surface in an adult with quiet breathing, amounted to 80 square meters. N. Zuntz,[2] assuming the alveolar diameter of 0.2 millimeter, and the air volume of the lungs to be 3000 cubic centimeters, estimated the alveolar surface to be 90 square meters.

If we compare, first of all, the expired and inspired air, we shall find that the former is poor in oxygen and rich in carbon dioxide, in comparison with the latter. The outer air does not reach the alveoli in an unchanged condition. It is first saturated with water vapor, and warmed to the temperature of the body. It originally contains, on an average, 20.96 per cent oxygen, 78 per cent nitrogen, 1 per cent argon, and 0.04 per cent carbon dioxide by volume. We cannot, however, apply these values directly to the gas-exchange in the alveoli. For the absorption of oxygen, on the one hand, and the giving up of carbon dioxide, on the other, it is the composition of the *alveolar air* which alone comes into consideration. The latter is poorer in oxygen, and richer in carbon dioxide, than the expired air, which contains 16.4 per cent oxygen and 4.1 per cent carbon dioxide by volume. This is because only a part of the inspired air reaches the alveoli. A part of it remains unused in the air-passages, where it is mixed there with the alveolar air and expired. The carbon dioxide and oxygen content of the alveolar air at the moment it leaves the alveoli may be computed, if we know the volume of a single inspiration, and the size of the air-passages which contain the unchanged inspired air (nose, pharynx, trachæ, and bronchi). Such computations, it is true, are not accurate, partly because this latter value is not known closely enough, and

[1] Der Bronchialbaum der Saugetiere und der Menschen, p. 90, Leipzig, 1880.

[2] In Hermann's Handbuch der Physiologie, **4**, 90 (1887).

because the composition of the expired air varies according to the depth of the inspiration. In this way average values of 14.6 per cent oxygen and 5.6 carbon dioxide, by volume, have been found for the alveolar air when it leaves the alveoli. During inspiration, its composition tends to approach that of the inspired air. Some idea as to the extent of the changes in the composition of the air in the alveoli, during inspiration, may be obtained by comparing the amount of inspired air with that remaining in the lungs at the end of respiration. In ordinary breathing there remain 2800 cubic centimeters of air (1200 cubic centimeters residual and 1600 cubic centimeters of reserve air). As the average inspiration amounts to only 500 cubic centimeters, of which about 360 cubic centimeters reach the alveoli, it is evident that the changes in the decomposition of the alveolar air as a result of inspiration are not very great. The necessity of knowing the composition of the alveolar air, in each case, has been realized only as a result of recent investigation. Upon this knowledge depends our judgment as to whether the gas-exchange in the alveoli, between the blood and alveolar air, follows simply the laws of gas absorption, or whether other forces must come into play. It is, to-day, still believed by many that the first explanation of the gas-exchange in the lungs is entirely satisfactory. Blood reaches the lungs, through the pulmonary arteries, having a greater carbon dioxide tension than does the alveolar air. An equilibrium must be established between these two gas-pressures, and as a result carbon dioxide diffuses from the blood into the alveoli. Similarly, on account of its greater tension in the alveolar air, oxygen passes into the blood. The hemoglobin in this way becomes saturated with oxygen, and is ready once more to enter the general circulation. This assumption is supported by the work of Wolffberg[1] and of Nussbaum.[2] If, namely, the gas-exchange in the alveoli of the lungs follows exactly the laws of gas diffusion, then, in a lobule, which is cut off by the closing of the bronchial tube leading to it, the alveolar air must be in equilibrium with the carbon dioxide tension of the blood. Similarly the arterial blood, flowing from this lobule, must have the same carbon dioxide tension as that of the alveolar air. Wolffberg and Nussbaum found, as a matter of fact, that the carbon dioxide tension in the alveoli was the same as that of the venous blood which flows to them. They introduced a double-walled elastic catheter into a branch of the bronchus of a tracheotomized dog, in which this portion of the lungs was shut off, by inflating a rubber enlargement of the catheter. After a short time a sample of the alveolar air was withdrawn through a tube in the catheter and its chemical composition determined. They found on an average that this isolated alveolar air showed a carbon dioxide tension of 3.84 per cent

[1] Pflüger's Arch. **4,** 465 (1871); **6,** 23 (1872).
[2] *Ibid.* **7,** 296 (1873).

of an atmosphere. The corresponding value for the blood of the right heart was 3.81 per cent.

According to the results of this experiment, it would seem that we were unquestionably justified in assuming that the gas-exchange in the alveoli of the lungs takes place in accordance with the well-known laws of gas-diffusion. Quite recently, however, and especially by the extended experiments of Bohr,[1] facts have become known which cannot be explained on this basis. Bohr desired, in each experiment, to know the composition of the alveolar air. A good idea of this can be obtained by analyzing the out-going air, obtained at the moment it passes the bifurcation of the trachea. Such air contains more oxygen and less carbon dioxide than does alveolar air, but, on the other hand, it contains less oxygen and more carbon dioxide than the expired air; it represents a mean between the two. It is essential in such experiments that the gas tension of the arterial blood should be ascertained at the same time and with the same individual. Bohr experimented with large dogs which he compelled to breathe through easily movable valves. A gas-meter measured the amount of the expired air, from which a sample was taken for analysis. Bohr noted the depth of each inspiration and determined, after the death of the animal, the volume of the trachea and the bronchial tubes. From these values he computed the composition of the air at the bifurcation. The partial pressure of the oxygen and carbon dioxide in this gas was thereby known. Simultaneously, the gas-pressure from the blood of an artery was measured, in order to establish normal relations as far as possible. Bohr prevented coagulation of the blood by injecting peptone solution, or leech extract, and carried the blood back through a vein into the general circulation, so that the result of the experiment was not influenced by loss of blood.

Carbon Dioxide Tension.		Difference between *a* and *b*.	Nature of Air breathed.
(*a*) Air at the Bifurcation.	(*b*) Arterial Blood.		
16.6	10.1	− 6.5	Atmospheric Air.
14.3	16.7	+ 2.4	" "
34.6	17.4	− 17.2	" "
14.8	27.6	+ 12.8	" "
40.0	29.7	− 10.9	4.9% CO_2, 18.8% O_2
28.5	0.9	− 27.6	3.2% CO_2, 20.0% O_2

The results of these experiments showed, on the one hand, that the oxygen tension of the arterial blood flowing out of the lungs is frequently more than that of the air at the bifurcation and, on the other hand, in

[1] Skand. Arch. Physiol. **2**, 236 (1891).

several cases the tension of the carbon dioxide in the blood was less than that of the air at the bifurcation. The following table gives an idea of the results obtained :[1]

Weight of Animal.	O_2 absorbed per Kilogram in 1 minute.	Oxygen Tension.		Difference between *a* and *b*.
		(*a*) Air at the Bifurcation.	(*b*) Arterial Blood.	
Kilograms.				
14.1	9.8	127	144	+ 17
15.5	10.6	131	105	− 26
18.9	14.1	95	101	+ 6
41.5	14.7	110	122	+ 12
26.0	13.6	116	106	− 10
14.7	7.1	130	144	+ 14

These results indicate that neither the passage of oxygen from the alveolar air to the blood, nor of the carbon dioxide from the blood to the alveolar air, can be accounted for by diffusion alone. Some forces must be at work which tend to make the oxygen more active towards its absorption by the blood than can be accounted for by the partial pressures of the oxygen gas, and at the same time these forces enable the blood to give up its carbon dioxide even when the pressure of this gas is greater in the alveoli than it is in the blood itself. Bohr compares the lung with a gland, and conceives of its activity as that of a secretion. He assumes that the lung-cells have the power of temporarily uniting with oxygen and with carbonic acid. In fact, P. Ehrlich[2] has proved that the lungs possess an extraordinarily strong reducing power. He injected alizarin-blue into animals, this being a dyestuff which becomes colorless on reduction. The lungs of an animal freshly-killed were then found to be colorless, the blue color being apparent only after exposure to the air for some time. Now the lung tissue, like all other tissue, has its own metabolism. It consumes oxygen and evolves carbon dioxide. Its reduction power, according to Ehrlich's results, however, is so pronounced that it seems perfectly plausible to speak of an *oxygen-secretion* in the sense meant by Bohr.

Bohr and Henriques[3] also made the discovery, which is of itself very remarkable, that the lungs take an uncommonly large part in the general

[1] Cf. Bohr: Handbuch der Physiol. p. 146. Bohr measured directly the oxygen tension at the lung surface and compared this with the oxygen tension of the arterial blood. There was in this case a more considerable excess of pressure in favor of the blood. Cf. L. Frédéricq: Zentr. Physiol. **7**, 33 (1893); **8**, 34 (1897). Haldane and J. Lorrain Smith: J. Physiol. **20**, 497 (1896); and **22**, 231 (1899).

[2] Sauerstoffbedürfnis des Organismus, Berlin, 1885.

[3] Oversigt. kgl. Danske Videnskabs-Selskabs forhandl. No. 1, 1897, Arch. physiol. **9**, 590 and 710 (1897).

metabolism. They estimate that about one-third of the total metabolism takes place in this organ. We can understand this active metabolism, by assuming that it is capable of performing a particularly intense kind of work, and it is indeed possible that it is here that the work of secretion comes into play.

It must be admitted that the views of Bohr have been vigorously challenged. His results have been attributed to an insufficient equilibrium being established between the blood and the alveolar air. Bohr himself would not admit this; and now that fifteen years have passed since his first important results were published, without the appearance of any data which conclusively disputes it, we are compelled to place his opinions in the foreground in our discussion of the gas-exchange which takes place in the lungs; although the older assumption that the laws of gas diffusion are sufficient to explain this phenomenon is, on account of its greater simplicity, very attractive. It is, of course, not impossible, but on the other hand extremely probable, that diffusion does in part account for some of this exchange of carbon dioxide between the blood and the alveolar air. Other factors are probably active at the same time, so that there is a very active penetration of oxygen into the blood and removal of carbonic acid from the latter.

Bohr calls attention to the following observations in support of his assumption. In the *Amphibia* it is well known that, besides the lungs, the skin serves as an important organ of respiration. In the case of frogs, simultaneous determinations of the gas-exchange of skin and lungs showed that the taking up of oxygen by the skin is independent of the total extent of the metabolism. It is almost constant, and amounts to 43 to 60 cubic centimeters per kilogram of body weight in an hour. The carbon dioxide elimination on the other hand showed variations of from 92 to 179 cubic centimeters per kilogram in an hour. The gas-exchange taking place in the lungs is different. Much more oxygen is absorbed through the lungs than by the skin, and the variations are much greater (51 to 390 cubic centimeters per kilogram in an hour). The elimination of carbon dioxide may, in winter when there is a considerable absorption of oxygen, sink nearly to zero. Krogh,[1] who first mentioned these facts, found further that a carbon dioxide tension of but a few per cent, in the atmosphere surrounding the skin, caused a considerable increase in the amount of oxygen taken up by the lungs alone, while at the same time the amount taken up by the skin might be diminished. This action of the lungs does not take place, however, if the cutaneous branch of the vagus nerve is cut. The respiration by the skin is, on the other hand, apparently indifferent to the nervous system. Evidently the gas-exchange by the skin takes place by diffusion, while pulmonary respiration is more in the nature of a secretion.

[1] Skand. Arch. Physiol. **16,** 378 (1904).

A very considerable secretion of gases takes place in the swimming-bladder of fishes. Biot,[1] who examined the gases in fishes which live at great depths, found sometimes as much as 80 per cent of oxygen. Whereas the oxygen tension of water at a depth of, say, 1500 meters, amounts to only about one-fifth of an atmosphere, the partial pressure of this gas in the swimming-bladder is equivalent to that of 90 atmospheres. Moreau[2] has shown, moreover, that the oxygen content of these gases in the swimming-bladders of fish depends upon the depth at which the fish lives. Those living near the top of the water often contain a lower oxygen pressure than that of the atmosphere. If the same fish be placed at a greater depth, it is no longer in equilibrium with its surroundings. Equilibrium is restored, however, by more oxygen being secreted in the swimming-bladder. If the bladder is emptied by means of a trocar, it refills with oxygen after a time. This secretion, furthermore, is under the influence of the nervous system. On severing the pneumogastric (vagus) nerve, the gas secretion entirely ceases. Then, on artificially emptying the swimming-bladder, it does not refill with oxygen. The epithelium of the bladder itself is impermeable to oxygen. The oxygen passes out through the so-called *oval*.

These observations are sufficient to prove beyond question that the animal organism possesses cells whose function it is to secrete gases. It is true that these results cannot be applied immediately to higher organisms, but it gives undoubted support to Bohr's opinions.[3] By means of this active taking up of oxygen, the animal organism obtains a certain supply of this important gas, so that air containing but little oxygen suffices for its support within certain limits. Thus, for example, muscular effort requires an increased oxygen supply by an increase in the blood circulation. In a unit of time more blood passes through the lungs. If, by an artificial restriction of one of the pulmonary branches, more blood is made to pass through one lung than through the other, there is more oxygen taken up in the lung with the more blood, although the effect upon the elimination of carbon dioxide is not so marked.[4]

An interesting question, but one not so easy to answer, is whether the lungs of mammals are dependent upon certain nervous influences. This is known to be true in the case of the tortoise. With the *Testudo græca*, the trachea divides so high up in the neck that, without fear of injuring the important nerves, cannulas may be placed in the bronchi, and thus either lung be observed independently. If the vagus branches to one lung are cut, the absorption of oxygen by that lung is lessened, while that of

[1] Mémoires de la société d'Arcueil, 1807.
[2] Mémoires de Physiologie. Paris, 1877.
[3] C. Bohr: J. Physiol. **15**, 494 (1894).
[4] V. Maar: Skand. Arch. Physiol. **15**, 1 (1903); **16**, 358 (1904).

the other lung is increased.[1] The carbon dioxide elimination is similarly affected.

	Oxygen Absorption in —		
	Right Lung.	Left Lung.	Total.
Effect of severing the right vagus	15.4	17.1	32.5
	30.0	5.3	35.3
Effect of severing the left vagus.	29.1	5.2	34.3
	21.4	14.9	36.1

Stimulation of the vagus leads to the opposite effect. It is not so easy to decide how much these results are due to an influence upon the lungs, and how much is due to the influence of the vasomotor fibres. Apparently the latter is not sufficient to account for the whole phenomenon. The influence of the vagus nerve has also been observed with mammals. Stimulation of this nerve tends to make the respiratory quotient approach the value 1.

Now if we consider once more the gas-exchange in the lungs, we see that two processes are taking place side by side. Oxygen diffuses from the alveolar air, which is relatively rich in this gas, and saturates the venous blood with this element that is so important for the whole metabolism. Simultaneously, the blood laden with carbon dioxide gives up the latter to the alveolar air, which contains relatively less of it, and this takes place until the partial pressure of the carbon dioxide in the alveoli is equal to that of the blood, i.e., until equilibrium has been established. Now begins, without doubt, the activity of the epithelium of the lungs by means of which oxygen from the alveolar air is secreted in the blood-vessels, and has the effect of overbalancing the equilibrium between the oxygen tension of the blood and that of the alveolar air, in favor of the former. The carbon dioxide is eliminated with equal avidity, and given up to the alveolar air.

Oxygen now circulates anew with the blood to the tissues whose oxygen content is relatively less than that of the blood, so that oxygen is constantly diffusing from the blood, and first of all the oxygen is lost, which is merely dissolved in the blood. When this dissolved oxygen is lost, the reserve supply, i.e., that combined with the oxyhemoglobin, comes into play. The oxyhemoglobin now dissociates and oxygen is given up to the plasma, in order to keep the oxygen tension of the blood up to a certain value. Now the question arises whether this *internal respiration* takes place in accordance with the gas diffusion laws, or whether we must assume that here also a secretion, i.e., an active giving up of oxygen,

[1] V. Maar: Skand. Arch. Physiol. **13**, 269 (1902).

comes into play. At this point we meet with great difficulties in our search for knowledge. To answer this question we must know, in the first place, exactly how great the gas tension in the tissues is. We know, from the experiments of Strassburg,[1] what the oxygen tension of the lymph is, and as this surrounds all tissues and cells of the body, we get some idea as to the gas-pressure prevailing there. Strassburg found the oxygen tension of the lymph greater than one atmosphere. According to the general conception, the oxygen tension in blood is less than one atmosphere. If this be true, it is necessary to assume some special activity as the cause of the giving up of oxygen to the tissues. On the other hand, Strassburg found that the tension of carbon dioxide in the lymph was less than that of venous blood. From this fact we should speak of gas secretions in the tissues. Some idea of the consumption of oxygen by the tissues is obtained by tracing the oxygen tension of the blood in its transformation from the arterial to the venous condition.[2]

The oxygen supply of the tissues may be regulated in quite a number of different ways. The rate of the blood flow has an effect and again the change of the content of the blood in hemoglobin, whether it be due to the formation of new hemoglobin, or a relative increase by elimination of plasma. By means of such changes combined with variations in the intensity of work of the organs of respiration, the animal organism is, within certain limits, independent of quite considerable variations in oxygen and carbon dioxide tensions. It is highly interesting that each lung has its own independent gas-exchange, and yet is able to mutually compensate the other. If there is a greater oxygen tension in the air supply of one lung, then a greater absorption of oxygen takes place in this lung than in the other. At the same time, the other lung absorbs less than the customary amount of oxygen, so that the total absorption of oxygen by the two lungs remains about the same.

If the partial pressure of the oxygen in the inspired air becomes lower,[3] then naturally that of the alveolar air becomes similarly affected. The degree of change in the composition of the latter depends materially upon the amount of oxygen absorption and the ventilation of the lungs. This is an extremely important fact. It is for this reason that with the same oxygen partial pressure, the alveolar air of two different individuals may have a quite different composition, according as to whether one breathes more deeply than the other, so that the lungs have a greater ventilation.

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[1] Pflüger's Arch. **6**, 65 (1872).

[2] Cf. Ch. Bohr: Handbuch d. Physiol. p. 196. Loewy and von Schrötter, Z. exper. Path. u. Ther. **1**, 197 (1905).

[3] Cf. Paul Bert: La pression barométrique. Paris, 1878. Fränkel and Geppert: Ueber die Wirkungen der verdünnten Luft auf den Organismus, Berlin (1883). A. Loewy: Untersuchen über die Respiration und Zirkulation bei Aenderung des Druckes und des Sauerstoffgehaltes der Luft, Berlin, 1895.

The lower limit for oxygen tension in the alveolar air lies a little above 30 millimeters. This corresponds to an oxygen content of about 4.5 per cent, assuming a total pressure of 710 millimetres (= 1 atmosphere at the body temperature). Moreover, this is true only for a period of rest, and not for one of active work. In the latter case an oxygen pressure of 30 millimeters is not sufficient.

Paul Bert, Fränkel, and Geppert have shown that the amount of oxygen absorbed by the blood becomes equal to one-half the normal amount, only when the total pressure of the surrounding atmospheric air is less than 300 millimeters. This is interesting, because it gives us some conception as to the nature of the behavior of the gas-exchange during passage into a more rarefied atmosphere, i.e., in balloon ascensions, or in mountain climbing. In these two examples naturally the requirements upon the blood-gases are quite different. In the former case, there is practically no work to be performed, so that aeronauts reach a much higher altitude than do mountain climbers, before they experience difficulty in breathing. The fact that different individuals are affected differently at one and the same height is explained, first, by the fact that the lung ventilation and amount of air breathed is, as already mentioned, quite different, so that, in one case, the blood has more oxygen at its disposal than in another. It has been found, moreover, that the animal organism possesses an extremely delicate mechanism of regulation, which energetically opposes any deficiency of oxygen in the system. To this belongs the increase in the number of red corpuscles, and thereby of hemoglobin, which unquestionably takes place when men and animals pass from a locality into one of higher altitude. The increase disappears as soon as the original level is again reached.[1] The object of this is plain. No matter whether we assume that the absolute amount of these red corpuscles is increased, or that the increase is merely relative, brought about perhaps by the passing out of plasma, there is in a unit of blood more hemoglobin passing through the lungs in a unit of time than is normally the case. The way this increase in the red corpuscles caused by ascending high mountains is effected, has not been satisfactorily explained. It is remarkable that the change takes place suddenly, and in fact without any indication of there being any new formation of blood (red corpuscles with nuclei, etc.), and that on reaching a low level again, the reverse change takes place without any of the usual indi-

[1] Cf. Paul Bert: *loc. cit.* Die histiochemischen und physiologischen Arbeiten von Fr. Miescher, Vol. II, p. 328, Leipzig, 1897. Abderhalden: Z. Biol. **43,** 125 and 443 (1902); Medizin, Klinik, No. 6 (1905); Pflüger's Arch. **110,** 195 (1905). von Schrötter and Zuntz: *ibid.* **92,** 479 (1902). van Voornveld: *ibid.* **92,** 1 (1902). Otto Cohnheim: Ergeb. Physiol. (Asher and Spiro) II, 612 (1902). Durig and Zuntz: Arch. Anat. Physiol. Suppl. **1904,** p. 417. Jaquet: Ueber die physiologische Wirkung des Höhenklimas, Basel, 1904. Zuntz, Loewy, Müller, and Caspari: Höhenklima und Bergwanderungen in ihrer Wirkung auf den Menschen, Bong et Cie, 1906.

cations of a diminution in the number of red corpuscles. There is absolutely no doubt that after staying at a mountain height for some time, an acclimatization takes place in the sense that a new formation of hemoglobin results. It remains undecided how much this is due to an absolute increase in the number of red corpuscles, and how much to a merely relative increase. It seems reasonable to believe that this may be brought about by the adjustment of the vascular tonicity to definite pressures of the atmosphere.[1]

It remains for us to decide whether, besides the lungs, other organs of the body take part in the gas-exchange. We have already seen that with *Amphibia* respiration on the part of the skin plays quite an important part. In higher vertebrates this cutaneous respiration does not seem to be hardly worth considering. Schierbeck[2] estimated that in man there is an elimination of carbon dioxide amounting to 9 grams per 24 hours, or somewhat less than 1 per cent of the total gas-exchange. If there is an increased secretion of sweat, it may rise as high as 30 grams in 24 hours. The absorption of oxygen is much less. The following table prepared by Krogh[3] gives a good idea as to the extent of this cutaneous respiration on the part of man and certain animals:

	O_2		CO_2	
	Maximum.	Average.	Maximum.	Average.
Man	...	0.50	...	1.18
Man	...	...	3.1	0.94
Pigeon	0.92	0.47	1.1	0.60
Tortoise	0.1	...	0.15	...
Rana fusca	1.8	1.51	5.3	3.0
Rana esculenta	2.1	1.62	4.4	3.1
Eel	1.05	0.74	...	...

The above values refer to the amount per hour and per square decimeter of the skin. The volumes of the gas are given in cubic centimeters.

It was for a long time believed that the skin took part in the elimination of the gaseous products of metabolism. It had been observed, for example, that if the skin of an animal were varnished over it soon died. This, however, has more recently been found to be caused not so much by the retention of waste gases, as by the greatly increased amount of heat, caused by the crippling of the means for regulating the body tempera-

[1] Mountain sickness has been attributed to various causes, one of which is undoubtedly a faulty regulation of the vascular tone.

[2] Arch. Anat. Physiol. **1893**, 116. Cf. Aubert: Pflüger's Arch. **6**, 539 (1872).

[3] Skand. Arch. Physiol. **16**, 378 (1904).

ture. If this increase in temperature be prevented, the animal will not die.[1]

We recognize certain kinds of fish, especially the loach, *Cobitis fossilis*, in which there is a peculiar intestinal respiration. The middle intestine of the *Cobitis* contains an abundant supply of capillary blood-vessels and a peculiarly transformed epithelium. These fish swallow air, and discharge gases through the rectum. The gas which leaves the body contains less oxygen and more carbon dioxide than that entering.[2]

With the remaining members of the animal kingdom, the intestine plays no part in the gas-exchange. To be sure, the alimentary canal contains gas, resulting, in part, from swallowed air, which with the food, the saliva, and drink, is constantly being introduced, and largely from bacterial decomposition, fermentation, etc. Furthermore, carbonic acid is set free in the neutralization of the carbonates of sodium in the intestinal secretions by the hydrochloric acid from the stomach. The oxygen of the swallowed air is taken up little by little by the intestinal walls; and similarly, on account of its partial pressure, the carbon dioxide diffuses to some extent into the intestinal walls and into the blood-vessels, and in other cases these gases are given up from the vascular system, if the amount present in the intestine is slight. Likewise other gases, such as hydrogen, methane, sulphureted hydrogen, and nitrogen, are absorbed according to the laws of gas absorption, and can be eliminated by the lungs.

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[1] Cf. Laschkewitsch: Arch. Anat. Physiol. **1868,** 61. R. Winternitz: Arch. exper. Path. Pharm. **33,** 286 (1895). E. Babák: Pflüger's Arch. **108,** 389 (1905).

[2] Baumert: Chemische Untersuchungen der Respiration des Schlammenpeizgers, Breslau, 1855. D. Calugareanu: Pfluger's Arch. **118,** 42; **120,** 425 (1907).

LECTURE XIX.

ANIMAL OXIDATIONS.

In the last lecture we attempted to trace the path of oxygen on its way through the animal organism from the time of its being acquired from the alveolar air to its being given up to the tissues and their cells, and, on the other hand, we found that carbon dioxide is to be regarded as the end-product in the action of oxygen upon the nutriment. Meanwhile, we have failed to touch upon one point of greatest moment, namely, why the oxygen attacks and consumes this cell-nutriment. Outside the animal organism, if we expose albumin, fats, or carbohydrates to the action of oxygen at the body temperature, even for a long time, there is no perceptible oxidation of these materials. Within the animal organism, on the contrary, these substances are oxidized in a short time, and the chief products of the oxidation are carbon dioxide, water, and urea. Consequently, conditions must prevail within the organism which facilitate the action of oxygen upon the material exposed to its action.

We are acquainted with quite a number of facts which compel us to assume that, even within the animal tissues, oxygen as such is not able to act upon the unchanged food. Under no circumstances should we imagine for a moment, that the oxygen supplied to the tissues, at once of its own accord, begins to oxidize the different substances present in the cells. If this were the case, it would be absolutely impossible for us to account for quite a number of processes taking place in the animal organism. Above all, it would then be unintelligible, why oxygen is brought to the cells, together with the newly absorbed nourishment, without any oxidation taking place until the cells are reached. The fact that the blood contains the greater part of its oxygen chemically united with the hemoglobin, does not suffice to explain this fact; for it would be expected that the oxygen, which was merely dissolved in the plasma, would be replaced, as soon as consumed, by the oxygen in the hemoglobin. Again, it would be inexplicable why, in the combustions taking place in the cells, it is only the fuel that is consumed, and not the cell-substance itself. On the other hand, we have seen that the organism can lose the power of oxidizing certain substances, such as carbohydrates, for example, which are ordinarily consumed easily, while other oxidation processes are not affected in the slightest. We know now that in diabetes substances hard to oxidize are consumed without difficulty; whereas, unchanged *d*-glucose alone has ceased to be a

food, because the organism has lost the power of being able to utilize the energy stored up in it. If, on the other hand, the grape-sugar is slightly changed before its introduction into the organism of the diabetic, then the tissues are capable of completely oxidizing it.

If the animal oxidation took place merely as a result of the coming together of oxygen and nutriment, it would be expected that when an increased amount of the reacting substances was present, a more vigorous oxidation would ensue. This is, however, not the case. Under normal conditions, it is not possible to increase the amount of oxidation taking place in the tissues by increasing the supply of oxygen and the amount taken up by the blood.[1] Similarly, we are not able to increase the total consumption of material very much by increasing the supply of carbohydrate or fat. Only of albumin do we know that the amount present governs somewhat the extent of the transformation.

We know, to be sure, of compounds which are not attacked by oxygen in neutral solutions, but are attacked in the presence of alkali. Pyrogallol absorbs oxygen in alkaline solutions so vigorously that it is used for the detection of small quantities of this gas. To be sure, we do not have free alkali present in our tissues, but merely alkali carbonates. These also favor such oxidations. Thus it is known that a solution of glucose and soda absorbs oxygen from the air,[2] although the amount taken up is but slight. Schmiedeberg [3] has shown, moreover, that benzyl alcohol in the presence of water is not attacked by atmospheric oxygen, but is transformed to benzoic acid when it is in a sodium carbonate solution exposed to the oxygen of the air. This compound is also oxidized by the oxygen in the blood. If benzyl alcohol is conducted, with blood containing oxygen, through the kidneys or lungs of dogs or pigs, benzoic acid is formed; while if salicylic aldehyde is employed in the above experiment, salicylic acid is formed to some extent. The amount of acid obtained in each case is very small. It is necessary to state in this connection that these experiments are by no means sufficient to account for the combustion of the nutriment. They merely show us that easily oxidizable substances are more readily acted upon by oxygen, when contained in alkaline solutions, than in neutral or even acid ones. They in no way refer to the oxidation of the more difficultly oxidizable foodstuffs.

We are compelled to assume that either the oxygen is changed in the tissues to a form in which it is more active than usual, or that the nutriment is in some way changed by the activity of the cell, so that it is more readily acted upon by oxygen. Or these two processes may take place side by side in the cell.

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[1] See Schaternikoff: Arch. Anat. Physiol. **1904**, Suppl. 135.

[2] M. Nencki and N. Sieber: J. pr. Chem. **26**, 1 (1882).

[3] Arch. exper. Path. Pharm. **14**, 288 (1881).

The fact that the oxygen in the condition in which it is given up to the tissues is not capable of consuming the unchanged nutriment, enables the cell to adjust the metabolism to its requirements. Above all, this fact enables the cell to utilize a certain particular material for definite functions when it so desires. We should also not forget that we know altogether too little concerning the relations of the separate organs to one another, and of the different kinds of cells in one and the same organ, to be able to judge whether under all conditions, the combustion of the fuel is entirely effected by the cell that begins the work, or whether one cell merely carries the oxidation to a certain stage, and another cell carries the combustion farther, until finally the material is completely oxidized. Such an assumption seems extremely probable from the observations of Bohr and Henriques,[1] who found that extensive oxidations take place in the lungs. Oxygen is consumed there and carbon dioxide evolved to an extent sufficient to lead one to presume that incompletely oxidized, metabolic products reach the lungs together with the blood, and that the combustion is completed by the lungs. On the other hand, according to the assumption that the respiratory exchange takes place as a sort of secretion process, it is probable that the lungs have a certain amount of work to do which requires the expenditure of energy, so that this supply of fuel is necessary for its function. Bohr and Henriques found that about one-third of the total metabolism taking place in the body was effected in the lungs. The separate observations varied from 0 to 66 per cent.[2]

We must emphasize the fact at the start that we are not yet able to give a perfectly clear explanation as to the nature of animal oxidation processes. At present, we recognize merely the initial products, food and oxygen, and the final products of the combustion. We are therefore forced to rely upon assumptions. In fact, the great number of hypotheses which have been brought forward, show clearly upon what an insecure foundation they all rest. Here we will only attempt to mention only the more important theories, and emphasize only those which rest, to some extent, upon experimental observations.

The question to interest us first of all is this: Is the oxygen changed in form so that it attacks the nutriment more readily? The formation of ozone has been suggested. We know that ozone is a stronger oxidizing agent than ordinary oxygen, and oxidizes compounds which are not affected by the latter. Schönbein[3] carried his studies on ozone to the phenomena of cell life. He attributed numerous oxidations taking place in the plant organism to the primary formation of ozone. The plant tissues, he assumed, contain some substance which possesses the power of

[1] Arch. de Physiol. **1897**, 590. See Lecture XVIII, p. 432.

[2] These high figures have not been generally accepted. — Translator.

[3] Poggendorff's Annalen, **65**, 171 (1845).

ozonizing atmospheric oxygen. The cells take up the ozone, and then as the latter breaks down to ordinary oxygen, the extra atom of oxygen attacks some oxidizable substance. The assumption that as a matter of fact there is an ozone formation in the tissues, meets with certain difficulties. Relatively small amounts of ozone are poisonous to the cells. It has never been possible to detect the presence of ozone in either plant or animal organisms. So this hypothesis was soon abandoned. The assumption that *active* oxygen is present in the tissues, is far more probable. Hoppe-Seyler,[1] who suggested this hypothesis, based it upon the fact, that in the animal tissues energetic reduction processes take place, side by side with the oxidations. In this way, reducing substances are formed which unite with one atom in the oxygen molecule, setting the other atom free in an active condition. The butyric acid fermentation of sugar is an example of such a reduction process. Hydrogen is set free:

$$C_6H_{12}O_6 = C_3H_7COOH + 2\,CO_2 + 2\,H_2.$$

A support for the assumption that active oxygen causes the animal oxidations, is furnished by the theory of nitrification. In this case also, it is assumed that the organism, to whose activity the formation of nitrate is due, first of all produces readily oxidizable substances, which then decompose the atmospheric oxygen molecule, and thus form active (nascent) oxygen for the oxidation of the nitrogen.

The formation of such readily oxidizable substances which under normal conditions are immediately oxidized further, is likewise indicated by the so-called " spontaneous combustion " of hay. In this case, on account of insufficient ventilation, such readily oxidizable substances collect in considerable amount, and are suddenly oxidized as soon as fresh air enters.

If we are to believe that the oxidations in the animal organism take place in this way, we must assume that first of all the food is hydrolyzed, and that easily oxidizable compounds are formed which are oxidized by the oxygen, received by the tissues from the blood, and at the same time a part of this oxygen is activated. This nascent oxygen unites with the more difficultly oxidizable substances. The first stage in the oxidation of the food is, therefore, a hydrolysis; and inasmuch as the last-mentioned process is brought about by means of ferments, these play a part in the entire phenomenon of oxidation. This assumption has much in its favor, and corresponds to certain discoveries. With its help we are able to understand, for example, why the diabetic cannot oxidize *d*-glucose, and only this one substance. We have simply to assume that the ferment is absent which hydrolyzes this sugar so that oxygen does not come in contact with

[1] Pflüger's Arch. **12,** 16 (1876).

the cleavage-products of this substance. To be sure, this does not explain why the nascent oxygen set free in the combustion of other foodstuffs, is not able to act upon *d*-glucose. At all events, the assumption that oxidation is preceded by a hydrolysis, gives to the cell the power of utilizing its nutriment at the time it is needed. We know that the action of the ferments is specific, i.e., that they are able to act only upon certain definite compounds. If it be assumed that the cells do not contain the ferment in an active condition, but that the ferment is activated only when its action is needed, we are able to understand very clearly much about the economy of the cells. We begin to understand how the cell can have the food, the ferment, and oxygen all present together without any combustion taking place, until at a given moment the ferment becomes activated. The following phenomenon harmonizes well with such an assumption.

The animal organism consumes without difficulty the cleavage-products of the food which it obtains as such. Thus the decomposition products of albumin, such as glycocoll, alanine, etc., are readily oxidized to urea.[1] This is true, however, only of those amino acids which are present in albumin, i.e., those having the same configuration. If, for example, instead of feeding a rabbit with *l*-leucine, we administer the racemic form, *d-l*-leucine, only a part of the molecule, namely the *l*-leucine, is oxidized, while the other half of the racemic substance molecule, the right-rotating leucine, appears unchanged in the urine. This is evidently because the animal cells are not adapted to the combustion of *d*-leucine, which under ordinary conditions is foreign to it, so that it possesses no ferment to attack it. The oxygen, therefore, is not able to attack it. Furthermore, it is not strange that when the combustion is very vigorous a part of this substance is actually oxidized. We may indeed assume that oxygen activated by some other decomposition process can cause such oxidation.

Interesting as this hypothesis appears, we must not forget to state that in this simple form it does not suffice to explain all the phenomena pertaining to animal oxidation. The actual relations are far more complicated than we have indicated. Above all, we would expect that in the absence of oxygen there would tend to be a piling-up of these readily oxidizable substances, and to a considerable degree, particularly as now the entire energy required by the organism must be furnished by the hydrolytic decompositions. We have already mentioned experiments performed in this direction.[2] G. von Bunge[3] has shown that ascarids are able to exist for several days without any oxygen supply. During this time they move about quite actively. The energy necessary in such cases must be pro-

[1] Cf. Lecture XI, p. 228.

[2] Lecture IV, p. 74.

[3] Z. physiol. Chem. **14**, 318 (1890).

duced by means of partial decompositions. We should expect, therefore, that hydrogen and other easily oxidizable substances would be formed.

This was, however, not the case. The presence of hydrogen could not be detected, nor did oxygen disappear, if oxygen was supplied after the worms had existed for a day without it. Now we have no right to apply the results of such experiments to human beings, or to other animal organisms. The parasites of the intestine are accustomed to get along with a very limited supply of oxygen. It is perfectly possible that their metabolism takes place entirely differently than is the case with the remaining animal organisms. Perhaps hydrolytic decompositions take place in them without forming any easily oxidizable substances. Furthermore, it is conceivable that these little worms normally have active oxygen at their disposal, formed, at least to some extent, by the energetic reduction processes which take place in the alimentary canal. On the other hand, we must remember that the assumption of an *activation* of oxygen, by means of the formation of reducing substances in the tissues, has never gotten beyond the hypothetical stage. It is remarkable too that the animal organism itself is able to keep easily oxidizable substances in its tissues, such as phosphorus, for example, in an unchanged condition for a considerable length of time. It is not easy to understand how such a substance as this escapes the active oxygen unless it be assumed that during its transport through the animal tissues, it never actually comes in contact with nascent oxygen. Such an assumption seems strained, for we know of countless examples in which the animal organism is protected from poisons by their being oxidized. The oxidation does not always take place to such an extent, but frequently it serves to prepare a new substance which can become harmless by conjugation with something else, whether it be glycocoll, sulphuric acid, glucuronic acid, or urea. On the other hand, it is a fact that a reduction process may facilitate the combination of the poison with one of the above compounds. At all events, it is extremely interesting to find that these oxidations are quite different in various cases. At one time the compound may be completely oxidized, whereas, in another case, merely an atom of oxygen may be added to its composition. We can, indeed, believe that the cells, as already mentioned, may regulate their decompositions by means of their ferments; but it remains an enigma, according to the assumption of the presence of active oxygen, why the oxidation should stop at a certain stage in the case of readily oxidizable substances, whereas in other cases a total oxidation takes place.

Unquestionably, we would prefer an hypothesis which in itself includes this regulation of the extent of oxidation. The first suggestion of such an hypothesis was made by Moritz Traube.[1] He pointed out the importance

[1] Theorie der Fermentwirkungen. Berlin, 1858.

of *oxygen carriers*. In the study of inorganic chemistry, we know of various processes which take place in the presence of a carrier of oxygen, but will not take place from direct contact with oxygen. An example of such a process is in the oxidation of glucose by potassium indigo sulphonate, or by copper oxide. If a solution of glucose is warmed with alkali carbonate in the air for some time, practically no oxygen is taken up. As far as we can see, the glucose remains unaffected. Now if we add a little potassium indigo sulphonate to the solution, in the course of a short time the blue solution becomes decolorized, and the amount of unchanged glucose remaining in the solution gradually becomes smaller and smaller. The glucose has become oxidized. If now we shake the solution, it soon turns blue again, but again on standing it becomes colorless. At the same time, more of the glucose becomes oxidized. This process may be repeated again and again, until finally no more glucose remains. A very small amount of potassium indigo sulphonate suffices to cause the oxidation of a large amount of glucose. Copper oxide exerts a perfectly similar effect. An ammoniacal solution of cupric oxide becomes decolorized on warming it with glucose. The cupric oxide is converted into cuprous oxide, or, in other words, the copper is reduced. Oxygen has been furnished to the glucose molecule. If the decolorized solution is exposed to the air for some time, gradually, and first at the places where the solution is in direct contact with the air, it turns blue again. Oxygen is thus being taken up by the cuprous oxide, which may be given up subsequently to more glucose. The whole process can be accelerated considerably by shaking the liquid with air.

It is not difficult to conceive that substances are present in the animal tissues which act as carriers of oxygen for the more difficultly oxidizable substances. As we have said, a very small amount of such substances suffices to accomplish an indefinite amount of oxidation. The carrier itself is in the same condition at the end of the process that it was at the beginning. Now we know that ferments play an important part both in the animal and vegetable kingdoms. The thought naturally arises that perhaps certain of these ferments may act as carriers of oxygen. Traube speaks of oxidizing ferments. Recently Schmiedeberg[1] has mentioned the possibility of certain definite oxygen-carrying ferments; while Jaquet[2] has followed up this suggestion and succeeded in proving that extracts of the organs serve as carriers of oxygen, and that in fact the *principle* which causes this action may be precipitated by means of alcohol without its losing any of its power of causing oxidation. Heating to 100° C., however, destroys it.

To-day, no one doubts that a great many such ferments are actually

[1] Arch. exper. Path. Pharm. **14**, 288 (1881).
[2] *Ibid.* **29**, 386 (1892).

present, both in the animal and vegetable kingdoms. It is easy to detect their presence by means of certain chemical reagents. If, for example, an organ extract is shaken with an alkaline solution of α-naphthol plus p-phenylenediamine, the formation of blue indophenol is soon apparent.[1] Without the addition of the organ extract, the formation of this color takes place very much more slowly. A reaction made use of by Schönbein is the blue color obtained with tincture of guaiacum. Its indication of the presence of oxidizing ferments is, however, not altogether reliable. The guaiacum reaction is also brought about by the presence of numerous other oxidizing agents, such as ferric chloride, chromic acid, chlorine, bromine, etc. By means of these organ extracts salicylic acid may be converted into benzoic acid, and benzyl alcohol into benzoic acid.[2] Formaldehyde is similarly converted into formic acid,[3] and arsenious acid into arsenic acid.[4] α-naphtylamine is changed into violet-blue oxynaphtylamine, and benzidine into a brownish-violet substance.[5] Likewise phenolphtalin is changed to phenolphthalein.[6] These are a few examples of the oxidations which may be brought about readily by means of organ-decoction or organ-extract; and, in fact, with plants the oxidizing action of certain of their organs, the roots for example, may be demonstrated directly by allowing them to grow upon strips of paper moistened with solutions of the above-mentioned reagents.

By means of these discoveries, the whole question of animal and vegetable oxidations, the latter not differing from the former in its essential particulars, has been turned in an entirely new direction. Although, at the present stage of its development, it is perhaps going too far to speak of a perfect explanation of the oxidation processes in the tissues, still, on the other hand, it is true that numerous processes which would be otherwise beyond our comprehension, are now better understood. As we shall see later, we have every reason to believe that a given ferment always acts upon only quite definite compounds. Thus the proteolytic ferment, trypsin, attacks only proteins, and not carbohydrates. It might have been assumed *a priori*, that the oxidizing ferments are exceptions in this respect, and are in general capable of serving as carriers of oxygen. This is, as a matter of fact, not the case, as we know from numerous observations. Thus it is only certain definite oxidizing ferments which are capable of causing the above-mentioned oxidation of α-naphthol and p-phenylenediamine to indophenol in alkaline solution. In the liver, for example,

[1] F. Röhmann und W. Spitzer: Ber. **28,** 567 (1895).
[2] Jaquet: Arch. exper. Path. Pharm. **29,** 386 (1892).
[3] Pohl: *ibid*, **38,** 65 (1896).
[4] W. Spitzer: Pflüger's Arch. **71,** 596 (1898).
[5] M. Raciborski: Anzeiger der akad. der Wissensch. zu Kraukau, **1905,** 338.
[6] Kastle and Shedd: Am. Chem. **J. 26,** 527 (1901).

the ferments belonging to this organ are capable of converting aldehydes (e.g., salicylic aldehyde) into the corresponding acid, but they are incapable of effecting the indophenol synthesis. Jacoby[1] was unable to effect the oxidation of such compounds as acetic acid, stearic acid, etc., by means of the ferment which changes salicylic aldehyde into salicylic acid. Again, ferments are known which will turn tincture of guaiacum blue, but have no action upon salicylic aldehyde.[2]

Particularly in the vegetable kingdom we meet with these ferments possessing a quite specific oxidation power, which are designated with particular names according to the work that they perform. In the sap of plants we often find the so-called *tyrosinase*,[3] i.e., a ferment which transforms tyrosine into colored products. The same, or at least a very similar ferment, also occurs in animal organisms. Thus the stomach juices of starved meal-worms act upon tyrosine.[4] A similar action explains an old observation. As is well known, the blood of insects is nearly colorless, but becomes dark as soon as it leaves the body. Von Fürth and Schneider[5] have found that this so-called *melanosis* is the result of the action of an oxidizing ferment. They proved this in the following manner: They obtained, by piercing the pupæ of *Deilephilia elepenor* and *euphorbiæ*, a greenish-colored liquid, from which they obtained a precipitate upon the addition of ammonium sulphate. This precipitate, on being dissolved in 0.05 per cent soda solution, and added to a solution of tyrosine, soon caused a violet coloration, which gradually turned black. Eventually dark flocks of a precipitate were thrown down. The tyrosinase thus obtained acts upon other aromatic compounds, containing hydroxyl groups, such as catechol, chinol, etc. In the insect blood itself, tyrosine is not present, but a chromogen, which is evidently closely related to it. It may be assumed safely, that tyrosinase is very abundant in nature, and undoubtedly plays an important part in the formation of pigments. In the blood of the river-crab and in the ink-glands of the *cephalopoda* a tyrosinase is found.

Tyrosinase is much more widely distributed in the vegetable kingdom in which it was discovered. Bertrand[6] has found besides the tyrosinase a second ferment, laccase, which acts upon only quinol and pyrogallol.

Closely related to these ferments is the glucolytic ferment, which we have already discussed,[7] but whose existence, however, has not been posi-

[1] Virchow's Arch. **157,** 235 (1899).

[2] Abelous et Biarnès: Compt. rend. soc. biol. **49,** 175, 285, 493, 559, 596; **50,** 495.

[3] E. Bourquelot and G. Bertrand: J. pharm. chim. (6) **3,** 177 (1896); Bull. soc. mycol. France, **1896,** 18 and 27.

[4] W. Biedermann: Pflüger's Arch. **72,** 105 (1898).

[5] Hofmeister's Beiträge: **1,** 229 (1901).

[6] Compt. rend. **122,** 1132, 1215 (1895); **123,** 463 (1896).

[7] See Lecture IV, p. 73, and V, p. 87.

tively established. The oxydase which takes part in breaking down the nucleins also belongs here.[1]

In this class of oxidizing ferments we must also reckon the ferment of the acetic acid bacteria, discovered by E. Buchner and J. Meisenheimer.[2] Quite independently of the other activity of these microbes, this ferment changes alcohol into acetic acid:

$$CH_3 . CH_2OH + O_2 = CH_3 . COOH + H_2O.$$

Unquestionably, a great many other oxidations are to be traced to the action of oxidizing ferments, and we find in the literature numerous other ferments described under particular names. We must not forget, however, that the identification of ferments is an extremely difficult task, and it is very seldom that in any case it is absolutely proven a new one is at hand. Thus we know from the investigations of Bertrand [3] that in the juice of the berries of mountain ash a hexatomic alcohol, sorbitol, is present which under certain conditions is changed to the hexose, sorbose. It would be unjustifiable from this fact alone to assume that this evident oxidation is to be attributed to the presence of a ferment. It has been, in fact, established that a certain species of bacteria, *Bacterium xylinum*, which is introduced into the berries by means of a tiny red flea, *Drosophila funebris*, causes this transformation. This bacterium will likewise oxidize mannitol to fructose, xylose to xylonic acid, etc. Although it is indeed very likely that these bacteria act side by side with oxidizing ferments, still, on the other hand, it is not right to assume their presence until the ferment itself has been isolated.

We have intentionally made this digression in order that we may gain some idea as to the way in which such oxidations are effected, and as to the abundance of such ferments. Of course this has not told us much concerning the "oxidative" breaking down of the most important foodstuffs. At present we do not know exactly how and when the oxidation takes place in the case of protein, fat, or carbohydrate. We can make assumptions, but we know nothing with certainty.

We have up to now concerned ourselves merely with the empirical knowledge, and have not said anything as regards the way in which these ferments act. Perhaps a glance at the mechanism of their action may give us some idea of the oxidation as it takes place in the tissues. Unfor-

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[1] See Lecture XIII, p. 293.

[2] Ber. **36,** 634 (1903).

[3] Compt. rend. **122,** 900 (1896); **126,** 842, 984 (1898); **127,** 124, 758 (1898). Concerning the oxidation of glucose to gluconic acid, and of gluconic acid to oxygluconic acid, see Bontroux: Ann. Pasteur, **2,** 308 (1887); Compt. rend. **127,** 1224 (1898). Oxidation of quinic acid to protocatechuic. Emmerling and Abderhalden: Zentr. Bacteriol. Parasitenkunde und Infectionskrankheiten, **10,** Abt. II, p. 337 (1903).

tunately, we must again state that we are now dealing entirely with hypotheses, and that we are not yet in position to explain with certainty the exact nature of the processes which are brought about by the action of oxidizing ferments.

Traube [1] suspected the formation of hydrogen peroxide. In the oxidation of readily oxidizable substances, instead of the oxygen molecule being merely split and active oxygen set free, he imagined that water is first split off. The hydroxyl group in the latter then combines with the oxidizable substance, R, and the free hydrogen atom combines with neutral oxygen, forming hydrogen peroxide:

$$R + 2\,H_2O + O_2 = R(OH)_2 + H_2O_2.$$

Hydrogen peroxide is a strong oxidizing agent, and will attack the difficultly oxidizable substances.

The above process may be supposed to take place somewhat differently. Perhaps the readily oxidizable substance (R) combines with the oxygen, and forms itself a peroxide. The latter can then give up its extra atom of oxygen to some difficultly oxidizable substance (R_1).[2]

$$R + O_2 = RO_2. \qquad RO_2 + R_1 = RO + R_1O.$$

In this case the peroxide takes the place of the hydrogen peroxide, and has the same effect. According to this theory it is not that we have *active* oxygen present, but rather that the oxygen in the peroxide is held in a loosely combined condition from which it is easily set free.

The question now arises, What connection do the ferments have with this peroxide formation? We can imagine that the ferment itself combines with oxygen, and by forming a peroxide thus acts as a carrier. In fact, such an action has been assumed, and A. Bach with R. Chodat [3] has supported the assumption by quite a number of experimental observations. These authors carried out their investigations with plants. They separated the ferments which take part in oxidations into three classes. First, there are albumin-like substances which form peroxides from the oxygen that is brought to the tissues by the blood. Such ferments they designate as *oxygenases*. Then Bach and Chodat identified *peroxydases*, ferments which have the power of increasing the peroxidation power of the former. Finally, in each cell there are present, according to these authors, *catalases*, which decompose hydrogen peroxide catalytically with evolution of oxygen.

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[1] Ber, **10**, 1111 (1886); **10**, 1115; **22**, 1496 and 3057 (1889); **26**, 1471 and 1476 (1893).

[2] C. Engler and W. Wild: Ber. **30**, 1669 (1897).

[3] Biochem. Zentr. **1**, 417 and 457 (1903). A. Bach: Berichte, **38**, 1878 (1905); **37**, 3785 (1904). Bourquelot: Compt. rend. de la soc. biol. **49**, 402 (1897). Batelli and Stern: Compt. rend. **140**, 1197 and 1352 (1905); **141**, 139 (1905).

Substances which act catalytically upon hydrogen peroxide have been known in nature for a long time. It was even known to Thenard [1] that fibrin and the tissue of the kidneys and lungs were capable of decomposing hydrogen peroxide into water and oxygen just as energetically as is done by platinum, gold, and silver. In milk [2] also, and in blood,[3] there is a hydrogen-peroxide-catalase. The catalases are evidently of quite common occurrence.[4] Their significance is differently explained. It is held that they tend to prevent the appearance of *active oxygen*. It may be shown, for example, that urea and xanthine cannot be oxidized by hydrogen peroxide in the presence of a catalase. They decompose hydrogen peroxide with the formation of *molecular* oxygen.[5] In fact, the catalases are not oxidizing ferments at all. They are not of themselves able to turn tincture of guaiacum blue, nor in the presence of hydrogen peroxide. Thus the cells evidently possess a means of restricting and regulating the activity of the oxidation processes taking place within them.

The action of the oxygenases appeared to have a very simple explanation, when it was found that their ash contained substances which of themselves could act as carriers of oxygen. Thus Bertrand [6] found 2.5 per cent of manganese in the ash from laccase. Manganese salts are very active carriers of oxygen. In other oxidases iron is present. Bertrand compared 0.1 gram of ferment in 50 cubic centimeters of quinol solution in its action first with manganese alone and then with a manganous salt plus the ferment.

Manganous salt alone	0.3 c.c. oxygen absorbed
Laccase, from Lucerne, alone	0.2 c.c. oxygen absorbed
Laccase plus manganous salt	6.3 c.c. oxygen absorbed

These inorganic constituents have been assumed to combine with albumin, and form a dissociable compound. The metal constituent serves to carry the oxygen and in much the same way as we described for the oxidation of sugar in alkaline solution by means of copper oxide. The manganese is, according to this view, originally present in the divalent or manganous form, which takes up oxygen from the tissues with the formation of tetravalent manganese (corresponding to the manganese dioxide type), and the other atom in the oxygen molecule is carried to the oxidizable

[1] Ann. chim. et physiol. (2) **11,** 85 (1819).

[2] P. Raudnitz: Zentr. Physiol. **12,** 790 (1899); Z. Biol. **42,** 91 (1901).

[3] George Senter: Z. physikal. Chem. **44,** 257 (1903), and **51,** 673 (1895); Proc. of the Roy. Soc. **74,** 201 (1894).

[4] O. Loew: Z. Biol. **43,** 256 (1902).

[5] Philip Shaffer: Am. J. Physiol. **14,** 299 (1905).

[6] Compt. rend. **124,** 1032, 1055 (1897).

substance. Again, the manganese dioxide decomposes with loss of oxygen, and in this way the splitting off of oxygen by the ferment containing the manganese is effected. Such views are, however, very improbable, because, according to the researches of Chodat and Bach, the oxidases containing manganese obtained from plants are inactive in the absence of peroxidases. The peroxidases themselves have the function of activating the oxidases, for in the great dilution in which they are present in the juices and cells, the latter do not readily give up their oxygen from the peroxide formation. The action of the peroxidases may be compared with the decomposition of peroxides by means of ferrous salts.

With the help of these hypothetical representations we are able to see how the cells are not only able to regulate carefully the hydrolytic decompositions caused by ferments, but also the oxidations as well. The latter are directly dependent upon the formation of the peroxidases. Now the observations upon which this line of reasoning is advanced have been made upon plants, but there is little doubt that the animal cells conduct their oxidations in much the same way. All this, however, is purely hypothetical. It should always be taken into consideration that all experiments conducted in the study of the action of oxidases have been with substances which are oxidized without much difficulty. The combustion of such important foodstuffs as albumin, fat, and carbohydrate is still an obscure process. We do not know definitely in what manner the oxidizing destruction takes place. It is clear to us, from this discussion, that the metabolism which takes place within the cells is infinitely complicated, and that in the establishment of the fact that oxygen is taken up and carbon dioxide eliminated, nothing whatever was determined as to the real root of the matter. We are now beginning to understand at how many places the mechanism concerned in the cell-decompositions may be disturbed and how diverse these disturbances may be.

In the light of our present information concerning the breaking down and combustion of the separate foodstuffs in the animal organism, we may safely assume that the preliminary stage of practically all decompositions is a hydrolysis. First of all the foodstuffs are subjected to hydrolytic cleavage. According to all our present knowledge, amino acids are formed from albumin, glucose from glycogen, and glycerol and fatty acids from the fats. We must assume that the other substances which play a part in the metabolism of the organism are prepared for combustion in an entirely analogous manner. We know that the nucleins are decomposed into albumin and nucleic acid, and that these are again broken down into their simpler components. Purine bodies are thus formed,[1] which we believe first lose their nitrogen group and are then prepared for oxidation. In this case we can establish very accurately the moment at which oxygen

[1] See Lecture XIII, p. 292.

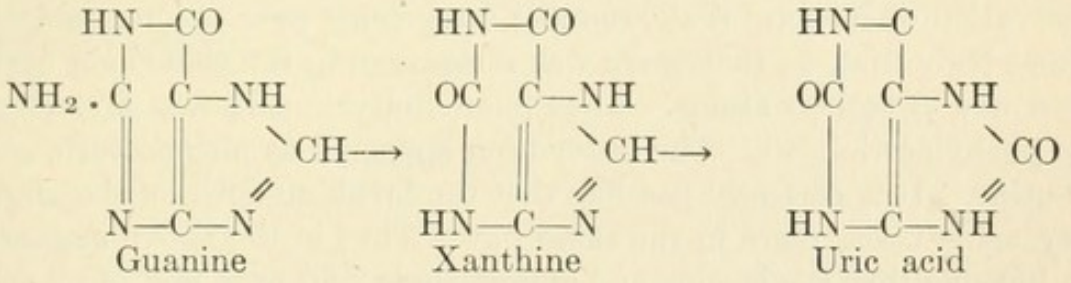
attacks the molecule. Lecithin is also decomposed into its separate constituents. In short, an intermediate metabolism takes place in the tissues, which is caused by processes perfectly similar to those taking place in the alimentary canal. There the decomposition serves the purpose of converting substances which are naturally foreign to the organism, but are contained in the food, into substances which the tissues can assimilate and incorporate. Hydrolysis is in all cases the preliminary stage to combustion. The fact that oxygen itself in any form is not capable of acting directly upon the cell-nutriment, i.e., that it cannot directly ignite this fuel, makes it possible for the cells to satisfy the demands for energy within quite wide limits without regard to external conditions. The cells are able at all times to utilize certain cleavage-products for building up new cell-material while they make use of other less valuable substances merely as fuel. They can adjust their own economy according to their individual requirements. The breaking down of the nutriment can take place from time to time along different lines, as we explained in the case of sugar. Only at a certain given moment does oxidation ensue. Again, it is remarkable that, as far as we know at present, we meet with specific actions. Not every oxidase is capable of oxidizing tyrosine. Here certain doubts arise as to whether the oxidation takes place exactly as we have represented, or whether, for example, the oxygen given up by the activated oxygenase actually of its own accord attacks the difficultly oxidizable substances without further assistance and consumes it. We meet with objections to such an assumption, especially as there are many classes of compounds known of which only one optical isomer is oxidized, while the other is not. We have already indicated the behavior of the amino acids. If a rabbit is fed with leucine it is chiefly the *l*-leucine which is oxidized, while the greater part of the *d*-leucine which does not occur in albumin is eliminated as such.[1] A great many similar examples are known. We need merely refer to the observations made with carbohydrates. C. Neuberg and J. Wohlgemuth[2] injected *d*-, *l*-, and *dl*-arabinose into rabbits, and found that 7.1 per cent of the *l*-arabinose, 36 per cent of the *d*-arabinose, and of the *dl*-arabinose 31 per cent of *dl*-arabinose plus 9.6 per cent of *d*-arabinose were eliminated unchanged. The remander of the material was oxidized. On the other hand, A. Brion[3] found that of levo- and mesotartaric acid 93.6 to 97.3 per cent were oxidized, of racemic acid only 58.1 to 75.3 per cent, and of dextrotartaric acid 70.7 to 74.4 per cent. We will discuss these facts later. Here they

[1] J. Wohlgemuth: Ber., **38,** 2064 (1905). Schittenhelm and Katzenstein: Z. exper. Path. Ther. **2,** 560 (1906). Abderhalden and Samuely: Z. physiol. Chem. **47,** 346 (1906).

[2] Ber., **34,** 1745 (1901), and Z. physiol. Chem. **35,** 41 (1902); **37,** 530 (1903).

[3] Z. physiol. Chem. **25,** 283 (1898).

are merely cited to show that the oxidation processes taking place in the animal organism are by no means to be considered as *direct* in nature, i.e., the cleavage-products as such (the amino acids, dextrose and the fatty acids), are of themselves not susceptible to oxidation in the tissues under the prevailing conditions, unless it be assumed that, for example, *d*-leucine does not enter into the metabolism of the cells and does not come in contact with the carriers of oxygen. There is, however, no support to such an assumption. On the contrary, we know that the muscular cells of the diabetic are constantly being offered *d*-glucose. The cells consume albumin and fat, or rather their decomposition products, just as well as ever. Glucose alone they allow to pass on unchanged. The key is lost which can unlock the energy stored up in the glucose molecule. Now of course an assumption that the oxydase which is capable of offering oxygen to the dextrose is absent, helps us here. The fact that the diabetic can complete the oxidation without difficulty, if the glucose is previously converted into a more readily oxidizable form, does not necessarily prove, however, that the glucose molecule must be opened up in some way before oxidation. It is indeed possible that the glucuronic acid and saccharic acid offered to the diabetic may be consumed at an entirely different place in the organism, and not utilized at all for the work of the muscular cells.[1] At the same time the empirical knowledge that we possess, indicates that the foodstuffs as such are not at once suitable for oxidation. Something must be taken out of the complex molecule before the oxygen in the cells tissues can act upon it. The investigations of Schittenhelm point in this direction. Guanine, a cleavage-product of the nucleic acids, is first of all changed into xanthine:



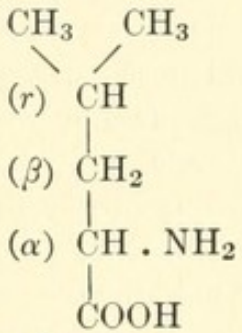
This transformation takes place with loss of ammonia; a hydrolytic ferment is active. Now for the first time the molecule is ready for oxidation, and uric acid is formed from it. The latter is then acted upon with the aid of another ferment in a manner unknown to us. We thus see that a whole chain of different processes is necessary in order to completely consume a relatively simple substance, a purine base.

We must regard the breaking down of the amino acids as taking place

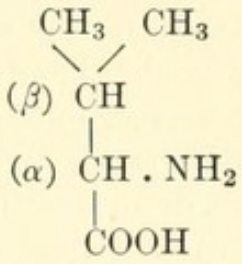
[1] See Lecture XIII, p. 292.

in a quite similar manner. Here, evidently, first of all the NH_2 group is removed by the action of a hydrolytic ferment, and then combustion takes place.

The fact that we are now getting closer to the realization of the actual conditions is shown by the recent investigations of Embden, Salomon, and Schmidt.[1] They conducted through the liver of a dog, right after killing it, blood to which the different amino acids had been added, and found, for example, that leucine caused a considerable increase of acetone in the circulation. Shortly previous it was established by Embden and Kalberlah [2] that the liver normally produced acetone. Now all of the amino acids do not yield acetone. Thus, for example, it is not formed from aminovaleric acid, although a glance at the formulas of these two acids shows they are closely related compounds:



Leucine



Aminoisovaleric acid

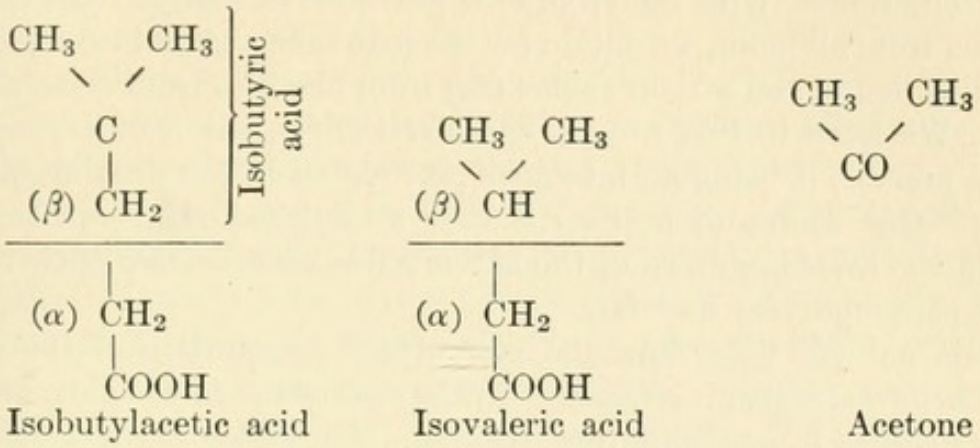
Perhaps the different behavior of these two amino acids gives us an indication as to the manner in which at least a part of the decomposition products of albumin is acted upon in the tissues. Embden recalled the observation of Knoop [3] that aromatic fatty acids were decomposed in the animal body first, so that there was a cleavage in the side-chain between the α and β carbon atoms. Thus phenylbutyric acid was first changed into phenylacetic acid. The latter then appeared as phenaceturic acid in the urine. It is perfectly possible that the breaking down of the aliphatic fatty acids takes place in the same way. Thus in the above instance we can imagine that the leucine and aminovaleric acid were first of all robbed of the amino group. From the former isobutylacetic acid is formed, and from the latter isovaleric acid. Then, by loss of carboxyl and oxidation, isovaleric acid is formed from the isobutylacetic acid, and isobutyric acid from the isovaleric acid. Next cleavage takes place between the α and β carbon atoms. Now if these ideas are correct, we must expect that

[1] Hofmeister's Beiträge, **8**, Heft 3/4 (1906).

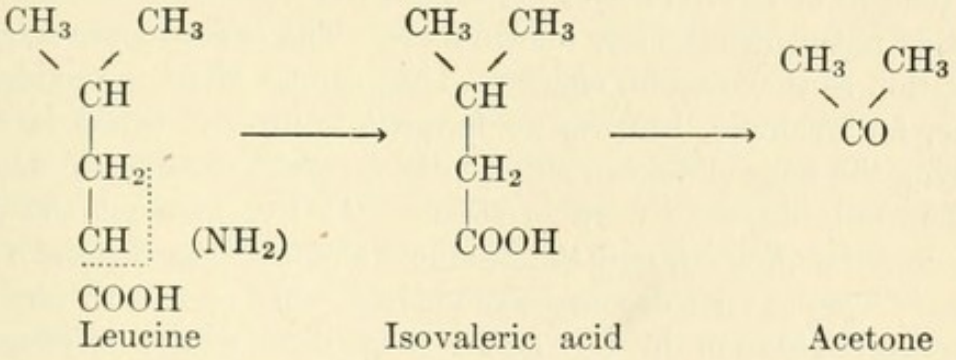
[2] *Ibid.*

[3] Der Abbau aromatischer Fettsäuren im Tierkörper. Habil.-Schrift, Freiburg i. B. 1904.

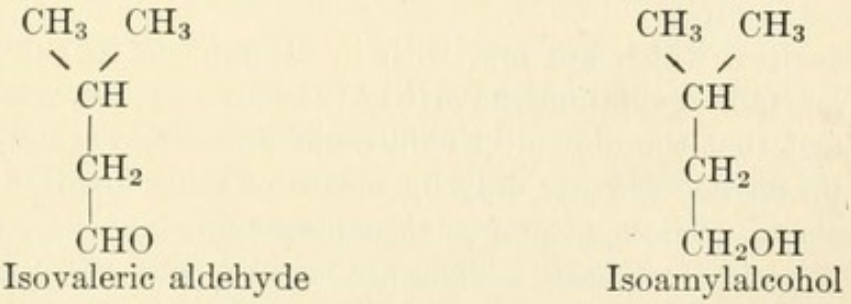
isobutyl acetic acid itself is not broken down into acetone, while this will be the case with isovaleric acid, as the following formulas show:



Direct experiment confirms this explanation. The decomposition of leucine, therefore, may be represented as taking place in the following stages:



In place of the isovaleric acid, naturally the corresponding aldehyde or alcohol may appear:



We have intentionally gone into this hypothesis at length in order to emphasize the complexity of such a process in contrast to the simple idea of combustion in the tissues that is generally assumed. Many observations speak for the above conception of the breaking down of the amino acids. We have discussed this with tyrosine and phenylalanine. On the other hand, we must admit that we are not yet able to understand why the animal organism can split off a carboxyl group from leucine, but not

from isobutylacetic acid. There seems to be an intimate relation to the urea formation. The NH_2 and CO groups leave the molecule of the amino acid at one time. With the proof of the acetone formation from products obtained from albumin, we obtain for the first time a clear idea concerning the utilization of the carbon chains free from nitrogen from certain amino acids. We learn in this way to consider the formation of acetone as a normal process, it being an intermediate product in the decomposition of leucine. One source of acetone is thus established. Perhaps from this stage in the breaking down of the amino acids we can trace their relation to the carbohydrates and fats.

We do not yet know how the preliminary preparation of the glucose molecule for oxidation is effected. In the case of the fatty acids, however, we are justified in assuming that oxidation is preceded as above described by a cleavage between the α and β carbon atoms. It seems probable that the eventual oxidation always takes place with products having but few carbon atoms in the chain. With these presumptions, we can at least draw a picture of the oxidation processes in the animal organism, which will at least not contradict any known facts. The cells prepare substances for oxidation as they require energy. They cannot effect the oxidation of *d*-leucine, for example, because no ferment is present which is capable of breaking the molecule down sufficiently to make the oxygen accessible to it. The cells do their work in stages. At any moment the decomposition may be stopped, and the products already formed, used for new syntheses. They do not decompose suddenly. We cannot by any means compare the oxidation in the animal organism with a conflagration. Everything is regulated to the most minute detail. Cleavage and oxidation take place alternately, so that the cell can utilize the energy it obtains from step to step, and only in this way is it possible to regulate so carefully the heat supply.

The difficulties which are met with in attempting to explain animal, as well as vegetable, oxidations, have led to various hypotheses which depend upon the fact that the difficultly oxidizable substances are made capable of taking up oxygen directly only by means of some function exerted by the protoplasm. These attempts at explanation, however, do not rest upon any experimental basis. They are far in advance of our knowledge concerning the nature of the cell-protoplasm. It is for this reason that it is so difficult to submit them to experimental proof. O. Loew [1] traces the oxidation to the unstable condition of the albuminoid in protoplasm. It transfers the lively movement of the atoms in the active albumin molecule to the oxygen and the oxidizable substance. In this way there is a loosening up of the molecule, so that the atom of oxygen is offered a point

[1] Ber., **35**, 2487 (1902).— cf. E. Wolff: Die chemische Energie der lebenden Zellen. Munich, 1899.

of attack. We shall not enter here into a discussion of this or a number of similar hypotheses. It may be merely said concerning them that they are not very helpful, because we know practically nothing concerning the chemistry of the protoplasm.

Oxygen has, as we have seen, not only the function of enabling the organism to make use of the energy stored up in the decomposition products of the food, but it also serves frequently to prevent the cells from suffering injury. It is entirely wrong to assume that oxygen, after it once enters into reaction, immediately burns up the substance completely. We are certain that in many cases only a partial oxidation takes place; i.e., the oxidation takes place in stages. This fact also precludes any simple explanation of animal or vegetable oxidations. The cell must in each individual case determine the degree of oxidation. With activating of the oxygen, or by the carrying of oxygen by means of a peroxide formation, the course of oxidation in the animal tissues is by no means explained. Exactly as the chemist may choose special oxidizing agents, and, by establishing the conditions, he may regulate the degree of oxidation, so in the same way the cell is capable of regulating the oxidation according to its requirements. Particularly instructive examples are shown by the behavior of certain foreign substances, injurious to the cells, from whose action the organism protects itself, as we have repeatedly seen, in a number of different ways. Rudolph Cohn [1] has shown that methylquinolin is for the most part entirely oxidized, and that the same is true of *o*-nitrobenzaldehyde.[2] With santonin,[3] $C_{15}H_{18}O_3$, on the other hand, the oxidation is not carried so far but it is changed into oxysantonin, $C_{15}H_{18}O_4$. The animal organism prepares a large number of such substances by coupling them with certain substances such as sulphuric acid, glycocoll, glucuronic acid, and urea. The first two of these substances just named come from albumin, while glucuronic acid results from carbohydrates. Sulphuric acid combines with a great many substances of a phenolic nature; thus, ordinary phenol when introduced into the body leaves it in the form of potassium phenyl sulphate:[4]

$$C_6H_5OH + HO \cdot SO_3K = C_6H_5 \cdot O \cdot SO_3K + H_2O.$$

In this case, which was first noticed and is the simplest of all, the coupling takes place directly. Sometimes, however, the phenol is first oxidized. In this way quinol is formed, which then unites with the sulphuric acid:[5]

$$HO \cdot C_6H_5 + O = HO \cdot C_6H_4 \cdot OH$$
$$HO \cdot C_6H_4 \cdot OH + HO \cdot SO_3K = HO \cdot C_6H_4 \cdot O \cdot SO_3K + H_2O$$

[1] Z. physiol. Chem. **20**, 210 (1895).
[2] *Ibid.* **17**, 274 (1893).
[3] M. Jaffé: *ibid.* **22**, 538 (1896–97).
[4] Baumann and Herter: *ibid.* **1**, 244 (1877–78).
[5] Baumann and Preusse: Z. physiol. Chem. **3**, 156 (1879).

Here, the object of the oxidation is not so apparent, because the combination with sulphuric acid takes place just as easily before as after. In other cases it is necessary for the poisonous substance to be oxidized first, in order to make it possible for the combination with sulphuric acid to take place. Thus we know that benzene is first changed into phenol,[1] indole to indoxyl, and skatole to skatoxyl.[2] Acetanilide appears in the urine as *p*-aminophenol, as *p*-acetylaminophenol, and as the anhydride of hydroxyphenylcarbamic acid partly united with sulphuric acid and in part with glucuronic acid.[3] In the group of glycocoll conjugates we have xylene converted to toluic acid:[4]

$$CH_3 . C_6H_4 . CH_3 + 3\,O = CH_3 . C_6H_4 . COOH + H_2O.$$

Mesitylene is changed into mesitylenic acid,[5] and cymene to cumic acid.[6]

For conjugation with glucuronic acid, similarly, the cells act upon poisonous substances in various ways. Thus, for example, *o*-nitrotoluene is changed to nitrobenzyl alcohol, which then unites with glucuronic acid:[7]

$$NO_2 . C_6H_4 . CH_3 + O = NO_2 . C_6H_4 . CH_2OH$$

$$NO_2 . C_6H_4 . CH_2OH + C_6H_{10}O_7 = NO_2 . C_6H_4 . CH_2O . C_6H_9O_6 + H_2O$$ [8]

We have distinguished in the case of all the organic foodstuffs two different functions which they have in the cells of the animal organism. On the one hand they may serve as sources of energy, while on the other hand they may serve as material for the construction of new tissue. Likewise it is possible for the inorganic salts to develop energy by physical methods; but their chief use, however, is in the formation of new material, whether it be due to an intimate union with organic material, or whether the unchanged inorganic salt is in an indispensable constituent of the cells and that their activity only results from its presence. Of oxygen we have spoken of but one function, that of making energy available. The question naturally arises whether oxygen itself is not used as building material in the construction of new cells? There are, as a matter of fact, certain observations which seem to indicate that the entrance of oxygen into the contents of the cell, the protoplasm, plays an important part in the excitability of the cell. Kühne recognized the fact that the protoplasm of *Amœbæ*, *Myxomycetes*, and the filament hairs of *Tradescantia*, lost its

[1] Schultzen, Naunyn, and Munk: Du Bois' Arch. **1876,** 340, and I. Munk: Pflüger's Arch. **12,** 142 and 148 (1876).

[2] Baumann and Brieger: Z. physiol. Chem. **3,** 254 (1879).

[3] Jaffé and Hilbert: Z. physiol. Chem. **12,** 295 (1888). Mörner: *ibid.* **13,** 12 (1889).

[4] Schultzen and Naunyn: Du Bois' Arch. **1876,** 353.

[5] Nencki: Arch. exper. Path. Pharm. **1,** 420 (1873).

[6] Nencki and Ziegler: Ber. **5,** 749 (1872).

[7] Jaffé: Z. physiol. Chem. **2,** 47 (1878–79).

[8] Cf. Fromm: Die chemischen Schutzmittel des Tierkörpers bei Vergiftungen. Strassburg, 1903.

motion and excitability when in hydrogen, but became active when placed in oxygen again.[1] Max Verworn [2] confirmed these observations on *Rhizopoda* of the Red Sea. It is difficult to perform such experiments upon the cells of more highly organized forms, because they contain combined oxygen, which enables the muscles, for example, to work for some time with production of carbon dioxide in an atmosphere devoid of oxygen.[3] If, however, a muscle is allowed to act until exhausted, it is found that it becomes active again, only when provided with a new supply of oxygen. Such relations have been established for the muscles of the heart. Finally, as is well known, E. Pflüger [4] has shown that frogs which were kept at low temperatures in pure nitrogen gradually lost their excitability, but regained it again, even after remaining twenty-four hours in this atmosphere, on being placed in the air once more. We have to thank Max Verworn [5] for a very interesting experiment in this direction. He replaced all the blood in a frog with a physiological salt solution, containing 0.6 to 0.8 per cent. The salt solution was free from oxygen. If now by injection of strychnine the ganglion cells of the spinal medulla were excited, as much as possible, then the neurons worked (under the constant streaming of salt solution) until finally all the oxygen stores had been exhausted. In less than an hour the reflex excitability is lost. After this the strongest irritation produces no reflex action. If now a salt solution containing dissolved oxygen is caused to circulate through the blood-vessels, the frog revives within a few minutes, and again shows the increased excitability caused by the strychnine. Every time the circulation of the salt solution containing the dissolved oxygen is stopped, the ganglion cells at once become unexcitable. The experiment may be repeated over and over again, with the same frog. Since in this experiment the ganglion cells are not provided with fresh nutriment, it is not possible to explain the action of oxygen by the simple assumption that its absence prevents the complete combustion of the decomposition products, while at the moment oxygen enters the energy becomes available to the cells. To be sure, we are quite ignorant concerning the work of the ganglion cells. We do not know what their expenditure of energy is. It may be very small. If we remember that the oxygen of itself is not able to attack the decomposition products from the food, but requires assistance on the part of the cell before the oxygen can find a point of attack, then it is not perfectly clear why the oxygen

[1] Untersuchungen über das Protoplasma und die Kontraktilität, Leipsic, 1864. Z. Biol: **36**, 425 (1898).

[2] Die Bewegung der lebenden Substanz. Jena, 1892. Cf. also Die Biogenhypothese. Jena, 1903.

[3] Kronecker: Ueber die Ermüdung und Erholung der quergestreiften Muskeln (1871). Joteyko: La fatigue et la respiration élémentaire du muscle. Paris, 1896.

[4] Pflüger's Arch. **10**, 251 (1875).

[5] Arch. Anat. Physiol. **1900**, Suppl. 152.

should nevertheless revive the excitability as shown by the oxidation and development of energy. It is not necessary in every case that all of the hydrolytic products should be consumed. The cell is able to be very economical with its fuel. On the other hand, it is perfectly possible that oxygen under these conditions may play an unusual part in the mechanism of the cell work. We cannot work out such problems successfully until we have a more accurate insight into the oxidations of the cells and tissues, and as long as our physical and chemical conceptions of the protoplasm are still vague. We meet here with a great many riddles, which for the present are unanswerable — for the present, we say, because we can scarcely doubt that before long the rapid progress of biological chemistry will bring clearness to our conception of these complicated processes. An advance in the science is only possible, however, when it is clearly and sharply recognized where the facts end and the hypotheses begin. We can only build upon the former, and the latter serve merely as a framework which is of value only when it is possible to replace the fantasies of the brain little by little with facts verified by experimentation.

LECTURE XX.

FERMENTS.[1]

We have repeatedly encountered the conception *ferment* in our discussion of the transformations of our organic foodstuffs in the alimentary tract and in the tissues. We have seen that the proteins are subjected to the action of pepsin-hydrochloric acid in the stomach, and to trypsin in the intestine, and that the ferment diastase decomposes almost completely the complicated carbohydrates in the mouth, and to a much greater extent in the intestine, while the ferment lipase hydrolyzes the fats in the stomach and in the intestine. The ferments also participate largely in tissue-metabolism. We meet them wherever life processes occur. They are distributed as widely in the vegetable world as in the animal kingdom. Fermentation phenomena are so striking that they were recognized in very early times, alcoholic fermentation first attracting attention. Spallanzani,[2] in 1785, showed the solvent action of the gastric juice on protein, while Kirchhoff,[3] a few years later, called attention to the fact that fresh gluten will saccharify starch. Liebig and Wöhler [4] discovered emulsin, which splits amygdalin, and Bussy identified myrosin. If to these we add the discovery of the oxidases by Schönbein, and the preparation by Berthelot of yeast invertin, the action of which upon cane-sugar was known even to Mitscherlich, we shall include practically all of the most important fermentation phenomena known at the end of the nineteenth century. It is only during the last few decades that we have really made any great progress in our knowledge concerning the various kinds of ferments.

Before discussing the nature of the ferments and their action, we must state in advance that we have not yet succeeded in characterizing the ferments as chemical individuals. We know practically nothing concern-

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[1] In order to avoid confusion, we shall employ the term "ferment" in its original sense, — something which causes fermentation. It has been customary to distinguish between *organized* ferments and *unorganized* ferments, or enzymes; but it will be shown in the following pages that such a distinction was based upon a misapprehension. For a more complete orientation concerning ferments and fermentations, consult J. Reynolds Green: The Enzymes; and Carl Oppenheimer: Die Fermente und ihre Wirkungen, Leipzig, 1903.

[2] Lazz. Spallanzani: Versuche über d. Verdauungsgeschäft. Leipzig (1785).

[3] Schweiger's Journal, **14**, 389 (1815). Dubrunfaut: Soc. Agricult. Paris (1823.)

[4] Poggendorff's Ann. **41**, 345 (1837).

ing their constitution; in fact, we do not even know to what class of compounds they belong, or whether they form a class of their own. It has always been customary to classify the ferments with the proteins. There were various reasons for this assumption. Until recently, the composition and structure of the proteins were as little understood as that of the ferments themselves, while the fats and carbohydrates had been thoroughly investigated. Our knowledge concerning the compositions of the latter compounds makes it seem improbable that the ferments belong to either of these two classes. The proteins, on the other hand, with their complicated structure and their various elementary components, are far more likely to include the ferments, endowed as they are with such numerous and finely differentiated functions. We are, however, at present unable with our limited knowledge of the proteins to draw any conclusions regarding the structure of the ferments. It is often stated, in order to show their albuminous nature, that the *pure* ferments give the reactions of the proteins. Unfortunately, we have not yet succeeded in isolating any ferment in a pure state. In the first place, we have absolutely no criterion of purity. Pekelharing [1] and M. Nencki and N. Sieber [2] have recently attempted to purify the pepsin of the stomach, the former claiming to have accomplished the feat. Until we have some clear conception of the composition of the ferments, it is of little avail to discuss their nature. We are not even justified in assuming that the ferments belong to a single class of compounds. It is possible that the fats and carbohydrates also take part in their composition. For the present we are unable to state that the identification of this or that compound actually indicates that it belongs to the ferment. Such substance may simply be dragged down mechanically in the process of isolating the ferment. All of our methods for the preparation of the ferments are such that this assumption is justified.

The ferments are unquestionably closely related to the life-processes of the cells. They are to be directly looked upon as their secretion products. The ferments, until recently, were sharply divided into two groups, the *organized ferments*, or simply *ferments*, and the *unorganized ferments*, or *enzymes*. Those which were only active in the presence of the living cell were included with the former, while those which acted independently of the cells, such as diastase, pepsin, trypsin, etc., were placed in the latter class. The designation *organized* or *unorganized*, was to give the impression that, in the first case, the protoplasm of the cell was absolutely necessary for performing the function of the ferment, while the *unorganized* ferments were capable of accomplishing their work without any further assistance. It is true, however, that we are not at all acquainted

[1] Z. physiol. Chem. **22**, 233 (1896/97); **35**, 8 (1902).

[2] *Ibid.* **32**, 291 (1901).

with the ferments as such. We only recognize their presence by their activity, which alone distinguishes them. This, in principle, is the same for the *organized* as the *unorganized* ferments. The cell produces ferments, which it requires for its own economy, and others, which it sends out, to produce results that will directly or indirectly benefit it. The assumption that the ferments which were active when away from the cells had other powers than those remaining in the cell was entirely arbitrary. Especially, as it had never been found possible to isolate such a ferment, i.e., an *organized* one, from the cell, and bring it into activity in its isolated form, there was no logical ground for sharply differentiating between *organized* and *unorganized* ferments. We know that all ferments are more or less subject to external influences. Definite conditions must be fulfilled to develop their action. Thus, for most ferments the *optimum* temperature lies between 35° and 45° C. The reaction of the medium in which the development occurs is also of considerable importance. Pepsin, for instance, requires a hydrochloric acid solution, while trypsin acts in a neutral or faintly alkaline medium. When we find, furthermore, that the formation of ferments by the cells and their activation has recently been recognized as a complicated process dependent upon certain influences, we can readily understand why a definite ferment ceases to act when it has been torn away from its original sphere of activity. We do not know how many ferments the individual cell contains. It is very probable that the ferments are first produced in an inactive form, as *zymogens*, and only activated by the cells when they are needed. It is also possible that the individual ferments are very closely related in their *work*, i.e., they act together, and assist one another. We also know that many ferments have their action restricted by the decomposition products which they themselves produce. Such an accumulation of cleavage-products would hardly occur in the cell itself, for they are quickly acted upon by other ferments. If, however, such a cell-ferment were forced to develop its activity outside of the cell, we can easily see how it might soon cease to be efficient.

The discovery by E. Buchner,[1] that it is possible to isolate and separate from the cell structure, the ferment from yeast which converts sugar into carbon dioxide and alcohol, proved for the first time that the similar effects of ferment and cell activities are evidently due to the fact that the same agents are at work in each case. This discovery, which does away with our previous distinction of ferment and enzyme, was carried out in the following manner: Buchner [2] ground a kilogram of yeast with a kilogram of quartz-sand and 2.3 kilograms of infusorial earth. His purpose was to

[1] E. Buchner, H. Buchner and M. Hahn: Die Zymasegärung. München & Berlin (1903).

[2] Albert and Buchner: Ber. **33**, 266, 971 (1900).

destroy the cell walls and permit the release of the cell protoplasm. The whole mass was then subjected to a pressure of from 400 to 500 atmospheres. The expressed liquor had a slightly acid reaction, it was weakly opalescent, and contained albuminous material. It contained the active principle which converts sugar into carbon dioxide and alcohol. Buchner calls it *zymase*. It is very unstable, the filtered juice losing its activity after a few days. The zymase is destroyed at 40–50 degrees. It can be dried, and in this form keeps much better. It can also be precipitated from its solution by the addition of alcohol or ether,[1] by which we obtain a white powder, only partially soluble in water, but more so in glycerol.[2] The glycerol extract is very active. Zymase has recently been obtained directly from the yeast cell, by first killing the cell by contact with ether-alcohol or ether-acetone. The zymase remains active after this treatment. Such preparations, which may be kept for a considerable time, are called *preserved yeasts*.

A vigorous objection was quickly raised against classifying zymase with the unorganized ferments. Special attention was called to the fact that the zymase in the expressed liquor was active only for a short time. This was explained on the assumption that the zymase must be looked upon as a part of the protoplasm, which, on being separated from the remainder of the cell contents, possessed only a short period of activity. Quite aside from the fact that it is now possible to prepare zymase with better keeping qualities, this objection is met by what has been said above concerning the cell ferments and their dependence upon their environment and other ferments, etc. The expressed liquor contains not only zymase, but also other ferments, of which one, the so-called *endotryptase*, quickly destroys zymase. Zymase is, in fact, very susceptible towards proteolytic ferments. It is also perfectly clear, that those ferments contained within the cells would naturally be much more susceptible to unusual conditions than those other ferments which are given off by the cells, and are undoubtedly better equipped for battle with the outer world.

That, moreover, fermentation is not necessarily dependent upon living cells, was already indicated by an observation of Fiechter,[3] who showed that hydrocyanic acid completely arrests the vital processes of yeast, but does not at once stop the fermentation.

The tracing of the conversion of glucose to alcohol to a fermentation process is not the only case where a so-called "life-process" has been proved to take place independently of the living organism. Zymase, like all other ferments, must be regarded as a product resulting from the life-processes of the cells. The enigma of their formation and existence still

[1] R. Albert: Ibid. **33**, 2775 (1900).
[2] R. Albert: Ibid. **35**, 2375 (1902).
[3] Wirkung der Blausäure. Diss. Basel (1875).

remains even after they have been isolated. E. Buchner and J. Meisenheimer[1] have recently succeeded, by using the acetone method of procedure, in obtaining a preparation from *Bacillus Delbrücki* (Leichmann) which produced lactic acid from grape-sugar in the same manner as the bacillus itself. They also obtained preserved preparations from beer-acetic-acid bacteria, using the acetone method, which converted alcohol into acetic acid.

There is evidently no sharp dividing line between the individual cell-ferments and the free, unorganized ferments. There are some which are undoubtedly closely related to the cell contents, and others which are more loosely united to the cell contents than is the case with yeast zymase, and can consequently be more easily isolated from the cells. Finally, there are those ferments which are given off by the cells themselves.

The secretion by the gland cells can be followed directly by histological methods, and it is quite possible that certain visible changes of the gland cells may be related to the formation of ferments. The morphological changes in the cells of the pancreas have been studied in particular. Although the cells of the resting gland are but slightly distinguishable from one another, we observe sharp, generally double, boundary lines, at the instant when activity begins. The cells, and likewise the gland itself, change their form. They become filled out. We observe kernels, which belong to the inner zone of the cells, migrate towards the lumen of the gland, become smaller, and finally disappear. The cell-changes in the salivary glands, especially the parotid, have been very carefully studied during their activity. The cells decrease in size during secretion. The nucleus, usually angular, becomes rounded, and shows granules very distinctly. The clear, homogeneous substance, predominating when at rest, decreases in amount, while the granular substance increases. It is difficult to say whether the gland cells give up a part of their protoplasm during the secretion, or if, as seems more probable, products are formed which then go over into the secretion. It is possible that the granules mentioned possess some relation to the formation of ferments.

The fact that a great many ferments are not secreted as such, but in an inactive form, must be looked upon as of the greatest significance for the conception of fermentation processes. The activation results from the influence of another substance which is often produced at another place, and does not necessarily form a part of the ferment, and may even be of simpler composition. We call the secreted inactive ferment a zymogen or proferment. Thus, we know that the pepsin zymogen is activated by hydrochloric acid, while the trypsin zymogen requires the enterokinase. Undoubtedly there are innumerable other ferments which are secreted in

[1] Ber. **36,** 634 (1903).

an inactive form, both in the animal and the vegetable organisms. The cell is thus enabled to regulate its entire metabolism. It only activates the ferments when it needs them. The way this activating process is brought about is still unknown. We can imagine that the activating agent splits the zymogen, perhaps forming a smaller molecule, or possibly it breaks open an anhydride or lactone formation, thus permitting those groups to act which are able to force an entrance into the material to be acted upon.

Although the discovery of the wide distribution of ferments and their action, and the knowledge that the cell-functions in a narrow sense correspond to such processes, opens up new paths and points of view for Biology, although on the other hand we must not forget that the great mystery of cell-life remains unsolved. The living cell produces the ferment; of this there is no doubt. At this point we encounter the most important problems of the whole subject of biology. We should be making a great mistake if we were to say that the knowledge of fermentation reactions has solved the mystery of life. It has, to be sure, cleared up many processes which were previously obscure, and has given us a very much clearer conception of the whole subject of metabolism. If, however, we turn from fermentation to the ferments themselves, we immediately touch the unknown. The ferments point to the cells and their metabolism and functions. It would be equally short-sighted, and lead to a complete misunderstanding of biological chemistry, if we were to consider a solution of this mystery as impossible, and content ourselves with an undefinable conception of "the vital force." There can be no doubt that the rapidly advancing biological science will attack the problem of the chemistry of the ferments as soon as the time is ripe. The mystery of the ferments will disappear as soon as we are able to replace our conception with a chemical representation. In the attempt to explain biological processes more and more in accordance with the laws governing the exact sciences, it is never advisable to wipe away the boundary between the knowledge gained by exact methods and what has been established by mere hypotheses. The more sharply we separate the known from the unknown, the freer will be the development of our further investigations, and the more independently will the facts, as such, speak for themselves.

Let us review what we know about the nature of fermentation from this point of view. As a result of our unfamiliarity with the chemical nature of the ferments, we are deprived of one of the most important supports in any exact study concerning them, and are temporarily restricted to hypotheses in explaining their manner of action. The number of ferments is very large. We can here only refer to such as are in harmony with experimental facts.

Let us, at the start, ascertain what are the characteristics of the ferments themselves.

The first remarkable fact is that they are never found as end-products of the reactions. They remain unchanged. The smallest amounts suffice to repeat the same reaction a countless number of times. Thus, invertase is capable of inverting at least 200,000 times its own weight of cane-sugar,[1] rennin at least 400,000 parts of casein.

The action of the ferments should, theoretically, be an unlimited one. There is, however, as has been shown in the case of rennin,[2] a gradual loss of efficiency. This is not, however, caused by the reaction itself. We also know, as we shall see more in detail later, that the ferments have a specific action, i.e., trypsin, for instance, only attacks protein, and never the carbohydrates or fats; while diastase never acts on albumin, nor does lipase ever have any effect upon carbohydrates or albumins. We know, furthermore, that the ferments are produced by living cells, some of them being given up by the cells, while others are retained in the cell itself. Finally, we are, in most cases, acquainted with the end-products, and are thus able to draw conclusions regarding the nature of most reactions which they cause to take place.

Now there are in inorganic chemistry a great many facts known, which remind us very much of the behavior of ferments. We refer to those chemical processes in which the presence of a minimum amount of a given substance is sufficient to produce a marked acceleration. We speak in such cases of *catalyzers*, and call the whole process *catalysis*, or one of *contact* action. Ostwald[3] defines catalysis as *an acceleration of a slow chemical change brought about by the aid of a foreign substance.* According to this definition, the reaction in question is not started by the catalyzer, but whereas it takes place very slowly of itself — perhaps even to an imperceptible extent — the rate of change is accelerated by the presence of a specific substance. In support of this assumption we have the fact that increasing the amount of the catalyzer causes further acceleration of the catalysis. If the process served merely to start the chemical change, we should hardly expect that the amount of catalyzer present would have any influence, and at all events the speed of the reaction would be independent of the amount of contact substance present. We shall subsequently come back to this definition. We are interested here in the similarity between these contact substances and the ferments. One of the chief common characteristics is the fact that neither appears among the

[1] J. Chem. Soc. Trans. **57**, 834 (1890).

[2] Reichel and Spiro: Hofmeister's Beiträge, **6**, 68 (1904), and **7**, 479 (1905).

[3] Grundriss der allgemeinen Chemie, 3d ed. (1889), p. 514. Ueber Katalyse. Lecture. Hirzel. Leipzig (1902). Compare also, G. Bredig: Die Elemente der chemischen Kinetik, etc., Ergeb. Physiol. (Asher and Spiro) **1**, 134 (1902).

end-products of the reaction, and both are effective when present in very minute quantities. Thus, a very small amount of nitrous acid is sufficient to convert relatively large quantities of sulphur dioxide and atmospheric oxygen into sulphuric acid. $\frac{1}{10.000}$ to $\frac{1}{300.000}$ milligram of colloidal platinum or manganese dioxide, or $\frac{1}{3.000}$ milligram of gold, will cause the decomposition of more than one million times as much hydrogen peroxide. Ernst [1] has shown that $\frac{1}{10}$ milligram of colloidal platinum will catalyze 50,000 times as much oxyhydrogen gas without losing any of its efficiency. It is of great significance for the conception of the action of catalyzers to know that there are substances which have the opposite effect, and retard reactions which are already in progress. Bredig [2] calls these *negative catalyzers*. Thus, we know that traces of ethylene, alcohol, ether, oil of turpentine, and ethyl iodide will tend to prevent the oxidation of phosphorus.[3] Bigelow [4] for instance, has shown that the presence of 0.000,0014 gram of mannitol per cubic centimeter will reduce the rate of the oxidation of 800 times as much sodium sulphite in aqueous solution to one-half its former value. On the other hand, we also know of substances which will directly prevent catalysis. These are known as *anti-catalyzers* or *paralyzers*. As an example of this may be mentioned, that 0.000,000,001 gram of hydrocyanic acid per cubic centimeter will reduce the catalytic effect of 0.000,006 gram of colloidal platinum in the decomposition of hydrogen peroxide to one-half its original value.

Not only do we have the above analogies between the ferments and catalyzers, but there are other similarities, such as the effect of external conditions upon their activity, e.g. temperature, etc. Certain laws have also been worked out showing how the rate of the reaction depends upon the amount of ferment present and upon the temperature, etc., and these relations illustrate the analogy between the ferments and the above-mentioned catalyzers. It would be beyond the scope of these lectures to dwell longer upon these interesting observations. We can abandon the subject the more readily because if we were to attempt to apply these principles to the processes in the animal and vegetable organisms, it would further our insight into the cell processes but little. We must not be deceived by the little knowledge we have gained regarding catalysis, for, generally speaking, we know nothing at all concerning the way in which the catalyzers act. We know merely that their presence is necessary. We must admit that the relations between catalyzers and ferments are to be regarded only as analogies, there being no proof that their actions

[1] Z. physikal. Chem. **37**, 448, 454 (1901).

[2] Bredig and v. Berneck: *Ibid.* **31**, 324 (1899); Bredig and Ikeda, **37**, 1, 63 (1901).

[3] Centnerszwer: *Ibid.* **26**, 1 (1898).

[4] *Ibid.* **26**, 493, 503 (1898).

are identical. Even the application of the definition of catalysis to fermentation requires reflection. The ferment in such a case would merely serve to accelerate reactions which were already in progress. We would have to assume, for example, that albumin would be decomposed hydrolytically at 37 degrees in an aqueous solution or suspension, although the hydrolysis proceeds so slowly that we are unable to detect its progress. The addition of pepsin-hydrochloric acid, or of trypsin, accelerates the reaction to such an extent that we are able to recognize the hydrolysis in a short time, on account of the appearance of cleavage-products. We cannot deny that such an explanation is hardly satisfactory. It is not susceptible to direct proof, and leads to a forced assumption. It is not at all clear why pepsin-hydrochloric acid should hydrolyze the albumin molecule so much less and possibly in an entirely different manner than trypsin does, or why the latter should split off definite amino acids so much quicker than others, and furthermore leave certain complexes entirely unaltered. We know, furthermore, that grape-sugar may be decomposed in various ways, which must be regarded as fermentation processes. In butyric acid fermentation, it yields butyric acid, carbon dioxide, and hydrogen:

$$C_6H_{12}O_6 = C_4H_8O_2 + 2\,CO_2 + 2\,H_2.$$

In lactic acid fermentation, it is decomposed as follows:

$$C_6H_{12}O_6 = 2\,C_3H_6O_3.$$

Finally, we know, that zymase splits it into ethyl alcohol and carbon dioxide:

$$C_6H_{12}O_6 = 2\,C_2H_5OH + 2\,CO_2.$$

The two latter processes are unquestionably pure fermentations. In these cases, the ferment not only accelerates reaction already existing, but it also determines the direction and progress of the same. The fact that the ferments act in such a specific manner, i.e., only attack substances of definite configuration, leaving other closely related compounds entirely untouched, indicates very clearly that we cannot be satisfied with the definition of Ostwald, at least as far as fermentation processes are concerned. We cannot expect to understand perfectly the action of ferments until we have become better acquainted with their chemical composition. As long as we deal with them only as conceptions, we can hardly expect to make any progress regarding the nature of their action. We do not wish to underestimate the value of physical chemistry in this direction; we merely wish to differentiate between those facts which we can consider as proved, out of the numerous investigations of recent years, and those results which are only based upon hypotheses.

One of the most interesting properties of the ferments is their specific action. The animal, and also the plant organism, as we have mentioned many times, works almost exclusively with optically active carbon compounds, i.e., with compounds which have at least one asymmetric carbon atom. The asymmetry of the elementary constituents of the cells begins at the moment of the assimilation of carbon-dioxide[1] by the parts of the plants containing chromophyll, and is transferred directly by the herbivora, indirectly by the carnivora, into the animal organism. From a compound containing only one asymmetric carbon atom we can imagine two optical isomers and one racemic compound formed by a union of the two.[2] Even Pasteur[3] was acquainted with the fact that if we inoculate a solution of ammonium tartrate, containing a small amount of nutrient salts, with traces of *Penicillium glaucum*, a peculiar change takes place. The solution, which was at first entirely inactive, becomes optically active during the development of this mold, rotating towards the left. The lævorotation continues to increase, and only assumes a constant value when the dextrotartaric acid, the optical isomer of the lævotartaric acid, has been entirely consumed by the mold. This interesting phenomenon can be explained on the assumption that the mold evidently only utilizes one of the optical modifications of tartaric acid, while lævotartaric acid remains unchanged. After this observation of Pasteur, which was attributed to the action of an organized ferment, others followed. Thus, by the aid of *Penicillium glaucum*, the following optically active forms were obtained from the racemic compounds: *d*-mandelic acid, *d*-aspartic acid, *d*-leucine, *l*-tartaric acid, *l*-mannonic acid lactone, *l*-glutamic acid, and *l*-glyceric acid.[4]

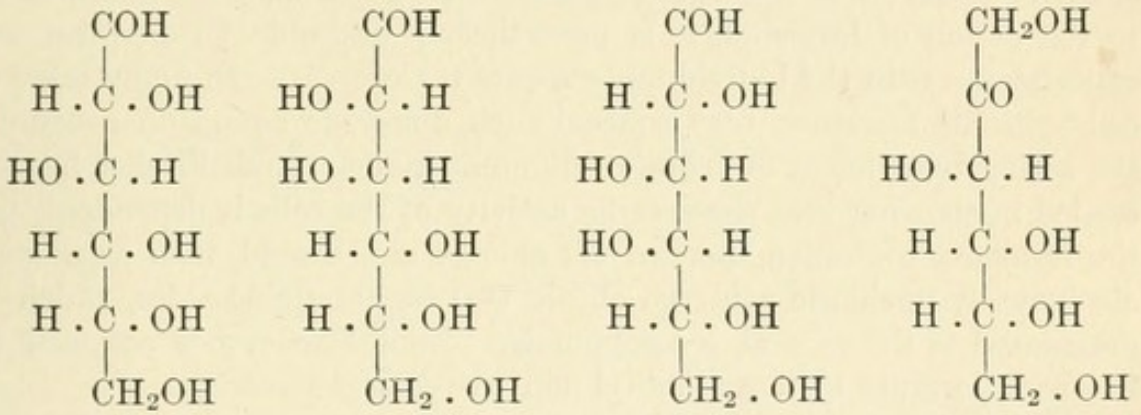
Felix Ehrlich[5] has made an interesting discovery in this direction. He permitted a pure culture yeast to act upon synthetically prepared, racemic leucine, in the presence of cane-sugar. After a time a distinct odor of fusel oil was noticed. Isoamyl alcohol was separated from the liquid by fractional distillation. All the leucine present was not used up in this process, but only the *l*-leucine. The *d*-leucine could be recovered unchanged from the liquid. The same experiment, using *d*-isoleucine, resulted in the formation of *d*-amyl alcohol. The splitting of racemic bodies into the optically active components by means of lower organisms has become an important method for the preparation of such compounds, and has become of great significance in identifying synthetically produced substances with those which occur naturally. As different organisms decompose different parts of the racemic bodies, it is possible for us to obtain in this

[1] Compare Lecture IV, p. 54.
[2] Compare Lecture II, p. 15.
[3] Compt. rend. **51**, 298 (1860).
[4] Compare C. Winther: Ber. **28**, 3000 (1895).
[5] Z. Vereines Deut. Zuckerind. **55**, 592 (1905).

way either one of the optical isomers, by selecting the proper organism. Emil Fischer,[1] who early recognized the significance of stereo-chemical influences in biological processes, studied the alcoholic fermentation from this point of view. He found that, of two isomers, yeast will ferment only one; in fact, the following:

Ferments	*Does not Ferment* [2]
d-Glucose	*l*-Glucose
d-Mannose	*l*-Mannose
d-Galactose	*l*-Galactose
d-Fructose	*l*-Fructose

The configuration of these compounds has been explained by the researches of Emil Fischer,[3] and it is possible from the structural formulæ at hand to determine the influence of the configuration upon the attack by yeasts. The formulæ of the four fermentable sugars are as follows:



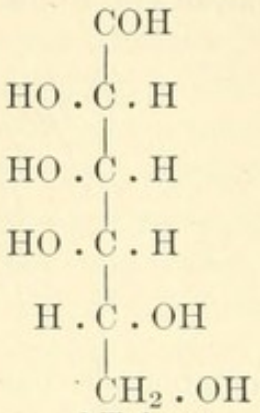
It is clear from these formulæ that *d*-fructose, *d*-glucose, and *d*-mannose closely resemble one another in their stereo-structure. The OH and H groups are arranged alike in three of the four asymmetric carbon atoms. It is interesting to note that these three sugars also possess close chemical relations, as is indicated by many of their transformations, and especially by the fact that they go over into one another, simply on heating with alkali. The *d*-galactose, in its configuration, does not stand so close to the sugars mentioned. The same also applies to its behavior. It is more slowly fermented; in fact, some varieties of yeast, such as *Saccharomyces apiculatus* and *productivis*, do not act upon it at all. All of the other known aldose and ketose sugars remain unacted upon by yeasts. The

[1] Z. physiol. Chem. **26,** 60 (1898–99).

[2] Ber. **23,** 382, 2620; **25,** 1259; **27,** 2031; **27,** 2985; **27,** 3479; **28,** 1429; **27,** 2035 (1894).

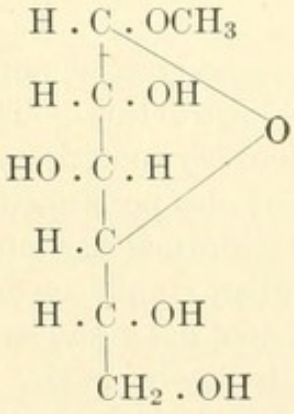
[3] Compare Lecture II, p. 17.

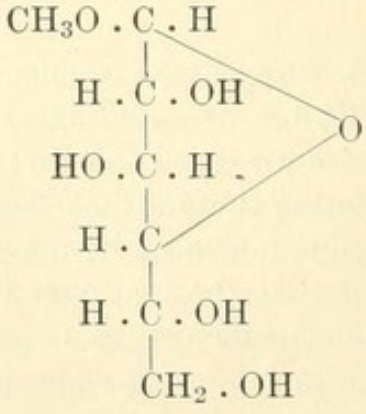
following formula of a non-fermentable sugar, *d*-talose, will show what a slight difference in the configuration in the molecule may prevent the action of the yeast:



d-Talose

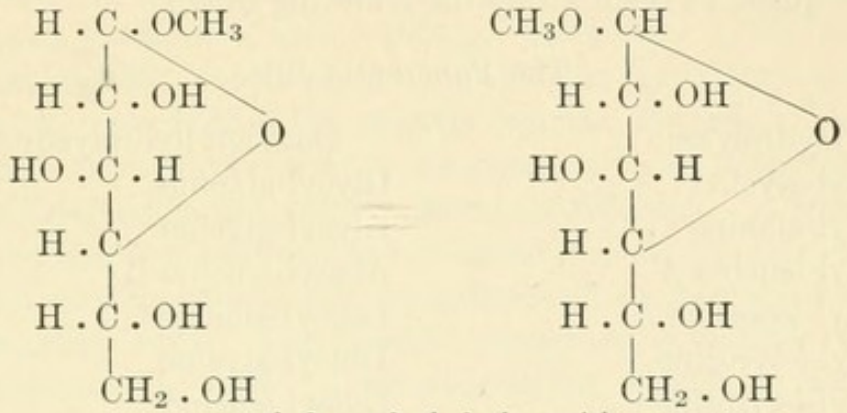
Although, from what was said about zymase, we can conceive of the selective behavior of the cells of *Penicillium glaucum*, *yeasts*, etc., as due to the action of ferments, it is nevertheless desirable to perform such experiments with the individual ferments themselves. In many cases the cell, with its ferments, may conceal such a specific action on account of the acting together of ferments of different kinds. Emil Fischer has succeeded in showing that the specific activity of the cells is dependent upon the ferments contained therein. If aldoses are heated with very weak, alcoholic hydrochloric acid, we obtain two isomeric glucosides, which are designated as the α- and β-compounds. Emil Fischer has assigned the following formulæ to these methyl derivatives of *d*-glucose:



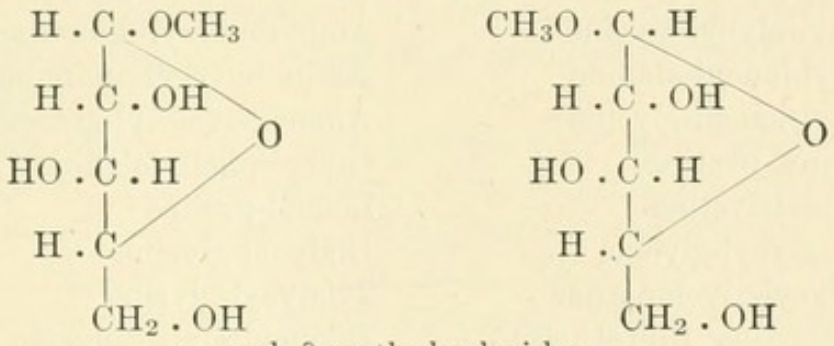


Only one of these two compounds, in fact the β-form, is hydrolyzed by emulsin into methyl alcohol and grape-sugar; the α-form remains unacted upon by this ferment. On the other hand, ferments obtained from yeast do not attack the β-glucoside, but hydrolyze the α-form. The stereoisomers of the above compounds, α-methyl-*l*-glucoside and β-methyl-*l*-glucoside, are not attacked by either emulsin or the yeast ferments.

Moreover, the ferments are so sensitive that they can detect differences in chemical compounds which are, as far as our present knowledge of structure and stereo-chemistry shows, perfectly analogous. This is clearly shown by comparing the α- and β-methyl-*d*-glucosides with the α- and β-methylxylosides.



α- and β-methyl-*d*-glucosides



α- and β-methylxylosides

While the former are split by either emulsin or the yeast ferments, the xylosides are not acted upon by either of them.

Emil Fischer made analogous observations with the polysaccharides. Some of these are also unfermentable, and their different behavior is undoubtedly due to differences in the configuration of the molecules.[1]

For investigating the relations of the action of ferments to the configuration of the individual compounds, Emil Fischer by his extensive syntheses in the protein group has opened up recently a new field which in its diversity far exceeds the relations with the carbohydrates. This scientist has, as we have already seen, made the cleavage-products of the proteins unite together in various anhydride-like combinations. The number of chains which it is possible to unite in this manner is very large. The number of possible isomers which can be obtained by using different amino acids and in different sequence, and also by employing the racemic amino acids, is greatly increased on account of the fact that all of the known cleavage-products of albumin, with the exception of glycocoll, contain at

[1] Compare also A. Kalanthar: Z. physiol. Chem. **26**, 89 (1898).

least one asymmetric carbon atom. Now it was of great interest to trace the behavior of the synthetic polypeptides in the presence of proteolytic ferments, in order to ascertain whether the fine distinctions which were noticed with the carbohydrates would also apply here. The synthetic polypeptids do, in fact, show a decidedly different behavior toward the pancreatic juice, as is shown by the following table: [1]

The Pancreatic Juice

Hydrolyzes:	Does not hydrolyze:
*Alanyl-glycine	Glycyl-alanine
*Alanyl-alanine	Glycyl-glycine
*Alanyl-leucine A	Alanyl-leucine B
*Leucyl-isoserine A	Leucyl-alanine
Glycyl-*l*-tyrosine	Leucyl-glycine
Leucyl-*l*-tyrosine	Leucyl-leucine
*Alanyl-glycyl-glycine	Aminobutyryl-glycine
*Leucyl-glycyl-glycine	Aminobutyryl-aminobutyric acid A
*Glycyl-leucyl-alanine	Aminobutyryl-aminobutyric acid B
*Alanyl-leucyl-glycine	Aminoisovaleryl-glycine
Dialanyl-cystine	Glycyl-phenylalanine
Dileucyl-cystine	Leucyl-proline
Tetraglycyl-glycine	Diglycyl-glycine
Triglycyl-glycine-ester (=Curtius' Biuret-base.)	Triglycyl-glycine
	Dileucyl-glycyl-glycine

If we examine these two columns, we observe that the pancreatic ferment has various points of attack. In the first place, the structure of the individual compounds must be taken into consideration. A good example here is the behavior of alanyl-glycine:

$$CH_3 . CH(NH_2) . CO . NH . CH_2 . COOH,$$

and its isomer, glycyl-alanine:

$$NH_2 . CH_2 . CO . NH . CH(CH_3) . COOH.$$

The former is hydrolyzed, the latter is not. The nature of the individual amino acid is also important. Thus, those dipeptides are susceptible to hydrolysis in which the alanine acts as acyl, examples being: alanyl-glycine, alanyl-alanine, alanyl-leucine. The hydroxy acids, tyrosine and isoserine, have a similar effect when they are at the end of the chain. It is

[1] Emil Fischer and Emil Abderhalden: Z. physiol. Chem. **46,** 52 (1905). Compare also, Sitzber. Kgl. Preuss. Akad. Wiss. **10** (1905), and Emil Fischer and Peter Bergell: Ber. **36,** 2592 (1903); **37,** 3103 (1904).

possible that the electro-negative character of these compounds plays a part. It has not yet been decided whether the easy cleavage of the two cystine derivatives is due to this cause, or that the length of the chain is a factor. It is very noteworthy that the dipeptides, which contain α-amino-butyric acid, and leucine as acyl, offer great resistance.

The influence of configuration is also clearly indicated in this case. The polypeptides in the above table which are designated by an asterisk, are racemic compounds. In all of these cases, the hydrolysis is an asymmetric one, i.e., only half of the racemic body is attacked. The products resulting from the hydrolysis were the same as those active amino acids which are contained in the natural protein-substances. A special case is shown by the contrast between the alanyl-leucine A. and alanyl-leucine B. In these two racemic compounds are present all four combinations of the four active amino acids. One racemic compound is *d*-alanyl-*d*-leucine + *l*-alanyl-*l*-leucine; the other, *d*-alanyl-*l*-leucine + *l*-alanyl-*d*-leucine. Of these four combinations, the pancreatic juice will attack only that corresponding to *d*-alanyl-*l*-leucine. This fact is of great significance. It supplies us with a means for determining directly the configuration of the synthetic polypeptides.

The number of the amino acids contained in the complex molecule is also of influence. The glycine chains give us a distinct example of this. Glycyl-glycine, diglycyl-glycine, and triglycyl-glycine are not hydrolyzed, while tetra-glycyl-glycine is acted upon. Leucyl-glycine, also, is not decomposed, although leucyl-glycyl-glycine is. The reason that dileucyl-glycyl-glycine is not decomposed, lies probably in the configuration of the dileucyl group.

If the cleavage takes place along asymmetric lines, the beginning of hydrolysis in the previously inactive digesting liquid is established by the appearance of optical activity.

We may here include the observation of O. Warburg,[1] who showed that the ester of racemic leucine is saponified asymmetrically by the pancreatic juice. We do not know what ferment produces this result. Lipase may be the active principle, as is suggested by an analogous observation of H. D. Dakin.[2]

Closely related to these discoveries, is the fact previously mentioned,[3] that the animal organism utilizes only one-half of certain racemic substances, the other optical isomer being eliminated unchanged. These discoveries show very clearly the similarity of "organized" and unorganized ferments, and justify us in concluding that all ferments must be considered from the same point of view.

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[1] Ber. **38**, 187 (1905).

[2] Proc. Chem. Soc. **19**, 161 (1903); **J. Physiol. 32**, 199 (1905).

[3] Compare Lecture XIX, p. 452.

The fact that the ferments act asymmetrically leads to the assumption that they themselves are asymmetrically constituted. The ferment must be exactly fitted to act on the compound which is to undergo cleavage. Possibly the assumption, so often made, that the ferment temporarily combines with the substance to be hydrolyzed, will account for the specific behavior of every individual ferment. If this be true, we can easily understand that only a definite ferment can act upon a given compound. This assumption is supported by the observation, that pepsin and papain, for example, form such strong combinations with fibrin that they cannot be removed by washing. It has also been found that the inversion of cane-sugar by ferments is, as a rule, the same during equal intervals of time.

Further light has been thrown upon this problem by tracing the optical behavior of solutions containing optically-active polypeptides after the addition of a solution containing ferments.[1] All results indicate that the ferment temporarily unites with the substance which it splits. It is important that the cleavage products tend to prevent the further cleavage of the substance, in accordance with the mass-action law.

A peculiar significance of the ferments has recently been indicated by certain observations. Morgenroth[2] found that, after subcutaneous injection of rennin in small doses, the serum of the animal so treated, contained a substance which prevented the curdling of milk. This phenomenon is analogous to the production of anti-toxin by the animal organism after the injection of a toxin. In one case we obtain an *anti-toxin*, in the other an *anti-rennin*. Two per cent of the strongest immunizing-serum which Morgenroth obtained, added to milk, prevented its curdling even when the ferment was present to the extent of 1:20,000. Without the addition of anti-rennin, the curdling took place when the ratio was as low as 1:3,000,000. Even normal serum is supposed to contain some anti-rennin. Such *anti-ferments* have also been prepared which act against pepsin, trypsin, fibrin-ferment, tyrosinase, lactase, and urease. These experiments are of great importance in two directions if they can be confirmed. In the first place, this discovery will serve to unite a purely biological process with another which, up to the present time, has not been studied by itself. The acquirement of immunity and the formation of anti-ferments may prove to be analogous phenomena; and the toxins, which resemble the ferments in many respects, may belong to the same class of substances. A further analogy lies in the fact that the ferments

[1] Abderhalden and Koelker: Z. physiol. Chem. **51**, 294 (1907); Abderhalden and Michaelis: *ibid.* **52**, 326 (1907); Abderhalden and Gigon: *ibid.* **53**, 251 (1907); Abderhalden and Koelker: *ibid.* **54**, 363 (1908).

[2] Zentr. Bact. **26**, 349 (1889); **27**, 721 (1900).

have a toxic effect when injected subcutaneously. Hildebrandt[1] found that the lethal dose for a medium-sized rabbit was 0.1 gram for pepsin, invertase, and diastase; 0.05 gram for emulsin and myrosin; and 2 grams for rennin. All of the injected ferments caused rise of temperature. Dogs, which had had ferments injected, would not eat, showed thirst, trembling, restlessness, an unsteady gait, and eventually coma. Rabbits showed principally emaciation, weakness, and sometimes extensor convulsions. Such observations necessarily have only a relative value, owing to the fact that the nature of the ferments is still unknown, so that their purity cannot be estimated.

On the other hand, the formation of the anti-ferments and the observed normal occurrence of these, gives us an indication of the *rôle* which, for example, the ferments absorbed by the intestines perform in their circulation through the body. We can easily imagine that the organism paralyzes the activity of the absorbed ferments by the production of the anti-ferments. The presence of such substances also suggests an explanation of how the living tissue is protected against self-digestion.[2] We can also imagine that this protection is obtained by the cells altering the material which they require for constructive purposes, and do not wish to consume, so that the ferments are unable to find any point of attack. It is certainly not without significance that the connective tissue, e.g., elastin and substances like spongin and silk-fibroin, which are not considered as nutrient materials, contain in large quantities just those amino acids which make hydrolysis difficult. The substances are, as a matter of fact, hardly attacked by pepsin-hydrochloric acid or by trypsin. The cell has only to cause a slight rearrangement of the atoms in the molecules of the assimilated products to form modifications which the ferments are not able to attack, or it may cause them to combine with other cell-components.

An unsolved problem is the origin of the ferments. It has often been suggested that they bear a definite relation to the food. In fact, many observations indicate a close connection between the production of ferments and the assimilation of the food. We merely do not understand the more intimate relations existing between the two processes. Brown and Morris[3] have shown that the leaves of many plants contain the most diastase in the morning, the amount decreasing during the day. If the assimilation is carried out in the sunlight, the formation of diastase ceases

[1] Virchow's Arch. **121,** 1 (1890); **131,** 26 (1893).

[2] E. Weinland, Z. Biol. **43,** 86 (1902), has found such anti-ferments in cell-free extracts of parasitical worms. Extracts from the mucous membranes of the stomach and intestine, as well as erythrozytes, are credited with retarding the solution of fibrin. Z. Biol. **41,** 1, 146 (1902).

[3] J. Chem. Soc. **62,** 604 (1893); **57,** 493 (1890).

entirely. The dormant seeds do not contain any ferments, i.e., not in an active form; they do not become active until the beginning of germination. The barley embryo does not produce diastase when absorbable sugar is available for it.

The formation of ferments among the molds is likewise influenced by the nature of the nourishment. If they are provided with albumin, they will produce proteolytic ferments; if they are cultivated upon starch, they will form diastase. Furthermore, it is known that yeast, for example, which has been cultivated for a long time on a specific substratum, can be " taught " to utilize definite compounds if we gradually withdraw the other nutriment.

Closely related to these observations is the fact that the plant cells, in the presence of definite products, also produce the ferments which will decompose them. This is usually true of the glucoside-splitting ferments. Thus we find amygdalin together with the ferment emulsin in bitter almonds; while the glucoside, sinigrin, is accompanied by the ferment myrosin in black pepper.

There are numerous analogous observations in the animal kingdom. It was known to Claude Bernard [1] that the larvæ of *Musca lucilia*, a kind of fly, possessed large stores of glycogen, but did not produce any diastase. The latter appears only when these stores are required by the pupa.

Numerous relations between the kind of food and the amount and nature of the secreted digestive fluids have become known by the extensive researches of Pawlow. Nervous influences dominate the production of the ferments. This was evident long ago from the investigations of Claude Bernard on the decomposition of glycogen in the liver. Pawlow has studied this subject more carefully, as we shall see later.

From all of these statements, we involuntarily obtain the impression that fermentation processes play a large part in the whole economy of the individual cell, the tissues, and finally the entire organism. This view is supported by the common occurrence of such processes throughout the whole plant and animal kingdoms. The fact that their activity begins as soon as the life processes start indicates their importance. The ferments are found in very early stages of human and animal embryos. Langendorff [2] detected trypsin especially early. Pepsin is entirely absent among the carnivora just after birth, but is found in the herbivora. Diastase is absent from human beings and rabbits previous to birth. The ferments, evidently, play a part not only in physiological relations, but also in pathological ones. Thus, we observe that fibrin from the bronchial tubes during croupous-pneumonia is gradually dissolved and finally disappears.[3]

[1] Revue scientifique (1873), p. 515.

[2] Arch. Anat. Physiol. **1879**, 95.

[3] F. Müller: Verhandl. XX. Kongresses in. Med. zu Weisbaden (1902).

In this case, apparently the migrant leucocytes which are present in large numbers furnish the ferment and cause a normal digestion in the lungs. Some insight into the cell processes, and the ferments which come into action thereby, was believed to be obtained from Salkowski's discovery that organs, even when kept perfectly sterile, gradually dissolve of themselves.[1] Cleavage-products are formed at the same time which suggest the presence of trypsin-like ferments. To be sure, it is generally stated that the disintegration of the cell contents and especially of the albumin, in this process which is known as *autolysis*, is not the same as that which takes place in a true trypsin digestion. It is true that the same end-products (amino acids, purine bases, etc.) are formed, but we do not know whether the decomposition takes place in the same way, and with the formation of the same intermediate products. It is questionable whether we are justified at present in making deductions regarding normal cell metabolism from the autolytic processes which result several days after death.[2]

Thus far we have considered cell-metabolism and fermentation from only one point of view. We have mentioned only the *splitting* ferments. Now we know that extensive syntheses take place in the animal organism. Since Wöhler, in 1824, proved that benzoic acid administered to the animal organism was neither consumed nor excreted as such, but that a nitrogenous acid, richer in carbon, appeared in the urine, namely hippuric acid, which is composed of glycocoll and benzoic acid, a great many other syntheses have been proved to take place. We need only to recall the fact that fats are split into glycerol and fatty acid in the alimentary tract, only to reappear on the other side of the intestine as fats, and also that the albumins and complicated carbohydrates are disintegrated into simple components only to be reconstructed, to realize that synthesis is another established function of the animal cell. Although, as far as our present knowledge shows, these syntheses are for the most part simple ones, and usually consist of the union of two or more molecules with elimination of water, we must not conclude that the animal cell is incapable of effecting complicated syntheses. Certain recent results lead us to suspect that the animal organism is capable of building up complicated structures. The synthetic processes of the plant and animal organisms were for a long time hidden in obscurity. Indeed, on purely theoretical grounds, by comparing ferments with catalyzers, the conclusion was drawn that fermentations must be reversible processes, and that they may be endothermic as well as exothermic reactions. As a matter of fact, a whole series of syntheses has been carried out by the aid of ferments. Thus we may refer to the formation of isomaltose from concentrated *d*-glucose solutions by means of

[1] Z. Klin. Med. **17**, Suppl. 77 (1890).

[2] Cf. Lecture XII, p. 265.

yeast maltase, and to the production of isolactose from glucose and galactose by kephir-lactase, and to the synthesis of amygdalin from mandelonitrile glucoside and grape-sugar with the aid of yeast maltase.[1] From these observations there can be no doubt that fermentations are reversible processes. This does not prove, however, that the conditions in the tissues and cells are such that the known ferments are active in this direction to any great extent. It is very tempting to refer all metabolic processes to fermentation. At one place there is a decomposition, at another, construction, according to the requirements of the cells. The ferment acts as an intermediary. Many enigmas would thus be solved at one stroke, and many apparently different processes referred to one simple basis. It is certainly possible that such a significance really belongs to the ferments, and that they dominate the entire metabolism. We must, however, confine ourselves to the facts, and build on them alone as foundation. We are, in the first place, impressed with the fact that all the fermentation syntheses so far carried out satisfactorily do not give rise to products to which the ferment is accustomed, with the possible exception of the amygdalin synthesis. We do not obtain maltose from grape-sugar, but an isomer, isomaltose; nor do we obtain lactose from glucose and galactose, but only isolactose. These facts are certainly not without significance. Can the maltase again decompose isomaltose, or kephir-lactase hydrolyze isolactose? These were unsolved problems until recently. Thanks are due E. F. Armstrong [2] for thoroughly studying these fermentation syntheses, and especially the formation of maltose and isomaltose. Armstrong started with the fact which we have not previously mentioned, that *d*-glucose can exist in stereo-isomeric forms. If we crystallize grape-sugar from alcohol, we only obtain the α-form. If this is kept for several days at 105 degrees, it goes over into the β form. The two modifications have different optical behaviors. It has been attempted to represent this type of stereo-isomerism by means of formulæ, although a satisfactory explanation is still lacking. If glucose is dissolved in methyl alcohol, containing hydrochloric acid, glucosides corresponding to both forms are produced. As we have already seen, only one of these varieties is split by maltase, the other only by emulsin. The variety hydrolyzed by maltase is the α form. If we apply these observations to maltose and isomaltose, we can regard the former as glucose-α-glucoside, and the latter as glucose-β-glucoside. In this case also, the maltase is only capable of splitting the α form, while the β-glucoside remains unattacked. Now maltase produces synthetically β-glucoside, i.e., the glucoside which it cannot decompose. The following experiments completely cleared up these relations. If glucose was treated

[1] Cf. Lecture III, pp. 37, 38.

[2] Pro. Roy. Soc. **76** (B), 592 (1905); **19**, 209 (1903); Jour. Chem. Soc. **83**, 1305 (1903).

with concentrated hydrochloric acid, and hydrochloric gas acid passed into the liquid at 0 degrees, it was shown, after long standing at 10 degrees, that the fluid contained both glucosides, maltose and isomaltose, as well as unchanged glucose. The hydrochloric acid makes no distinction; it works with the α as well as the β form of glucose. That both bioses had, in fact, been formed, was proved by the circumstance that maltase as well as emulsin produced glucose from it. If glucose was kept at 25 degrees for two or three months in the presence of yeast maltase, it was shown after removing the unchanged glucose by means of *Saccharomyces intermedians*, that isomaltose was present. Emulsin produced *d*-glucose from it, but maltase was unable to do so. When glucose, on the other hand, was acted upon by emulsin, maltose was produced. These relations may be summarized as follows:

α-glucose ↓ glucose-α-glucoside (maltose) ↓ α-glucose — synthesized by emulsin.

α-glucose ↓ glucose-α-glucoside (maltose) ↓ α-glucose — hydrolyzed by maltase.

β-glucose ↓ glucose-β-glucoside (iso-maltose) ↓ β-glucose — synthesized by maltase.

β-glucose ↓ glucose-β-glucoside (iso-maltose) ↓ β-glucose — hydrolyzed by emulsin.

Each of these ferments, emulsin and maltase, builds up that biose which it cannot itself decompose. This synthesis by ferments is, therefore, different as far as our present knowledge goes from that produced by true catalyzer.

We have gone into these relations in detail in order to show that we are at present not justified in concluding that a single ferment in the cells can effect decomposition or synthesis according to the outer conditions. We have no reason for believing this. This does not, of course, exclude the possibility that these processes may be carried out differently in the cells and tissues. We are, however, not justified in regarding all processes of metabolism in the tissues and cells as being due to fermentation. It is absolutely necessary right at this point to confine ourselves to the facts, and not follow a plausible hypothesis, the value of which is especially problematical here, for there is a vast amount of research to be made. Although our knowledge of fermentation reactions constantly broadens, the great mystery regarding the origin and formation of the ferments remains, and the important question relating to their chemical structure is still unsolved.

Let us now turn to the classification of the ferments. We have often

encountered them in tracing the course of the organic nutrient materials in their passage from the alimentary canal to the tissues. It remains only to classify them in a comprehensive manner. We may divide the ferments into two main groups: (1) the hydrolytic, and (2) the oxidizing ferments. The former may be further subdivided according to the material to be attacked, e.g. (*a*) ferments which effect the decomposition of the carbohydrates, (*b*) the proteolytic ferments which act upon the proteins, and (*c*) the fat-splitting ferments. The diastatic ferments belong to class (*a*). They hydrolyze starch into dextrins and maltose. The decomposition of maltose into two molecules of dextrose is effected by *maltase*. *Invertase* splits cane-sugar into one molecule of dextrose (*d*-glucose) and one of lævulose (*d*-fructose). To this group belong a series of ferments whose characteristics have been less carefully studied, such as *cellulase*, which is supposed to decompose cellulose; *inulinase*, which acts on inulin; *seminase*, which disintegrates mannans and galactans; and finally *pectinase*, which is responsible for the hydrolysis of pectin. We might also mention *trehalase*, *melibiase*, and *lactase*, which decompose the sugars corresponding to their names. Special ferments are also known which hydrolyze the glucosides, as well as a urease that changes urea into ammonium carbonate. To class (*b*), the proteolytic ferments, belong pepsin, trypsin, rennin, the fibrin-ferment, and pectase which hydrolyzes pectin substances. The fat-splitting ferments form a class by themselves. Then there is lactic-acid ferment, which produces lactic acid from sugar. It attacks all of the simple hexoses, and some pentoses, but not cane-sugar or milk-sugar. It produces chiefly the α-hydroxypropionic acid, $CH_3 . CHOH . COOH$. It is still a question whether the lactic acid fermentation is to be looked upon as an independent process.

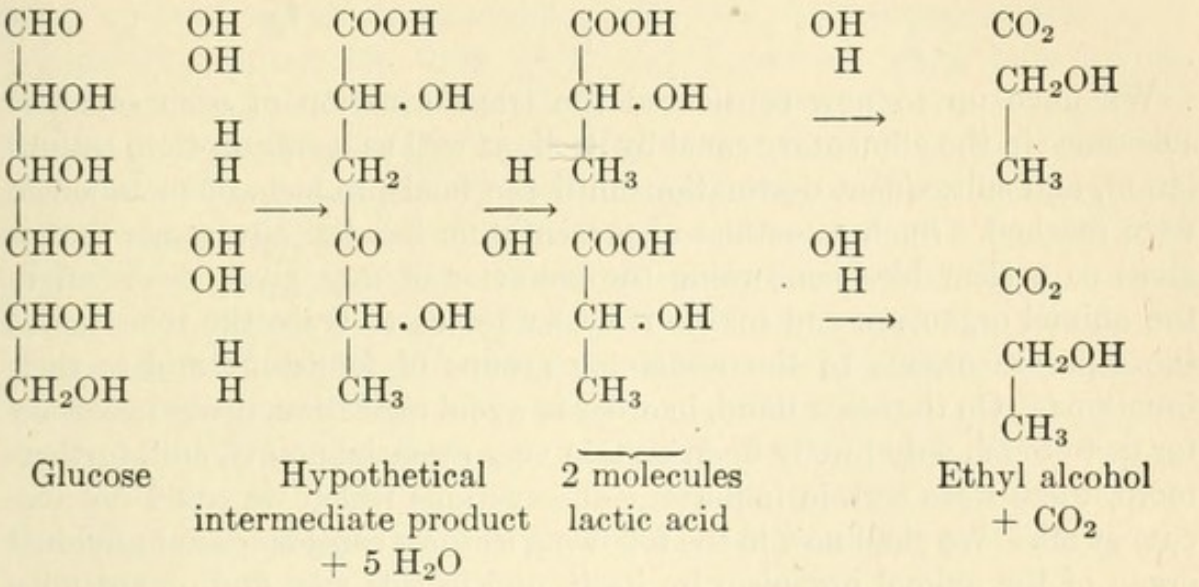
The second main group comprises the oxidizing ferments which have already been discussed. They obtain their oxygen either from the air or from decomposed hydrogen peroxide. To the former class belong the true oxidases, acting as oxygen carriers to the cells and tissues. Atmospheric oxygen is also utilized during the acetic acid fermentation of ethyl alcohol.

Alcoholic fermentation is quite a special process. It is a complicated affair, and the ferments producing it cannot, at present, be assigned to any of the above groups. As we have already shown,[1] alcoholic fermentation plays a part of which we cannot at present estimate the extent in plant and animal tissues, and inasmuch as an inspection of this process will give us some idea of the progress of a fermentation reaction, we will briefly discuss it. The reaction takes place according to the following general equation:

$$C_6H_{12}O_6 = 2\ C_2H_5OH + 2\ CO_2.$$

[1] Cf. Lecture IV, p. 74.

Until recently the whole process of alcoholic fermentation was surrounded with great obscurity. E. Buchner and J. Meisenheimer[1] have succeeded at least in indicating the way in which the decomposition proceeds. Thus, in their cell-free fermentations, they invariably found inactive lactic acid. They concluded that this must be a normal intermediate product, and formulated the alcoholic fermentation to take place as follows:



At all events, alcoholic fermentation is a very complicated process, and the question has now arisen, whether it is to be looked upon as brought about by one or by several ferments. One ferment may convert the sugar into lactic acid, while a second transforms this into alcohol. There are also by-products in the alcoholic fermentations. Glycerol, succinic acid, and acetic acid have been noticed among these. If we employ zymase, such products are not formed to any extent. They are evidently not a part of the alcoholic fermentation itself, but are due to other metabolic changes of the yeast.[2]

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[1] Ber. **37**, 417 (1904).

[2] For the older conception of alcoholic fermentation, cf. C. v. Nägeli; Theorie der Gährung. München (1879).

LECTURE XXI.

THE FUNCTIONS OF THE DIGESTIVE ORGANS.

I.

We have up to now considered the transformation of each separate substance in the alimentary canal by itself, as well as its absorption, assimilation, and subsequent destination until the final products of metabolism were reached. Such a method of presentation has the advantage that it gives us a clear idea concerning the behavior of any given foodstuff in the animal organism, and makes it easier for us to trace the relations of the separate organs to the remaining groups of foodstuffs and to their functions. On the other hand, in order to avoid repetition, it was necessary for us to touch only briefly upon certain very essential points, and, furthermore, there were certain important observations which we could not discuss at all. We shall now in the following lectures consider each individual organ of the animal organism by itself, and in this way find opportunity to mention what we have omitted, and, at the same time, to bind together certain apparently isolated facts with other analogous ones to a single unit. It is extremely difficult, and in fact impossible, to draw a sharp line between pure physiology and physiological chemistry. The time has long since passed when the latter branch of biological science could be considered as concerned chiefly with the investigation of the composition of the separate organs, the fluids of the body, and the excretions and secretions. It has been recognized for some time that the tracing of the relations of the different groups of foodstuffs to one another and their transformations in the organism, has introduced new problems into the field of physiological chemistry. With the solution of these problems, which are fundamentally important for the understanding of the entire metabolism, the limits of the working field of the physiological chemist are by no means reached, as we shall see. Physiological chemistry takes part more and more in explaining the functions of the various organs. Certain of the apparently-very-complicated processes have been brought nearer to our comprehension by the more recent investigations; and for some functions of which it was not supposed that there was any relation to chemical processes, newer observations have opened up entirely new perspectives, especially from a chemical point of view. Undoubtedly physiological chemistry must become more closely united with pure physiology in order that the fruits obtained in both fields may become fully ripe.

Such a point of view is particularly advantageous now that we come to consider the functions of the various organs in the digestive canal. They are of most diverse nature according to the part of the digestive stratum in which they are found. Let us begin with the functions of the upper end of the alimentary canal, the mouth. In it the food which has been prepared in various ways, is chewed up into small pieces and intimately mixed with the saliva. In this way the *morsels* are prepared for digestion. The saliva comes from the salivary glands and from the mucous membrane of the mouth. There are three pairs of the former, which may be distinguished chiefly by the nature of the fluids they secrete. The parotid (near the ear) gland produces a thin watery fluid containing chiefly albumin and salts. It is spoken of in general as an *albuminous gland*. Small glands of this nature are found as well in the mucous membrane of the nose and mouth. The other glands are the so-called *mucous glands*. They, in contrast to the former, furnish a glairy, or more ropy secretion, due to the mucin which it contains. To this class of glands belong, in the case of most animals, the submaxillary and sublingual glands. Small mucous glands are found distributed in the mouth, throat, and œsophagus. This distinction between two kinds of glands is not a sharp one. Thus the lower jaw of man contains glands which yield a thin secretion rich in albumin as well as one of a more mucous nature.

The secretion from each separate gland may be examined by constructing fistulas in the exit ducts, or more simply by introducing a canula into the mouth of the duct from where it discharges outward. Normally a mixture of the secretions from all of the glands comes into action. The extent to which the different glands take part in the formation of the saliva varies. The secretion of the glands is dependent upon quite a number of outside influences, as J. P. Pawlow [1] has quite recently called to our attention. In the exercise of their function, they are dependent upon nervous influences. The innervation of each gland is twofold. Cerebral and sympathetic fibers lead to each. The submaxillary gland contains fibers from the *chorda tympani*, and, on the other hand, fibers enter the blood-vessels of the gland from the *sympathetic system*. This double innervation also corresponds to the nature of the secretion. By stimulating the cerebral fibres, in other words, those of the *chorda tympani*, an abundant secretion of a thin liquid is produced; whereas, by stimulating the sympathetic nerve, only a few drops of a viscid liquid rich in mucin are obtained. The sublingual gland behaves quite similarly. The parotid gland is also partly dependent upon the cerebral nervous system (in this case the glossopharyngeal nerve) and partly on the sympathetic.

For some time it was believed that the influence of the nerves which reach these glands could be explained by an action upon the blood-vessels.

[1] Ergeb. Physiol. (Asher and Spiro) Jahrg. III., Abt. I, p. 177 (1904).

If the lingual nerve, which carries the cerebral fibers to the submaxillary gland, is cut, and then the peripheral stump stimulated, the blood-vessels in the gland become dilated. The blood streams from the veins with a bright red color similar to that of the arterial blood. At the same time there is an increased secretion of saliva. On the other hand, by stimulating the fibers of the sympatheticus, the blood-vessels are contracted. The blood passes more slowly, and the flow from the veins is small in amount and of a dark blue color. The activity of the secretion is diminished.[1] That, on the other hand, the innervation of the blood-vessels is not the sole function of the above-mentioned nerves, is shown by the experiments of Heidenhain.[2] He found that with the lingual nerve two kinds of nerve fibers enter the gland. If a dog was poisoned with atropin, then stimulation of the facial nerve fibers caused as before an acceleration of the blood-stream, while, on the other hand, there was no increase in the secretion of saliva. From this it is clear that the facial nerve, as well as the sympathetic, carries fibers to the submaxillary gland which have a specific action upon the individual cells of the gland. For the present we know but little concerning the details by which this stimulation is effected. It has not yet been found possible to establish beyond reasonable doubt the anatomical relations of the nerve fibers to the cells of the gland.

C. Ludwig and A. Spiess [3] have shown that with the activity of the cells the temperature also rises. They introduced a thermometer in the large vein of the submaxillary gland of a dog, a second in the exit duct from this gland, and a third in the carotid artery. If now the facial fibers were stimulated, then from the time of beginning of the activity of the glands the thermometers in the saliva and in the vein registered higher temperatures than that of the carotid.

It was at one time believed that the formation of the saliva could be regarded as a filtration process. It was soon found, however, that the separation of this liquid was evidently due to a specific activity on the part of the gland-cells. Even the chemical composition of the saliva indicates this. It is entirely different from the blood and lymph, and must have been formed only by means of a specific choice of the individual constituents of these liquids on the part of the gland-cells. In fact, it is even necessary for these last to produce for themselves certain substances. Here, we cannot treat in detail of all the evidence which has been brought forward against the assumption that the separating of the saliva is a result of a filtration process. We can only briefly touch upon it. Thus it has

[1] Claude Bernard: Compt. rend. **47,** 245 (1858).

[2] Pflüger's Arch. **5,** 309 (1872); **17** (1878). See also Barbèra: Bull. scienze med. di Bologna (8), **2,** 1 (1902).

[3] Sitzber. Wiener Akad. **25,** 548 (1857).

been found that if a mercury manometer be introduced into the exit duct, then after stimulating the cerebral secretory nerves, the mercury will in a short time rise 100 millimeters or more higher in this manometer than in another one that is placed in the carotid artery. There is, therefore, a considerable increase of pressure during the secretion of saliva.[1] It is also an important fact that stimulation of the secretory nerves has an effect even in animals from which the blood has been removed completely. That the cells of the gland are active during the preparation of saliva, is evident from a microscopic examination during a period of rest and one of action.[2] We have to thank Heidenhain[3] for this interesting information. He described the contents of the albuminous glands at rest and after secretion had taken place, using alcoholic-carmine preparations. In the first instance, a shrunken, finely granular substance is seen in a clear, uncolored background. The nucleus itself appears as an irregular, serrated structure without any distinct nucleolus. On making preparations in the same way of glands which have been in marked activity for some time, under nervous stimulation, there is a quite different appearance. The size of the cells has increased to a greater or less extent. The nucleus no longer appears serrated, but round. The nucleolus is now much more sharply outlined, and there is a considerable increase in the amount of substance in the vicinity of the nucleus so that the cells appear opaque. The glands themselves show similar changes. During a period of rest its gland-cells are large and clear. Their nuclei are flattened and parietal. The protoplasm is small in amount. The chief constituent in the composition of the cells is a clear substance which represents the secretion material of the gland-cells. When the gland becomes active the nuclei become round. The nucleoli become more distinct, and at the same time the nuclei are pressed more and more to the center of the cells. The cells themselves become smaller on account of loss of the above-mentioned clear substance. At the same time, there is an increase in the amount of protoplasm, evidently the beginning of the production of a new secretion. If we examine fresh material, instead of hardened preparations, we will

[1] We might mention in this connection the formation of retention cysts which often take place in the parotid gland when the duct from a lobule becomes stopped up for any reason; for example, in case of inflammation. If the secretion of saliva were to be regarded as a mere filtration process, it would be expected that the activity in the region cut off would soon cease. The secretion, however, continues. The amount of pressure developed in consequence is indicated by the swelling produced. Even if, later on, secondary changes appear, creating new conditions, still for the beginning of the formation of cysts our observation holds true.

[2] Cf. A. Noll: Die Sekretion der Drüsenzelle, Ergeb. Physiol. (Asher and Spiro) Jg. IV. p. 84 (1905).

[3] Zentr. med. Wissensch. **9**, 130 (1866). Hermann's Handbuch der Physiol. **5**, I. 64 (1883).

obtain quite corresponding results. Thus in place of the above clear substance, little granules are noticed which represent the secretion material produced by the cells. During rest these granules are being formed constantly to be given off during activity. There has been a great deal of discussion as to whether, in the secretion of the glands, the cells themselves are destroyed, or whether it is to be assumed that the cell, as such, retains its protoplasm and nucleus intact, and merely gives up the specific secretion produced by it. According to all known observations, the latter conception appears to be the correct one, for if the cells of the gland themselves should undergo breaking down, then there should be considerable evidence in the active gland of a renewal of cell material. As a matter of fact, there is but little sign of any such cell division taking place.

The cells of the various glands in the animal organism do not, to be sure, show any marked difference from the cells of the other tissues. We know that all sorts of different cells are constantly producing definite products which take part in metabolism and in the exercise of particular functions. We know, for example, that many cells give up ferments, while others produce compounds of simple constitution; for example, the cells of the suprarenal bodies produce adrenalin, and those of the intestine form secretin. The formation of such products as an immediate consequence of the activity of the cells escapes our observation only because the amount formed is so small, and partly, as is the case with the digestive ferments, because it is not these alone that are given up by the cells, but there is a much larger amount of other material set free simultaneously.

We may consider the formation of the secretion by the gland-cells in the same light, and trace it to the activity of the cells, and in a narrower sense to the protoplasm and cell-nuclei. We do not wish to speak — in order to avoid misconceptions — of a transformation of protoplasm into secretion, as is often done, but rather of the production of the latter by cell activity. We must remember that the gland-cells are constantly being supplied with new material by the blood, from which the secretion can be formed. This is taken up according as the cell requires such material, and by means of complicated processes the cell manufactures the secretion from it. The protoplasm itself remains behind in the cell together with the nucleus; both are preserved for a new formation of secretion. If we were to assume that the cells themselves were passing over into the secretion, it would be much more difficult to explain the process. There is no doubt that ferments play an important part in the formation of this secretion. Thus the cells of the gland have to break down to some extent the protein substance in the serum in order to construct the mucin which is contained in the secretion. The fact that evidently quite extensive transformations take place is shown by the fact that mucin contains a large amount of

glucosamine, while in the protein bodies present in serum there is but a small amount of this aminohexose. It is conceivable that the albuminous substances in blood-serum contain perhaps unknown preliminary stages in the glucosamine formation, but it is, however, also possible that we have here one stage in the process of the conversion of an amino acid into a sugar. At all events, the formation of mucin with its peculiar composition deserves considerable attention.

It is not to be assumed that during the activity of the gland, the cells must be entirely built up as well as the secretion formed. At the same time we should not think for a moment that these cells are permanent structures. We do not doubt that they are constantly being renewed like all other cells of the body, and that here and there a cell disappears to be replaced by a new one.

The decrease in the volume of the individual cells during their activity also has an effect upon the weight of the gland. If the submaxillary gland is brought into activity by stimulating the fibers of the facial nerve, it is found that the active gland decreases in weight.

Some attention is due to the question as to the manner in which innervation of the salivary glands is normally produced. The Russian physiologist Pawlow deserves great credit not only for having developed the operative technique so that it is possible under purely physiological conditions to trace the functions of the various digestive glands, but also for having shown in an entirely original way how the activity of the same is dependent upon definite external conditions. We have known for a long time that the activity of the salivary glands is influenced by sensations of taste and smell, and even by certain imaginations. Pawlow, however, deserves the credit for clearly demonstrating by experiment the remarkable ability that the salivary glands have for adapting themselves to their work. Among other things he called attention to the following observations: If a dog is fed with dry, solid nourishment, there at once takes place a considerable flow of saliva; while on the other hand, if the nourishment is liquid, there results but a slight flow. Chemicals which act as irritants, such as acids and alkalies, increase the flow of saliva in proportion to the irritation produced. The organism attempts in this way to protect itself from the action of such substances. It dilutes them and washes them out from the mouth as much as possible. If small quartz pebbles are placed in the mouth of a dog, the animal permits them to drop out gradually from his mouth, but without any flow of saliva. If, on the other hand, the same material is placed in the dog's mouth in the form of a powder, there is a considerable flow of saliva. The purpose of this is clear, — in this way the sand is washed out of the mouth, while in the case of the little stones the tongue alone can accomplish their removal. In all such cases we are struck with the utility of the whole mechanism. This is still more marked

when we find not only that the amount of saliva is regulated according to the requirements, but likewise its composition. At one time it contains considerable mucin, at another time relatively little, according to the conditions. Apparently the irritation, whether it be chemical, thermal, or mechanical, stimulates the apparatus at the end of the afferent nerves in the mucous membrane of the mouth. From here the impulses are transmitted to the central organ of the nervous system, and now by means of the efferent nerves the individual salivary glands are called into action. We speak of such a process in general as a *reflex action.*

It is of chief interest to us that it is possible by various outward effects to influence the activity of the glands in such a way that the composition of the saliva secreted is adjusted to the prevailing conditions. At present we can only imagine how this adjustment may be effected. The saliva itself always contains besides inorganic salts and water a certain amount of organic material, as we have seen. We also repeat that the saliva contains a ferment capable of hydrolyzing starch, the *diastase.* It is remarkable too that it always contains small amounts of alkali thiocyanate. We are wholly in the dark concerning the formation of this last compound, or as regards its use.

In this connection it seems fitting to mention some observations concerning secretions in the mouths of certain invertebrates. They are of particular value because they make it easier for us to understand the most important work of the gland-cells. Here we meet with the preparation of strong acids by means of cell activity from material which could not have contained the acid already formed. Long ago Troschel[1] noted, in examining a kind of snail, *Dolium galea,* that the animal squirted from its mouth a stream of liquid clear as water. The liquid showed a strongly acid reaction, and caused effervescence on coming in contact with the limestone lying on the ground. The secretion was produced from two large gland-like organs lying near the stomach. The ducts from it ascend on each side of the gullet and empty into the mouth. The secretion contains *sulphuric acid,* and, in fact, as much as 4.1 per cent of the free acid is present. Besides this, there is 0.4 to 0.6 per cent of hydrochloric acid. Other varieties of snails similarly produce acids. Some of these "acid snails" have been quite recently studied by Fr. N. Schulz.[2] He followed particularly closely the acid production on the part of the naked snail, *Pleurobranchia Meckelii* of the order *Opisthobranchi.* On being touched, the snail rolls itself up. If the animal is squeezed a little, its external surface after a short time becomes covered with a slimy secretion of acid reaction. This comes from glands which are very numerous in the skin.

[1] Poggendorf's Ann. **93**, 614 (1854); J. pr. Chem. **63**, 170 (1854); see also de Luca and Panceri, Compt. rend. **65**, 577 and 712 (1867).

[2] Z. allgem. Physiol. **5**, 206 (1905).

Besides this secretion the animal empties from its pharynx a very strongly-acid-reacting juice. This comes from a gland-like structure consisting of long coils enveloped in a highly complicated, contractile network. The chief constituent of the individual cells is a liquid, while the amount of protoplasm itself is relatively small. If the gland is stimulated, the above-mentioned network contracts; and the liquid secretion of the gland-cells, which is contained in larger or smaller vacuoles, is emptied into the exit duct. Evidently in this case the secretion is merely mechanically removed from the cells. After the relaxation of the contracted cell-coil, there remain in the cells at first only the shrunken protoplasm and the cell-nuclei, and then begins anew the formation of secretion. Several things indicate that the nucleus itself plays an important part in this process. The secretion contains free sulphuric acid. What is the source of this acid? It might come from sulphates, or from organic compounds containing sulphur, such as, for example, albumin. The latter is hardly to be considered as a possible source of sulphuric acid. It seems certain that most of the acid must be formed from sulphates, for the amount of acid produced is too large, and the amount of sulphur present in albumin too small, to account for the formation by the assumption of an oxidizing decomposition, as, for example, of cystine. It has been found that the secretion continues during starvation. If the albumin itself were the source of the sulphur, we would expect that the formation of sulphuric acid would soon cease in the starved organism. It has never been explained how the cells in the gland are able to produce the free acid. When we come to discuss the formation of hydrochloric acid in the human stomach, we shall find likewise that we are again in the dark. It has been attempted to explain the formation of the strong acid as a result of the mass-action law. We know that from salts of the mineral acids a small amount of acid may be set free by the action of large amounts of carbonic acid, for example. We also know that as a result of ionization a small amount of acid ions are probably set free in the organism. We shall not deny that perhaps a part of the acid in the secretion may be formed in some such way, and it is indeed conceivable that eventually all of the acid may be produced in such manner, if we assume that as soon as a little acid is set free, it is in some manner carried out of the range of chemical reaction, so that constantly more and more of the acid will be formed. But even such an hypothesis, which necessitates the further assumption of some means of removing the acid as fast as it may be formed, does not enable us to explain satisfactorily the whole process. At all events, the cells themselves must exert a specific action. In this case, the cells of the gland form sulphuric acid alone, while in the stomach only hydrochloric acid results. It might be assumed that the membrane of the cells is only permeable to certain ions. It has, for example, been asserted that the walls of the stomach are

impermeable to chlorine ions, and in this way the formation of hydrochloric acid in the stomach was explained.[1] It was soon apparent, however, that such a theory was untenable. If we stop considering the production of the acid by itself, but remember that the formation of the remaining products of secretion point to a specific and extensive activity on the part of the gland-cells, then evidently the formation of the free acid represents only one link in the chain of the entire secretion process. It is not any more remarkable than, for example, the formation of mucin in the cells of the salivary glands. Just as little as we are unable to account for the formation of the latter as a result of purely physical or chemical processes, is it possible for us to understand clearly the production of acid on the part of the cells.

The question next arises concerning the biological significance of the acid secretion in snails. Troschel, the discoverer of the presence of the acid, was inclined to believe that it was a means of protection against enemies. This is hardly correct in the sense meant. Although the *Dolium galea* is able to throw out this secretion when on land, the irritating effect of the acid would be lost when the animal is in the water, and, moreover, the water itself offers so much resistance that obviously the animal would not be able to send out a stream of secretion at any desired moment. On the other hand, it is possible that the acid formation, especially on the part of the glands in the skin of the *Pleurobranchi*, may serve indirectly as a means of protection in the sense that by reason of it these snails will be avoided as food by other animals. Unquestionably, the secretion of the large glands in the gullet must have some other significance. It has been suggested that they play a rôle in digestion. Careful investigation, however, has proved that this is not the case. Semon[2] believed that the sulphuric acid acts upon the lime skeletons of other animals upon which these snails feed. Thus calcium sulphate would be produced. The effect of this decomposition Semon studied on the skeleton of the star-fish. He found that the latter, after it had lain for some time in water containing sulphuric acid, could be broken up by means of the fingers. The direct examination of the intestinal contents of these snails did not lend any support, however, to the assumption of any such action. It is, therefore, improbable that the sulphuric acid is produced for the purpose suggested by Semon. Now we find in the animal kingdom repeated examples of contrivances by means of which one animal which eats another species for its nourishment is able to cripple its prey. This is the case with the poison glands of snakes. Perhaps in the case of the snails the acid forms a weapon of attack.[3] Many sea animals are extremely

[1] Cf. Köppe, Pflüger's Arch. **62**, 567 (1892), and Landislaus v. Rohrer: *ibid.* **110**, 416 (1905).

[2] Biol. Zentr. **9**, 80 (1890).

[3] W. Preyer: Naturwissensch. Wochschr. Berlin **5**, 481 (1890).

sensitive to acid. Thus the *Echinoderms* draw in their suckers when exposed to acid. They are then easily removed from the place to which they had attached themselves.

Let us now return to the saliva. We have already mentioned its most important functions. They are chiefly of a mechanical nature, — the food is surrounded by saliva and thus made easy to swallow. The saliva also is an important means of keeping the teeth clean. In this case also it exerts first of all a mechanical action, and, on the other hand, when of normal composition, it also tends to prevent decomposition by the bacterial flora of the mouth. It is probable that in many cases the formation of caries in teeth is due to an abnormal composition of the saliva. Tooth decay, moreover, usually results from a faulty formation of the tooth itself. The tissue composing the teeth is closely related to that of the bones. Three tissues are known to take part in the formation of teeth, of which one, the cement, corresponds to the bony tissue. Dentine also has a similar composition. We find that bones contain: calcium phosphate, magnesium phosphate, calcium fluoride, calcium chloride, calcium carbonate, ferric oxide, and an organic basal substance, which on boiling yields gelatin. Enamel, the third tissue of teeth, is characterized by its containing the least water and the most mineral matter of any substance in the human body. Under normal conditions it exerts great resistance to external influences, and, as long as it is intact, prevents infection by bacteria.[1] The enamel contains lime salts chiefly. At this place we need hardly mention the importance of the food being well chewed. It is perfectly clear that the subdivision of the food is of great benefit as regards the rapidity with which it is acted upon by the digestive ferments. By means of the teeth the food is changed into such a state that it can be acted upon readily by the digestive juices. It might be thought that on feeding with broth, the function of the teeth would be replaced. We shall see later on, however, that the manner in which the food is prepared has a great influence upon the function of the secretions in the digestive tract, especially that of the stomach. A uniform diet could not possibly stimulate our senses permanently.

In many cases the saliva acts as a solvent, and in this way the sensation of taste is obtained, for we can only taste substances which are in solution. The peripheral organs of the sense of taste are distributed throughout the whole of the mouth. We find them on the upper surface of the tongue, on the under surface at the tip of the tongue, in the mucous membrane of

[1] We should not fail to mention in this case the remarkable readiness with which wounds in the mucous membrane of the mouth, or indeed of the entire alimentary canal, are healed; and how seldom there is any infection here in spite of frequent exposure. We may perhaps speak of the immunity of cells in the mucous membrane and surrounding tissue. This immunity may be acquired by the fact that metabolic products of bacteria in the mouth are absorbed from the dilute solution there, and this creates immunity.

the soft and hard palate, the anterior pillar, the tonsils, the posterior pillar, the uvula, the epiglottis, and even the throat, itself. This wide distribution of the organs for taste perception is only noticeable during youth. In adults the sensation is more localized, although it varies greatly with individuals. The mucous membrane of the cheeks, the uvula, tonsils, and the middle of the tongue, are almost always incapable of taste perception in adults. The end-apparatus of the taste-nerves form the so-called taste-buds or taste-goblets. In man the *glossopharyngeal* and *trigeminal* nerves have been recognized as taste-nerves. The former innervate the back part of the tongue, the latter the front part. Individual peculiarities are noticed here, and sometimes one of these nerves alone provides for the whole region. The different sensations of taste may in general be attributed to four different qualities; namely, sweet, sour, bitter, and salt. We also speak of alkaline and metallic tastes. There is hardly room for doubt that these different sensations of taste are produced by the aid of different nerves, so that for the taste-nerves the *law of specific sense energy* [1] applies. According to this law, one and the same excitant when acting upon different nerves will always produce different sensations, while, on the other hand, all sorts of different excitants always produce the same sensation when acting upon the same nerve. The different quality of the sensation is therefore merely caused by the different nature of the end-apparatus in the central nervous system. Moreover, the peripheral reception apparatus is so specialized that it is only affected by certain definite excitants. It might be thought, and especially in the study of the sensation of taste, that the chemical constitution of a substance having a definite quality of taste might give us some idea of the way in which the definite end-apparatus is excited. Numerous experiments have been performed with this idea in mind, but without success.[2] Thus, for example, a great many amino acids taste sweet, while others are bitter. Glycocoll, alanine, and α-amino-valeric acid are sweet, while *l*-leucine, which occurs in nature, is bitter. It is remarkable that *d*-leucine has a sweet taste.[3] In *dl*-leucine the sweet taste predominates. The sense of smell is closely related to that of taste, and frequently they are confused with one another. It is, furthermore, interesting that both these sensations of taste and of smell may be produced by means of a very small amount of material. The organ of smell is in some cases especially sensitive. Emil Fischer and Penzoldt found, for example, that as little as 0.000,000,04 milligram of mercaptan in a

[1] Johannes Müller: Zur vergleichenden Physiologie des Gesichtssinnes. Leipzig (1826). Cf. R. Weinmann: Die Lehre von den specifischen Sinnesenergien, Hamburg and Leipzig (1895).

[2] Cf. Wilhelm Sternberg: Arch. Anat. Phys. **1903**, 538; **1904**, 483; **1905**, 201; Ber. **15**, 36 (1905).

[3] Emil Fischer and Otto Warburg: Ber. **35**, 3997, 4005 (1905).

liter of air is perceptible. We shall repeatedly come back to the influence of the sensatory impressions upon the functions of the digestive glands. The nerves of smell and of taste are important protective organs. They call attention to processes of decomposition, to decay in our food, and allow us to recognize the presence of many injurious substances.

From the mouth, the food is carried by the process of swallowing into the œsophagus, and from here directly into the stomach through the cardia. With the exception of a slight transformation of starch into sugar, the real process of digestion does not begin until the food reaches the stomach, where it proceeds energetically. We repeat that the diastase from the saliva can under certain conditions continue its action for some time, but, on the other hand, the stomach itself has its own ferments. As has been recently shown, it possesses, for one thing, a *lipolytic* ferment, although we have at present no means of estimating the extent of its activity. Its significance has been quite variously estimated and, in fact, its very presence has been doubted by some. Besides lipase, there are ferments present in the stomach which are capable of acting upon the protein of the foods. These are *pepsin* and *rennin*. We have already stated that the existence of the latter ferment has been quite recently questioned, and it has been assumed that the two properties of coagulating milk and dissolving albumin are due to the action of a single ferment. We can adopt this assumption that the action of rennin corresponds to that of pepsin, only when it has been verified by further investigation. Until this has been done, we will content ourselves with the older conception of the presence of both pepsin and rennin, although, we have already seen, there are a number of facts which speak in favor of Pawlow's hypothesis.

These ferments are produced by certain glands in the mucous membrane of the stomach. The stomach itself is not a physiological unit. Even the outer appearance shows a marked difference between the pyloric and fundus (or cardiac) portions of the mucous membrane. That of the former is pale and has a few deep folds, while the latter is of a reddish-yellow or reddish-gray color, and has numerous folds which are connected with one another by a sort of network. In these net-like little hollows between the folds end the stomach glands. There are two types of these glands, one of which contains but a single kind of cell, while the other contains two. In the pyloric end there is found but one cell form, while in the *cardiac* or *fundus* end the glands are of the latter type. There is no sharp distinction between them, however. These two types of glands, both of which are tubular in shape, are named according to the locality in which they are found, and are known respectively as *pyloric* and *cardiac* or *fundus glands*. The former contain a cylindrical epithelium, and the latter contain, in addition, other smaller cells irregularly distributed between the larger cells and the basement membrane. The first kind of

cells are designated in the cardiac glands as *chief, central, principal,* or *adelomorphous cells,* while the latter are called either *border, parietal,* or *delomorphous cells.* Between the gland-cells there are fine secretion capillaries which surround the border cells like a basket. For a long time it was believed that this histological distinction of two kinds of cells also corresponded to a physiological distinction. The fundus glands were supposed to secrete only pepsin, while the pyloric glands merely formed mucous. That this is not the case is shown by the fact that the secretion carefully collected from the latter portion of the stomach does actually contain pepsin. The pyloric and cardiac parts can easily be isolated, and in this way "small stomachs" are formed in the Pawlow sense, and by means of fistulas the contents of each may be studied by itself. At present we are still undecided as to the especial significance of the chief cells and of the border cells. It has been established that the former take part in the formation of pepsin and rennin, but the function of the border cells remains vague. From the fact that the pyloric part of the stomach produces little or no hydrochloric acid, it has been suggested that the border cells produce the acid, but there has never been any direct proof of this.

Whatever the functions of the cells may be, it is to be said that much the same changes take place in them during activity as in the cells of the salivary glands. During a period of rest the secretion is formed which is given up during the period of work.

All the secretions of the mucous membrane of the stomach and its glands taken together form the so-called *gastric juice.* It consists in part of mucous which is given off chiefly from the superficial surface of the mucous coat, together with the ferments, hydrochloric acid, inorganic salts, and small amounts of other organic substances. Exact studies concerning the formation of the gastric juice and of its dependence upon outward influences were first made possible by the operative skill of Pawlow and his pupils. His methods and his investigations gave us the first insight into the relation between the secretions of the stomach and physiological conditions. Pure gastric juice may be obtained by the establishment of a fistula, best in combination with one in the œsophagus. Thus the food swallowed by the animal may be removed before it reaches the stomach. In this way a fictitious feeding is obtained, and the gastric juice is not contaminated with the food, nor with the intestinal products. It was found advisable also to isolate a portion of the stomach, forming a little stomach, or blind sack, in which the secretion process could be studied by means of a fistula while digestion was going on in the rest of the organ. The gastric juice flowing out of such a fistula is, after filtering off the mucous, clear as water, odorless, and of an acid taste. This taste is due, as has been stated, to the presence of free hydrochloric acid. In

dogs there is from 0.46 to 0.6 per cent acid present, while in man the values vary greatly. Thus the amount present has been variously stated in the literature as from 0.05 to 0.57 per cent. We have already said that it now seems impossible to regard this free acid as taken directly from the blood, for the blood may be considered as of neutral reaction. A great number of experiments have been undertaken without definitely settling this question of the acid formation. We must rest content with the hypothesis that it is due to a specific activity on the part of the cells, and will state once more that it does not imply a process any more complicated than that of the formation of other substances which result from the specific activity of the cells in every gland. There is no justifiable ground for giving a peculiar position to the acid secretion.

The individual ferments are not found in a finished state in the mucous coat of the stomach; i.e., they are not given up by it in an active condition. This preliminary condition of the ferment is spoken of in general as that of a *zymogen*, and in the case of pepsin itself we have *pepsinogen*. The presence of such a substance may be shown by the following experiment: Pepsin itself is extremely sensitive to a solution of soda, by means of which it is soon destroyed. Pepsinogen, on the other hand, resists the action of soda much more strongly. If the mucous membrane of the stomach is cut up into small pieces and extracted with a dilute soda solution, there is obtained after filtering a liquid which of itself exerts no digestive action upon albumin, but does so on being acidified with hydrochloric acid. The acid serves to convert pepsinogen into pepsin. If, now, after the digestion has proceeded for a short time, the liquid is again made alkaline, a further addition of acid will no longer bring forth the digestive action of pepsin. The pepsin has been destroyed by the soda. We are at present unable to explain how the acid activates the zymogen, and, in fact, such processes will remain obscure until we know more about the chemical nature of the ferments themselves. We can indeed assume that the hydrochloric acid in some way causes a rearrangement of the atoms in the ferment molecule, possibly by the formation of an anhydride or something similar, and that in the new state the ferment is capable of exerting its characteristic action.

Rennin likewise is not present in the mucous membrane as such, but in the form of a zymogen. It is activated in precisely the same manner as pepsinogen, and in fact these two ferments are very similar in their entire behavior.

It is highly significant that the function of the mucous membrane and its glands is dependent upon definite kinds of stimulation. These can be effected in part in the stomach itself, or the stimuli may be transmitted to the stomach from other organs. The secretion of the gastric juice is brought about by reflex action. This may be demonstrated very prettily by making

œsophageal and gastric fistulas in a dog. Before the animal is fed, no gastric juice flows out of the latter. When the animal is offered food, first of all the dog chews it; on swallowing, instead of reaching the stomach, the food passes out through the fistula in the œsophagus. Thus there is no chemical, thermal, or mechanical stimulation produced upon the mucous membrane of the stomach. Notwithstanding, five or six minutes after the animal is offered food, there takes place regularly an abundant secretion of gastric juice. Pawlow and Schumow-Simanowskaja [1] have also succeeded in proving that the vagus nerve carries secretory fibers. The production of gastric juice is not entirely dependent upon the vagus as is evident from the fact that it continues after both the vagi have been severed, although the quality of the secretion is apparently changed; at least, there is less pepsin in it.

Highly important is the observation that psychic influences have the decisive effect upon the formation of gastric juice. It may be shown that the action of the nerves of taste and of smell alone do not suffice to cause a secretion from the lining of the stomach. Similarly the act of chewing is ineffective of itself. The reflex secretion of the stomach juices is only effected when the animal has the desire to eat. If, after the latent period of about five minutes, the secretion of gastric juice has once begun, it then continues for two or three hours. This secretion produced reflexively ceases immediately on cutting the two vagi nerves. It would be wrong to assert that the nerves of smell and of taste take part in the psychical stimulation of the secretion. To be sure, they may aid indirectly in many cases, by recalling to the imagination certain impressions which awaken the desires to eat. Of course these impressions may in some cases be first caused by the momentary stimulation of smell or taste.

By means of such experiments as those carried out by Pawlow in many directions, the great importance of the appetite has been clearly established. It is by no means a matter of indifference whether one eats with pleasure or from compulsion.

The importance of the gastric secretion produced reflexively by psychical influences is made very clear by the following experiment performed by Pawlow. He took two dogs, each having fistulas in the œsophagus and in the stomach, and introduced equal-sized pieces of meat through the gastric fistula of each dog in such a way that the animal was not conscious of the operation. One of the dogs was then given a piece of meat to devour. (In such cases, where the food devoured does not reach the stomach of the animal, we speak of a *fictitious* meal.) After some time had passed, Pawlow compared the pieces of meat in the stomachs of the two dogs. It was found that the meat in the stomach of the dog which had received the

[1] Arch. Anat. Physiol. **1895**, 53.

fictitious meal was much more thoroughly digested than the meat in the stomach of the other dog.[1]

It is an old experience that psychic influences may destroy the appetite. In this case there are great individual differences; often slight anger will suffice to destroy the appetite completely. These experiences can be shown experimentally to be well founded. Thus, among others, A. Bickel[2] has found that the gastric secretion stops at once when a dog is confronted with a cat. Undoubtedly the same relations exist in man. Naturally, in the latter case we do not possess such a rich field for observation. In man such experiments are influenced much more by secondary effects than is the case with animals, where reactions take place more in accordance with sensatory impressions. Certain imaginary effects are not so prominent in animals as in man. On the other hand, such experiments carried out with human beings are less valuable, because, when there is a gastric fistula, there is usually some pathological derangement of the stomach or œsophagus. Frequently there are tumors, especially cancers, to be considered. The latter are especially likely to cause a most deep-seated effect upon the whole metabolism of the cells and weaken the whole body, so that it is out of the question to speak of a normal function of the lining of the stomach and of its glands, even although the *carcinoma* may not have attacked the stomach itself. On the other hand, now and then the formation of a gastric fistula becomes necessary when there is no chance for the food to reach the stomach through the œsophagus, for example, as a result of strictures caused by injury to the mucous membrane. In such cases it is possible to carry out observations with human beings which are similar to those of Pawlow with dogs. Thus, Hornborg,[3] who studied a case of gastric fistula with œsophageal stricture, observed that it was not possible to detect psychic influences in all cases. The chewing of substances with pleasant taste produced a secretion, while chewing of indifferent or badly tasting substances had no effect. Chewing of itself appeared to act favorably upon the gastric secretion, while the mere sight of food had no effect. On the other hand, the secretion stopped if the boy was not allowed to eat at once something that tasted good; this evidently made the boy angry, and this feeling was indicated by a flow of tears.

The secretion of the gastric juice is produced not merely as a result of a reflex action, but we recognize certain other influences as well, which may be exerted within the stomach itself. Mechanical irritation does not suffice to start the flow of the juice. Chemical influences alone are to be

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[1] The highly interesting studies by Pawlow and his students have nearly all appeared in Russian only. His lectures have been translated into German by A. Walther, and published in 1898 by Bergmann of Wiesbaden.

[2] Deut. Med. Wochschr. **31**, 1829 (1905).

[3] Inaug. Dissert. Helsingfors (1903).

considered in this connection. Digestive activity is especially favored by broths, extracts, juice of meats and milk, and is somewhat stimulated by water and small amounts of alcohol. Fats, on the other hand, restrain the production of the digestive fluid, and also influence its qualitative and quantitative composition. These effects may be observed to best advantage by introducing the different substances into the stomach of an animal without its knowledge. The secretion under such conditions does not take place as quickly, usually not until after 15 to 30 minutes, and persists for various lengths of time according to the nature of the food. Experiments of this nature give one the impression that such a direct stimulation of the glands is incomplete in effect. A harmonious course of digestion is only assured when the secretion is strongly stimulated reflexively. We can indeed imagine that by means of digestion itself, substances are formed constantly which act as chemical irritants upon the lining of the stomach and the glands, so that after the flow of the juice is once started, it continues for a considerable length of time.

Pawlow and his students obtained very interesting results when they attempted to ascertain the effect of different kinds of food. It was found that the cells of the stomach did not produce the same juice in all cases. On the contrary, the composition of the digestive juice was suited to the nature of the food. First of all, it is of interest to know that the acidity of the juice remains constant in the secretion, while the amount of ferment present varies greatly. Now it is a well-known clinical fact that widely divergent degrees of acidity are found in the contents of the stomach. Such determinations, however, are of but a slight value, for many reasons; on no account should they be used to indicate the amount of hydrochloric acid normally present in the gastric juice. As we have already mentioned, it is not right to regard the contents of the stomach as a uniform mixture of the digestive secretion and the food. As Grützner [1] has recently proved, the newly-introduced food stands in the midst of that which has been in the stomach for some time, and does not at once come in contact with the walls of the stomach. In the *pars-splenica* of the stomach, the food may remain for hours without coming into intimate contact with the gastric juice. If now a part of such a digesting mixture be siphoned out of the stomach, it is obvious that a determination of the degree of its acidity might easily give rise to false conclusions. Quite a number of factors here come into play which may easily conceal the fact of the original uniformity in the acid content of the juices. It has been observed, for example, that with dogs in which there was a vigorous secretion, there was more acid in the gastric juice than when the production of the same took place more slowly. Similarly a higher acidity was noted if the juice

[1] Pflüger's Arch. **106**, 463 (1905).

was taken directly from the fistula than when the latter was closed for a time. The reason for this can be explained. The gastric juice flows over the walls of the stomach, which are covered with alkaline mucus, before it reaches the fistula. When there is a considerable amount of the juice being secreted, it is obvious that proportionately less hydrochloric acid will be neutralized than when only a small amount of juice passes over these walls; and similarly when the fistula is closed, the acid is more completely neutralized by the alkaline mucus than when it passes off freely. As Pawlow has stated, in a normal stomach as much as 25 per cent of the original acidity may be neutralized by the mucus. There are, to some extent, very complicated processes concerned in this neutralization the significance of which cannot be entirely disregarded. It is indeed possible that certain relations exist between the hydrochloric acid content of the stomach juices and the formation of the mucus by the membrane of the stomach, and that here again there is an adjustment corresponding to the nature of the different foodstuffs. Naturally the deviations in the acidity of the stomach juices vary much more greatly after the food has reached the stomach. At all events, any values obtained in this case should be very cautiously applied to the composition of the juice itself. The clinical practitioner must always have in mind all sorts of different relations, and should determine the combined hydrochloric acid as well as that which is still free. The careful physician should never be satisfied with a single observation, but should base his judgment upon examinations carried out under the most varied conditions.

Pawlow calls attention to the adaptability of the whole work of the stomach, and especially of its glands. This is shown in a number of little ways, and we are able to trace the functions of the stomach very well because we know the condition of the food as it enters. Such relations are much more difficult to establish in the study of the pancreas, and in some cases it is impossible, because its juices come in contact, under normal conditions, with an inextricable mixture consisting partly of decomposition products, and partly of unchanged food. Investigations have shown that a mixed diet, as well as the feeding of single articles, such as milk, bread, meat, etc., leads to a perfectly definite formation of the gastric juice. This is true not only of the composition of the fluid, but of the amount secreted and the duration of the secretion. First of all it is to be noted that the amount of gastric juice secreted is practically proportional to the amount of food. Thus 100 grams of raw meat caused the secretion of 26.0 cubic centimeters of juice; 200 grams = 40.0 cubic centimeters; 400 grams = 106.0 cubic centimeters. For a mixed diet, composed of milk, bread and meat, the following values were obtained: — 100 cubic centimeters of milk, 50 grams meat, and 50 grams of bread, correspond to 42.0 cubic centimeters of gastric juice, while double the

above quantity of the mixture caused the secretion of 83.2 cubic centimeters.

The digestive power of the gastric juice depends greatly upon the nature of the food. Pawlow and his students studied the power of digesting albumin on the part of both the gastric and pancreatic juices by Mett's method. This consists of sucking up the white of an egg into glass tubes of 1 to 2 millimeters bore and then coagulating it at a definite temperature. These tubes are inserted under entirely corresponding conditions into the digestive liquids, and taken out at the end of a definite period. Then, by means of a millimeter rule and a microscope, the amount of albumin that has become digested is measured. The following values are given as examples of such an experiment:[1]

At 8 o'clock the animal experimented upon was fed 200 grams of bread. It secreted the following amounts of gastric juice with varying digestive power:

Time.	Amount of Juice per Hour.	Digestive Power.
8–9 o'clock	3.2 c.c.	8.0 mm.
9–10 o'clock	4.5 c.c.	7.0 mm.
10–11 o'clock	1.8 c.c.	7.0 mm.

The same dog was then fed 200 grams of raw meat:

Time.	Amount of Juice per Hour.	Digestive Power.
12 o'clock	8.0 c.c.	5.37 mm.
1 o'clock	8.8 c.c.	3.50 mm.
2 o'clock	8.6 c.c.	3.75 mm.

Then 200 cubic centimeters of milk were fed to it:

Time.	Amount of Juice per Hour.	Digestive Power.
3 o'clock	9.2 c.c.	3.75 mm.
4 o'clock	8.4 c.c.	3.30 mm.

A control experiment showed that the values given were in no way caused by the order in which the food was eaten. It is evident from these figures that the juice secreted after feeding with bread possesses the greatest digestive power. Milk produces the weakest secretion of all.

[1] Cf. J. P. Pawlow: Die Arbeit der Verdaungsdrüsen, p. 42. P. Chigin: Arch. des sciences biol. III and Inaug. Diss. St. Petersburg (1894).

The total acidity likewise varies according to the nature of the food. It is greatest with meat and least with bread. It is interesting also to note that the duration of the secretion is also regulated according to the nature of the food; and in fact during the course of secretion, from hour to hour, the composition adjusts itself qualitatively to the conditions. We will give here the result of another of Pawlow's experiments:[1]

AMOUNT AND NATURE OF GASTRIC JUICE WITH DIFFERENT NOURISHMENT.

Hours.	200 gms. Meat, 200 gms. Bread, 600 c.c. Milk.					
	Amount of Fluid in c.c.			Digestive Power in mm.		
	Meat.	Bread.	Milk.	Meat.	Bread.	Milk.
1	11.2	10.6	4.0	4.94	6.10	4.21
2	11.3	5.4	8.6	3.03	7.97	2.35
3	7.6	4.0	9.2	3.01	7.15	2.35
4	5.1	3.4	7.7	2.87	6.19	2.65
5	2.8	3.3	4.0	3.20	5.29	4.63
6	2.2	2.2	0.5	3.58	5.72	6.12
7	1.2	2.6	...	3.25	5.48	...
8	0.6	2.2	...	3.87	5.50	...
9	...	0.9	...	...	5.75	...
10	...	0.4	...	...	...	...

From these figures it is evident that the maximum secretion is produced by meat during the first or second hour. In the case of bread, the maximum secretion is reached during the first hour, while with milk it is the second or third hour. With meat the juice secreted during the first hour has the greatest digestive power, in bread it is that of the second and third hours, while with milk the maximum digestive power is obtained much later.

These values do not merely correspond to a single experiment. They have been obtained again and again. At present we cannot say anything concerning the significance of these variations. We can indeed assume that one food requires more ferment for its hydrolysis than another, in order that its decomposition may take place to an equal extent within a given period. We must admit, however, that we are here confronted with many problems which cannot be solved until we know more concerning the nature of the ferments themselves and of the fermentation processes which they cause to take place. We introduce these interesting observations here, only to show how well organized are the functions of the digestive glands. In this way it is easier for us to understand, in considering

[1] *Loc. cit.* p. 44.

physiological processes, how great an effect is produced by any disturbance in the functions of the stomach. It is now clear to us that severe gastric disturbances may be brought about by purely nervous influences without there being organic changes. It is easy for us to believe that a hypersecretion may be produced by a condition of stimulation, caused, for example, by a supersensitiveness of the secretory fibers of the vagus. On the other hand, the experience of Pawlow and his school indicates the possibilities of conditions of restraint with a limited supply of secretion. The fact that the cells of the stomach glands are extremely sensitive to chemical stimulation, and adjust themselves to the nature of the nourishment with regard to their entire activity, enables us to understand that under pathological conditions it is not necessary for the amount of secretion to have become decreased or increased. Disturbances may arise which affect the production of some particular substance. It is perfectly clear that under such conditions the entire adjustment of the secretion to the nourishment would be affected. Thus, normally, for the digestion of bread, but little hydrochloric acid is present in the stomach during the entire duration of the secretion. There is, to be sure, a reason for this, for by this means the digestion of starch by the diastase in the saliva will continue much longer.

It is absolutely necessary that we should give prominence to the researches of Pawlow in our study of digestion in the stomach. By means of them, all the observations which have served for a long time to establish the existence of characteristic sense-nerves, may be applied likewise to the innervation of the intestinal canal and its glands. These organs also do not react fully by means of a single stimulation. Here again the stimulation is only taken up by certain definite cells and transmitted in a perfectly definite manner. The results of Pawlow's experiments are not at all astonishing. We may assume that purely chemical processes play a prominent part here. We may imagine that a certain kind of cell is adjusted so that it is susceptible to a given chemical stimulation, while a different cell is affected by another chemical substance. We may perhaps apply the facts that we have established in the study of ferments directly to the cells as a whole. The ferments are likewise products of the cells. The individual cells produce them in such a way that they possess certain groups which can react only with definite compounds corresponding to a characteristic grouping. Conversely, the cells may be so constructed that their function as a whole only appears when started by the action of certain definite substance.

The more extensive our knowledge becomes, and the more we enter into the secrets of the metabolism of cells, the better we become convinced that the cells themselves act by means of ferments. They do not part with such ferments, but retain them for their own use. These cell-

ferments may perhaps have a zymogen state of existence which requires an activator to bring its action into play. Every cell may possess several ferments. One substance may serve to activate a given ferment, while another activates a different one. Again, the gland-cells act by the aid of ferments. They are broken down and again built up until from the building material, which is of quite different composition, the specific secretion is produced. Now the cells of the glands in the stomach produce the secretion during a period of rest. They retain it until, by means of some sort of stimulation, they are made to give it up. We must not imagine that the act of secretion itself is a purely physical process. The material of which the secretion is composed does not exist in the cells in a condition capable of exerting the characteristic function. Probably during the secretion activity, a group of atoms is eliminated here and there and new combinations are effected. We do not yet know whether all these cells of a gland contain the same secretion, or not. All such assumptions are but speculations, without any experimental foundation. We mention them merely because the first glance at the results of Pawlow's investigations, which almost lead one to assume that the digestive glands are furnished with intelligence, must give one the impression that we are meeting with conditions here which are infinitely complicated, and which will be, apparently, inaccessible to further investigation. This is not really true. We have no doubt that from these experiments of Pawlow the first light will be shed upon the great obscurity which enshrouds the functions of the glands and their dependence upon nervous influences. To be sure, we are still far from the goal. Pawlow deserves our thanks for having at least pointed out to us the way this is to be attained.

We have up to now been concerned chiefly with pepsin, which is brought into activity by acid, and which is extremely sensitive to alkali. Now it is known that the pylorus part of the stomach secretes no acid. In spite of this fact, there is digestive power in the juice produced at this region of the stomach, as has been shown by experiments with an isolated blind sack in the pylorus. It is of much interest to find by the experiments of Pawlow and Parastschuk[1] that the proteolytic ferment of the pylorus digestive juice also requires hydrochloric acid to activate it. The activated juice shows a proteolytic and milk-coagulating action. It has been asserted that the pylorus portion of the stomach secretes a ferment which is active in alkaline solution.[2] If this be true, then we shall be forced to assume that a ferment other than pepsin is produced in this portion of the stomach. At present, however, we do not have sufficient ground for believing that there is actually a different ferment here, for it seems far more probable that the mucous membrane of the pyloric region, or the glands

[1] Z. physiol. Chem. **42**, 415 (1904).

[2] Karl Glässner: Hofmeister's Beitr. **1**, 24 (1904).

there, secrete pepsinogen, which comes into action only when brought into contact with the acid juices of the stomach. We do not yet have the means at our command for arranging the proteolytic ferments into classes with satisfactory exactness. We can, however, distinguish between ferments of the pepsin class and those similar to trypsin. The best way of establishing the class to which a ferment belongs, is, in this case, to allow it to act upon a polypeptide, and the results from the experiment are obtained most readily if we choose a polypeptide in the formation of which a difficultly soluble amino acid, e.g. tyrosine or cystine, takes part. Glycyl-*l*-tyrosine is decomposed in a short time by trypsin and similar ferments, but this dipeptide is not acted upon by pepsin. The secretion of the pyloric region behaves quite like the latter after it has been activated by hydrochloric acid.[1] It seems certain, therefore, that the ferment of the pylorus cells belongs to the pepsin group, as was assumed by Pawlow.

Now that we have become acquainted with the influence of the food, and its nature, upon the secretory relations in the stomach, it is time for us to turn to the action of the gastric juice upon the food itself. We have discussed this already at some length in considering the different classes of foodstuffs. Here we will merely repeat that pepsin, with the aid of hydrochloric acid, converts the albumins chiefly into peptones and in part to simpler cleavage-products; but on the other hand, a breaking down of the simplest cleavage-products, i.e. amino acids, cannot take place here, or at least only to a very slight extent. Again, the lipase causes the hydrolysis of a part of the fat, and in this way prevents, to some extent, the restraining influence which the fats have upon the gastric secretions. The carbohydrates, furthermore, may be decomposed somewhat while they remain in the stomach, but, to be sure, not by means of the ferment obtained from the gastric glands, but rather by means of the diastase from the saliva. This diastase, however, is destroyed on coming in contact with the acid of the stomach. Its period of action, therefore, depends upon the acidity of the gastric juice and the nature of the food. In case the food is in the form of a loose mixture which is easily moistened, it is evident that the action of the diastase cannot long continue.

Under the influence of the gastric juice, the food is changed into a pulpy mass known as the *chyme*. This consists, in part, of products formed from the decomposition of the food, and in part of unchanged food. Formerly it was thought that the muscular activity of the stomach had a great deal to do with the formation of this chyme. Doubtless in this way the contents of the stomach are thoroughly mixed and brought into intimate contact, layer by layer, with the gastric juice, but, on the other hand, this process takes place gradually, and not by means of violent muscular contractions, so that it is not right to speak of the food being

[1] E. Abderhalden and P. Rona: Z. physiol. Chem. **47**, 359 (1906).

kneaded in the true sense of the word. The innervation of the musculature of the stomach is partly provided by the vagus and partly by the sympatheticus. Since even the extirpated stomach contracts spontaneously, it has been assumed that the ganglion-cells in the walls of the stomach can cause this action.[1]

After the chyme is formed, the stomach has fulfilled its task. The pylorus then opens and the chyme enters the duodenum. This transference does not take place all at once. The time that the food remains in the stomach depends upon a number of factors. Purely physical conditions, such as the size of the food particles and the chemical nature of the contents of the stomach, both have an effect.[2]

Frequently we hear of a foodstuff being *easily digestible* or *difficultly* so without its being perfectly clear just what is meant by the term. As a matter of fact, it depends upon two factors. A food may be readily digestible, i.e., it can be readily acted upon by the ferments in the stomach, and yet appeal to us, according to its entire behavior, as difficultly digestible. This is due to the fact that although it may be easy for the ferments to act upon a food, still it may be converted into chyme only with considerable difficulty. The readiness with which a food may be converted into chyme should always be considered with regard to its digestibility. Digestion experiments in a test-tube cannot decide this. Many contradictions in theory and practice are to be traced to this point. Our present knowledge concerning the digestibility of various foods in the human stomach is still very vague.

As just mentioned, the stomach is not emptied all at once. It begins to be emptied very soon after the beginning of digestive activity. Thus when a dog is fed with meat, the first products of digestion appear in the duodenum after a few minutes. The stomach is emptied intermittently.[3] In feeding 100 grams of meat to a dog weighing 7 to 8 kilograms, all the chyme was emptied in the course of 2½ hours. It is difficult to get a correct idea of the time spent by the food in the stomach from such experiments. They are often very contradictory. We shall understand immediately why this is so, when we are told that Pawlow has proved that normally the opening and closing of the pylorus are regulated by the duodenum. If hydrochloric acid, or gastric juice, is constantly introduced into the duodenum through a fistula, a soda solution placed in the stomach will be retained during the whole course of the experiment. The period which normally follows the opening and closing of the pylorus

[1] Concerning the literature, see E. H. Starling: Ergeb. Physiol. (Asher and Spiro) Jg. I, Abt. **2**, 446 (1902). Extensive studies on the functions of the muscles of birds have been made by Mangold: Pflüger's Arch. **111**, 163 (1906).

[2] Cf. Moritz: Z. Biol. **42**, 565 (1901). von Mering: Kongress f. innere Med. Berlin, 1877 and Wiesbaden, 1893. A. Hirsch: Zentr. klin. Med. **47**, 993 (1892).

[3] Cf. Ludwig Tobler: Z. physiol. Chem. **45**, 185 (1905).

corresponds evidently to the time required by the alkaline juices of the intestine to neutralize the hydrochloric acid in the chyme. When this has been effected, then, reflexively, the pylorus is opened and a new portion of chyme passes out of the stomach. The suitability of such an arrangement is quite obvious. We shall see that the ferments of the pancreas can act only in neutral or alkaline solutions. If now the entire acid contents of the stomach were to be suddenly emptied into the intestines, then evidently the subsequent digestion would suffer.[1] In fact, only moderate amounts of chyme are to be found in the intestines of animals killed at various times after an abundant feeding. This is particularly remarkable when we compare the contents of the tightly stretched stomach at the beginning of digestion with that of the duodenum. The small portions of chyme as they leave the stomach are evidently at once further digested and absorbed. To the fat, also, has been ascribed an effect on the opening and closing of the pylorus. Enough has been said to show that the emptying of the stomach may take place with different degrees of rapidity according to the prevailing conditions. On the other hand, it enables us to understand why such contradictory statements are found in the literature concerning the time required. Almost all of the early investigators followed the course of the stomach's activity, by means of a fistula in the duodenum, in such a way that the chyme on leaving the stomach, in part at least, passed at once through the fistula opening, whereby naturally quite unusual conditions were created, leading to quite uncontrollable changes in the natural processes.

A question which has been much discussed is whether absorption begins to take place in the stomach. At present we can only answer this question in so far as we know that it is certain that the mucous membrane of the stomach does take up certain substances from the chyme. As soon as we possess further information concerning the amount and nature of the absorbed substances, we shall be able to close up this gap in our knowledge. Pure water is not absorbed perceptibly; but, on the other hand, aqueous solutions of sugar and peptone and of salts lose part of the dissolved substance and part of the water while they are in the stomach. The absorption of digested albumin in the stomach has been studied particularly carefully, but without its being possible to get any clear idea as to the extent that this takes place. We shall come back to this question of absorption when we speak of the functions of the remaining parts of the alimentary canal. We may, however, state in advance that it has not yet been found possible to refer these processes completely to physical or chemical laws.

[1] This fact must be considered in cases where there is an excessive secretion of gastric juice, and especially of hydrochloric acid. This tends to increase the time required by the stomach to empty itself.

Thus far we have concerned ourselves entirely with the stomachs of man and the carnivora; but, before leaving this part of the subject, we must pay some attention to a class of animals whose stomachs are much more complicated, namely the ruminants. The stomachs of these animals consist of four separate compartments connected with one another. The food at first passes into the *rumen*, or *paunch*, then into the *reticulum*, which is connected with the former by means of a wide opening. The reticulum itself has three openings. One, as just mentioned, leads to the paunch; a second to the third stomach, which is variously known as the *omasum*, *psalterium*, or *manyplies;* while the third opening connects the reticulum, also called the honeycomb, directly with the gullet. The psalterium provides the connection with the fourth stomach, the so-called *abomasum*, or rennet-bag. From the paunch and the reticulum, the food, which has already been mixed with saliva to some extent, is regurgitated, or thrown up into the mouth, in from 20 to 70 minutes after it has been first swallowed. This process is known as *rumination*, or *chewing the cud*. Each time only certain portions of the food are given up by the stomach. In the mouth the food is ground up extremely fine and kneaded together with saliva, after which it is swallowed again, and reaches, if it is already sufficiently pasty, the psalterium, through the so-called *œsophageal groove*. The latter leaves one side of the gullet at almost a right angle, and consists of a tube formed by parallel folds communicating directly with the psalterium. Only pasty and liquid materials can pass along this path. The solid and semi-solid constituents of the food fall from the gullet directly into the paunch and reticulum. A part of the food paste also reaches the psalterium through the narrow passage between it and the reticulum. In the psalterium the food is still more finely subdivided and intimately mixed. In the abomasum, the action of the stomach is completed, and, on the whole, the entire effect is similar to the process which takes place in other mammals. The first two divisions correspond to the cardiac portion of a single stomach, and the two latter to the pyloric end.

Finally, we must answer the question whether the stomach is an organ which is indispensable to life. This is not the case. The entire stomach has been completely extirpated from a number of dogs and the œsophagus connected directly with the duodenum without causing any disturbance in the health of the animal.[1] Recently the stomach has been successfully extirpated from human beings a number of times.[2] In no case have any symptoms developed which would indicate that the stomach is an organ

[1] Czerny: Beiträge zur operativen Chirurgie, Stuttgart, p. 141 (1878). M. Ogata: Arch. Anat. Physiol. **1883,** 89. Carvallo and Pachon: Arch. de physiol. 5 série T. **6,** p. 106 (1894).

[2] Langenbuch: Deut. Med. Wochschr. 1894, No. 52. C. Schlatter: Korrespondenzblatt Schweizer Aerzte **27,** 705 (1897).

indispensable to life, but these operations enable us to understand exactly what the duty of the stomach is in the economy of the whole organism. The stomach enables us to partake of our daily food within a relatively short time and at considerable intervals. It is to a certain extent a store-room. The stomach is also to be regarded as serving to protect the intestines. It prevents the injurious action of too hot or too cold foods. If the stomach has been removed, it is necessary to eat the food in small portions and at frequent intervals. The food must then be in a pasty condition before it is swallowed. It is interesting to find that if but a small piece of the walls of the stomach remain in the system after the operation, this enlarges and develops into a new stomach, which performs the functions, to some extent at least, of the original stomach.

LECTURE XXII.

THE FUNCTIONS OF THE DIGESTIVE ORGANS.

II.

From the stomach the food reaches the duodenum, and undergoes an energetic digestion. Here, as we have already repeatedly stated, the foodstuffs which in their composition are complex and unlike, as well as entirely unsuited for direct absorption by the tissues, are to a greater or less extent broken down into their simpler components. Thus complicated carbohydrates are transformed into the simplest sugar, the albumins into amino acids and polypeptides, and the fats eventually into fatty acids and glycerol. From these materials the body is able to construct the components of its tissues. Digestion serves not only to make the substances suitable for *absorption*, but, above all, for *assimilation*.

By means of this breaking down of the foodstuffs, the animal organism makes the cells of its tissues to a large extent independent of the nature of its food. It is to the cells a matter of indifference whether the food is of animal or vegetable origin; they will in all cases receive the same carbohydrates, the same fats and proteins from the blood. We may state in advance that evidently the walls of the intestine themselves play an important part in effecting the transformation of the separate foodstuffs. Within them takes place, according to our present knowledge, the building up of albumin and fat from the more simple components. Absorption takes place without doubt in proportion as this synthesis is accomplished. This fact makes it difficult to trace the exact relations of the foodstuffs to the intestine. Our knowledge ceases essentially with the taking up of the food by it. It might be thought that some idea of the complicated processes taking place in the intestine could be gained by causing, with suitable methods, an accumulation of the decomposition and synthetical products, e.g., in the examination of a surviving intestine. Up to the present time, however, such experiments have failed to give satisfactory results. The absorption of fats and their synthesis from the simple compounds has alone been followed to a certain extent by means of the microscope. With the proteins the relations are far more complicated. The walls of the intestine themselves consist chiefly of albumin. It is hard to tell what is new and what was originally present. As long as we are unable to differentiate sharply between the different albumins, there is practically no prospect of our being able to get direct proofs by means of

the paths already trod. We must not forget, moreover, that the action of the ferments, especially those of the cells, is extremely sensitively regulated. It is entirely dependent upon certain definite external conditions, such as, for example, the concentration relations. Every disturbance in this direction must force the entire course of the cell-work into other channels and quickly bring it to a halt. It is very important that digestion should be considered, at present, in a broad sense rather than to attempt to establish its significance in any definite direction. Only from this standpoint are we able to comprehend the nature of digestion in its completeness, and from thence new ways and new goals appear for future investigation in this infinitely complicated field.

In the duodenum, the chyme first comes in contact with the alkaline intestinal juices. The acid in the food mixture begins to be neutralized. This juice is partly obtained from glands placed in the mucous membrane at the beginning of the duodenum, and known as *Brunner's Glands;* but the so-called *Lieberkuhn's Glands* are more important. These are found in the mucous membrane of the entire small intestine. Even in the large intestine similar little glands are found, although these differ in the nature of the epithelium and in their functions from the corresponding glands in the small intestine.

The glands of Brunner have been considered by some as small pancreatic glands, while others have regarded them as similar to the glands in the pyloric region of the stomach. The work of Pawlow and Parastschuk [1] makes it seem probable that these glands yield a ferment which corresponds to pepsin. These investigators have shown, moreover, that, like pepsin, this ferment requires hydrochloric, or some other acid, to activate it. The secretion from these glands also has a milk-coagulating action. Here, in the same way as with regard to the juice of the pyloric region of the stomach, it is possible to show that Pawlow's assumption is correct.[2]

The secretion from the glands of Lieberkuhn has been the object of much careful investigation. It may be studied by the aid of a fistula, made in the small intestine. It has been shown that starving dogs produce no secretion, or at least but very little. Secretion sets in when the intestine is in any way irritated, whether by mechanical, chemical, or electrical means. Ingestion of food also causes the secretion. The secretion varies in different parts of the intestine. In the upper part it is less abundant than in the lower. The juice of the intestine reacts alkaline, and always contains sodium chloride and carbonate in apparently quite constant proportions. According to recent investigations, it contains a *fat-splitting* ferment,[3] and one with a slight *amyolytic* action. Furthermore,

[1] Z. physiol. Chem. **42**, 415 (1904).

[2] Abderhalden and Rona: Z. physiol. Chem. **47, 359 (1906).**

[3] W. Boldireff: Zentr. Physiol. **18,** 460 (1905).

an *invertase*, a *maltase*, and, in mammals, a *lactase*, have been found. Finally there is *erepsin*, already described, which has no direct action upon native proteins, with the exception of casein, but does act upon their hydrolytic decomposition products, the peptones.

The function of the mucous membrane of the small intestine is by no means restricted to the production of the intestinal juice. We shall soon see that substances are secreted by it which are of far-reaching importance for the functions of the pancreas and its ferment, trypsin.

The secretions produced by the Brunner and Lieberkuhn cells are unimportant compared with that of two more important accessory glands, the liver and pancreas. To be sure, this is not necessarily true of the physiological functions themselves, which in no case are to be judged entirely from the standpoint of quantity, but rather from that of quality. Particularly the more recent investigations have taught us that no matter how insignificant the function of any organ may appear, it must not be disregarded. All sorts of different processes are closely related to one another and influence each other reciprocally. Whether the particular link in the chain of the total processes is long or short is immaterial. We may well imagine that the secretion produced by the glands of Brunner is in many cases highly significant for the digestion of proteins. The pancreatic juice is not able, or at least only imperfectly, to attack connective tissue, for example, while pepsin in the presence of hydrochloric acid quickly accomplishes this. Now we know that when fat is present in the food the secretion of the gastric juice becomes considerably diminished, and it is very probable that in such cases the secretion from these intestinal glands is of great assistance.

One of the above organs, the liver, constantly gives up a peculiar secretion, the bile, which is continually passing through the bile-duct into the intestine. It should be mentioned at once that the formation of the bile is continuous, although the amount secreted varies. It continues during fasting, though in diminished amount. After eating, the secretion increases in amount; and, in fact, it has been found that the extent of the secretion depends, in part, upon the nature of the food. We shall soon come back to these relations.[1]

The bile, as it reaches the intestine, represents a mixture of the secretions of the liver-cells, the glands of the gall-bladder and the biliary passages. The latter yield mucous chiefly. The bile reacts alkaline to litmus. Its color varies in different animals and likewise in different individuals of the same species. In man the fresh bile is usually a golden yellow, but sometimes it has a greenish hue. It contains, besides salts, mucin and water, its own specific substances. These are the *bile-acids*, which are combined with alkali, and the *bile-pigments*. There are also constitu-

[1] Barbèra: Bull. della szienz. med. di Bologna (7) **9**, (1898).

ents which are found in other parts of the body as well. These are *cholesterol, lecithin, soaps, neutral fats,* and *urea.* Conjugated glucuronic acids have also been detected in the bile. The salts of the bile-acids are of chief interest to us. We shall come back to the bile-pigments when we come to consider the pigments of the blood from which the former result. That the bile-acids owe their formation unquestionably to the activity of the liver, is evident from quite a number of observations. If, for example, the liver be entirely extirpated from a frog, no more bile-acids can be detected subsequently in its tissues. If they were produced by other organs, the acids would be formed after the removal of the liver, unless it is to be assumed that the liver acts in conjunction with other organs in their production, and that it produces either the original stages or at least some essential stage in their formation. Although we are compelled to assume the existence of such reciprocal relations in a great many cases, still at present it may be regarded as proven that the liver is the sole place in which these bile-acids are formed. Any other assumption would appear less probable. In dogs also, it may be shown that the preparation of the bile is a function of the liver-cells. If the bile duct is ligated, there is first of all an accumulation of bile. Certain constituents of the latter then pass into the lymph, and are carried by means of the thoracic duct to the blood. Now if the thoracic duct be ligated as well as the bile duct, no more bile-acid can be detected in the blood.

The bile-acids belong to two groups; namely, the *glycocholic* and *taurocholic* groups. The members of the first group contain carbon, hydrogen, and nitrogen, and by their hydrolysis yield glycocoll and a non-nitrogenous acid; while those of the other group contain sulphur in addition to the above elements, and on hydrolysis they yield taurine, and similarly a non-nitrogenous acid. The constitution of the nitrogen-free acid which is contained in both groups of bile-acids, and which, moreover, appears to have a different composition in different bile-acids, has not yet been fully explained. In general, such an acid is known as *cholic* or *cholalic* acid. We will merely mention the fact that the acid has been assumed repeatedly to be related to cholesterol, but this relation has never been established satisfactorily.

The relative amounts of these two groups of acids vary according to the species of animal, and in fact one or the other group may be missing. *Glycocholic* acid, $C_{26}H_{43}NO_6$, is always present in human- and ox-bile. Besides this a second acid, *glycocholeic* acid,[1] is frequently found which differs from the first with regard to the "cholalic acid" which it yields; in this case the acid is known as *choleic acid.* The solubility relations of

[1] V. Wahlgren: Z. physiol. Chem. **36,** 556 (1902). O. Hammarsten: *ibid.* **43,** 109 (1904). H. P. T. Oerum: Skandin. Arch. Physiol. **16,** 273 (1904). C. Gundelach and A. Strecker: Ann. **62,** 205 (1847).

this last acid are different from the first cholic acid, and it has a higher melting-point. A *hyoglycocholic acid*[1] has been isolated from the bile of pigs.

Taurocholic acid[2] is found in the bile of man, carnivora, oxen, and a few other herbivora, and yields, on being boiled with acids or alkalies, *taurine* and *cholic acid*. It has the empirical formula $C_{26}H_{45}NSO_7$. In the bile of the goose the so-called *cheno-taurocholic* acid is found.[3]

In the bile of the shark, *Scymnus borealis*, Olof Hammarsten,[4] to whom our thanks are due for most careful investigations concerning the bile of different species of animals, found in place of the usual bile-acids, two ethereal-sulphuric acids which he called *scymnol-sulphuric acids*. They yield on hydrolysis, besides sulphuric acid, a non-nitrogenous acid, *scymnol*, which gives the characteristic color reactions of cholic acid.

As we have said, only one constituent of the bile-acids is understood in each case as regards its composition, and this is either glycocoll or taurine. These two substances originate, as we have already discussed in detail, from the proteins, and, in fact, glycocoll is recognized as one of the direct cleavage-products of albumin, while it is perfectly evident that taurine is formed from cystine.

The constitution of cholic acid is not yet clearly established. Mylius[5] has succeeded in obtaining from it a monobasic hydroxy-acid with one secondary and two primary alcoholic groups. By oxidation of cholic acid, the so-called *dehydrocholic* acid $C_{24}H_{34}O_5$ is formed; while by more energetic oxidation *bilianic* acid $C_{24}H_{34}O_8$ is obtained, perhaps more correctly a mixture of *bilianic* and *isobilianic acids*. By the oxidation of bilianic acid, the so-called *cilianic acid*, $C_{20}H_{28}O_8$, is formed. On reduction, cholic acid yields desoxycholic acid, $C_{24}H_{40}O_4$. According to our present knowledge, we can give to cholic acid the following formula:

$$C_{20}H_{31}\begin{cases}CH(OH)\\(CH_2OH)_2\\COOH\end{cases}$$

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[1] Severin Jolin: Z. physiol. Chem. **12**, 512 (1888); **13**, 205 (1889).

[2] Concerning the preparation of pure taurocholic acid, see O. Hammarsten, Z. physiol. Chem. **43**, 127 (1904), and Stefan Tengström: *ibid.* **41**, 210 (1904).

[3] Heintz and Wislicenus: Poggendorff's Annal. **108**, 547 (1859).

[4] Z. physiol. Chem. **24**, 323 (1898).

[5] Cf. Strecker: Annal. **65**, 1 and 130 (1848); **67**, 1 (1848); **70**, 149 (1849). F. Mylius: Ber. **19**, 369 and 2000 (1886); Z. physiol. Chem. **12**, 262 (1888). P. T. Cleve: Compt. rend. **91**, 1073 (1880). Olof Hammarsten: Ber. **14**, 71 (1881). Lassar-Cohn: *ibid.* **32**, 683 (1899). Z. physiol. Chem. **17**, 607 (1893). Fritz Pregl: Pflüger's Arch. **71**, 303 (1898); **72**, 266 (1898); Sitzber. kaiserl. Akad. Wissensch. in Wien, Math. naturw. Klasse, **111**, Abt. II b. October, 1902, and Z. physiol. Chem. **45**, 166 (1905). G. Bulnheim: Z. physiol. Chem. **25**, 296 (1898).

Choleic acid has also been oxidized, but this has not served to explain its constitution. We may mention, in passing, that a cholic acid described as *fellic acid*[1] $C_{23}H_{40}O_4$ has been obtained from human bile. In the polar bear another cholic acid has been isolated by Hammarsten[2] which he designated as *ursocholeic acid*, $C_{19}H_{30}O_4$ or $C_{18}H_{28}O_4$.

Our present knowledge does not tell us much concerning the formation and destiny of these cholic acids in the animal organism, and we are forced to rely upon assumptions. It is indeed perfectly possible that they are related to cholesterol, although this has never been established positively.

The composition of the bile varies not only in amount but also as regards its composition. We shall give a few figures showing the relative amounts of the different constituents. In 1000 parts of bile from the hepatic duct there were found to be present:[3]

Solids	25.20	35.26	25.40
Water	974.80	964.74	974.60
Mucin and pigments	5.29	4.29	5.15
Alkali bile-salts	9.31	18.24	9.04
Taurocholates	3.03	2.08	2.18
Glycocholates	6.28	16.16	6.86
Fatty acids from soaps	1.23	1.36	1.01
Cholesterol	0.63	1.60	1.50
Lecithin	0.22	0.57	0.65
Fats	0.22	0.96	0.61
Soluble salts	8.07	6.76	7.25
Insoluble salts	0.25	0.49	0.21

Of the inorganic salts present, sodium chloride predominates. Sulphates and phosphates are present only in small amounts. The bile from the gall-bladder shows a somewhat different composition from that taken directly from the bile-ducts. This is due to the fact that while the bile remains in the bladder it becomes thickened, owing to the absorption of some of the water, while at the same time mucin and other substances are given up by the mucous membrane of the bladder. The following analyses show the difference in the percentage composition of the bile from these two sources:[4]

[1] G. Schotten: Z. physiol. Chem. **11**, 268 (1887). Lassar-Cohn: Ber. **27**, 1339 (1894).

[2] Z. physiol. Chem. **36**, 525 (1902).

[3] Olof Hammarsten: A Text Book of Physiological Chemistry (Mandel), 1908, p. 327. Cf. also Ergeb. Physiol. (Asher and Spiro) **4**, 1 (1905), and Z. physiol. Chem. **32**, 435 (1901).

[4] Olof Hammarsten: Nova acta. Reg. Soc. Upsal, Serie III, 1894.

	Liver-bile.	From Gall-bladder.
Solids	2.06	16.02
Water	97.94	83.98
Mucin and pigments	0.28	4.44
Alkali bile-salts	0.85	8.72
Taurocholates	0.11	1.93
Glycocholates	0.74	6.79
Fatty acids from soaps	...	1.06
Cholesterol	0.08	0.87
Lecithin	0.03	0.14
Fats		0.15
Soluble salts	0.80	0.30
Insoluble salts	0.02	0.24

The part played by bile in the animal organism has been variously estimated at different times. Some have even regarded it as an excretion. According to this view, the bile serves merely to carry away the by-products which are formed as a result of the activity of the cells. We know that the liver plays an important part in the metabolism of the animal organism. Important decompositions and syntheses are constantly taking place in its cells. Its position between the blood-vessels of the viscera and those of the rest of the body make it easy to recognize its numerous important functions. We have spoken of the position it occupies with regard to carbohydrate metabolism, and have seen that at one time the liver cells construct glycogen from glucose molecules, while at another time it decomposes the latter into its constituents. Many discoveries indicate that the liver plays an important part in the transformation of fats and proteins into carbohydrates. Even in the metabolism of albumin it assumes a central position. In it is effected the formation of urea and of uric acid. The liver captures the ammonia set free in the alimentary canal and in the intestines themselves in order to make use of it in various ways, partly for the formation of urea, and partly for the neutralization of acid. The liver also stores up many substances injurious to the organism, or at least foreign to it. This is shown by the large amount of iron which accumulates here when large quantities of this element are taken into the system, and by the fact that many other substances are found in it which are otherwise foreign to the organism. The liver also effects the combination of many substances injurious to the system with sulphuric acid and glucuronic acid. It is indeed possible that in these processes, which by no means include the entire functions of the liver, waste products are constantly being formed which the cells of the liver no longer have any use for, and are consequently given up to the outside. This idea is supported by the fact that certain substances found in the bile are not indifferent to the organism. Thus we know that

the salts of the bile-acids lessen the frequency of the pulse. This is due to their action upon the heart. The latter is first of all stimulated, and for a short time there is an acceleration of the heart action, which, however, soon becomes retarded. Respiration also becomes less frequent. Thus we found in describing icterus, which results from the restricted discharge of the bile into the intestines, what severe pathological conditions appear if the secretions of the liver-cells are compelled to be eliminated through the kidneys, being carried thither by means of the lymph and blood-vessels. The fact that the bile undoubtedly plays an important part in digestion does not necessarily contradict any such assumption. There may be some adaptation here. It would not be altogether remarkable if we should find that the animal organism makes use of a definite function for different purposes. The secretion of bile does not necessarily assume a peculiar position because it has not yet been possible to find secretory nerves which govern the secretion. The liver behaves in this respect like the kidneys. The secretion of bile, according to this, is to be compared to the formation of the urine. In the case of all the other glands that we have studied up to this point, we have found secretory nerves. We should not, however, lay too much stress upon the fact that we have never found any nerves which in any way govern the secretion of the liver, for it was but a short time ago that such nerves were positively proved to exist for the stomach and pancreas. It is not altogether impossible that such nerves will be found in the case of the liver and possibly for the kidneys as well. Certain contradictory observations, and much that is not in accordance with the theory at present accepted concerning the secretion of the bile and urine, will at once become explicable if nerves can be found governing the action of these organs.

The experiments of Pawlow and his school have brought forward many observations showing the close relation between digestion and the secretion of bile, so that we are obliged to regard bile in the light of a specific secretion of the liver-cells. The constituents of bile are by no means waste-products of cell-metabolism, but they are much rather to be regarded, according to their formation and their entire functions, as true secretion products. It is indeed possible that the formation of the bile is accomplished by means of definite cells. It is also conceivable that their formation is, nevertheless, bound up with the intermediate metabolism in the liver to the extent that the cells of the liver utilize in a specific way certain decomposition products. The fact that the flow of bile is continuous is not contrary to any such hypothesis. Our knowledge concerning the destiny of the bile is still very limited. We do not know whether a part of it is constantly being resorbed. In fact, frequently the biliary cycle has been spoken of under the assumption that bile is constantly being resorbed and again secreted. It has also been observed that the

bile and its constituents, namely the bile-salts, accelerate the secretion of bile. Experiments carried out in this direction leave it uncertain for the present as to what significance we shall give to this resorption process.

Since it was not known precisely what rôle the bile played in digestion, it has been assumed to be quite different by different scientists. Some have held that the bile had an antiseptic action. It had been observed that animals with a biliary fistula showed increased putrefaction in the intestines. Direct experiments upon the bile itself have shown that it indeed tends to restrain the action of certain bacteria, but that it is by no means a good antiseptic agent. Furthermore, if fat be entirely excluded from the food, or only a limited amount of it given to animals with such a fistula, the intestinal putrefaction is not greater than under normal conditions. It was, therefore, not so much the absence of the bile that caused the observed putrefaction, but rather the faulty absorption of the fats.

Bile has, further, been thought to have an influence upon the peristalsis of the intestines. The extent of such action, under normal conditions, is still undecided. It has also been observed that if bile is added to a peptic digesting fluid a precipitate will be formed at once. Now normally the acid chyme passes out of the stomach mixed with pepsin into the duodenum. It might be thought that the action of the pepsin, which is no longer desired in this part of the intestine, is stopped by the bile precipitating pepsin with the albumin and its higher cleavage-products. It has, however, never been possible to establish satisfactorily the formation of such precipitates in the intestines, so that at present we are hardly justified in assuming that this test-tube experiment represents the normal condition. At the same time it does seem probable that the bile prevents the further action of the pepsin.

We have already touched upon one quite essential function of the bile, namely its rôle in the absorption of fats. We have seen that a large part of the fats, or perhaps even all, is decomposed into its components the fatty acids and glycerol. The bile is an excellent solvent for these fatty acids and the soaps (the salts of these acids), and on account of this fact a great importance has been ascribed to the bile in assisting the absorption of the fats and their cleavage-products. Before this action of the bile had been verified by direct experiment, it had been observed that if, for any reason, the flow of bile into the intestine was prevented, the fæces showed a pronounced pale color, and when a considerable amount of fat was present in the food it was at once obvious that undigested fat was present. Other facts indicate a faulty absorption of the fats in such cases. In animals with biliary fistula, where the bile was taken away, animals decreased rapidly in weight, although fed with the same nourishment that had previously agreed with them. It is clear that the loss of a

material as rich in caloric power as the fats will be quickly felt through the entire organism. If the nourishment of the just-mentioned animals was so chosen that they obtained sufficient calories from albumin or carbohydrates, then there was no further loss in weight. The absorption of the fat in such cases is not entirely prevented, but merely restricted. That an excessive amount of fat in the nourishment can act injuriously upon the absorption and digestion of albumin is clear, for the fat particles, from their mechanical action, can prevent, or make more difficult, the action of the digestive juice upon the proteins.

The bile not only takes part in the solution of the fatty acids and their alkali salts in the absorption of the fats, but its significance reaches much farther. We must remember that the fat is hydrolyzed by the aid of a particular ferment, called steapsin or lipase. This ferment is not present originally in the pancreatic juice as such, but in the form of a zymogen. The latter is activated by the bile. The bile also increases the fat-splitting action of the pancreas-lipase in a way which has never been satisfactorily explained.[1] Fürth and Schütz[2] have proved that the cholic acid component of the bile salts is the cause of this marked acceleration in the digestion of fats.

An exact idea concerning the conditions governing the secretion of the bile and its function was first made possible when Pawlow[3] instead of making use of a biliary fistula placed the entrance of the bile-duct into the duodenum on the outside of the body. He cut out the mouth of the bile-duct together with a piece of the intestinal membrane and sewed it into the wound in the body. He was then able to study the secretion of the bile under normal conditions. The observations made upon animals in which the bile flowed out of a fistula in the gall-bladder naturally could not represent normal conditions, for the bladder represents a reservoir for the bile, so that when it is constantly being emptied to the outside, the secretion of the bile must take place abnormally.

Pawlow succeeded in showing at once from the amount of bile flowing through this normal opening that the bile secretion depended upon the taking of food. He also showed the influence of the nature of the food upon the secretion. Thus we know that meat causes a particularly intense flow of bile, while for the carbohydrates a slight amount suffices. The fats stand intermediate between meat and carbohydrates. The nature of the secretion is also regulated by that of the nourishment. The maximum flow of bile does not correspond to the maximum amount of food, and it appears that there are specific differences here. The purpose

[1] M. Nencki: Arch. exper. Path. Pharm. **20**, 367 (1885).

[2] Zentr. Physiol. **20**, 47 (1906); Hofmeister's Beitr. **9**, 28 (1906) — cf. A. S. Loevenhart and C. G. Souden: J. Biol. Chem. **2**, 415, (1907).

[3] Pawlow: Le travail des glandes digestive (Pachon et Sabrazès). Paris, 1901.

of this adjustment to the different foodstuffs is easy to understand, when we point out that the bile assists the action of the pancreatic juice by accelerating the action of the ferments. It affects the fat-splitting ferments most, but it also increases the action of trypsin and diastase. Bile, consequently, is concerned not alone with the digestion of fat, but influences considerably that of the other foods. It assists the transference of the seat of digestion from the stomach to the intestines, probably by preventing the further action of pepsin.

The functions of the bile are not, in general, considered to be as important as would seem probable from the researches of Pawlow. It has been found, in fact, that the bile may be entirely excluded from the intestines without any serious disturbance taking place, provided the food be properly chosen. It would be wrong to conclude from this that the bile is of little importance in the digestive process, for even with complete extirpation of the pancreas, digestion does not cease entirely. The animal organism possesses ways and means of replacing, to some extent at least, lost functions. The ferments of the pancreatic juice can indeed perform their work without the aid of the bile, but it requires more time. Even then a part of the fat will be absorbed. There are no exact experiments to indicate the effect of the loss of the bile upon the metabolism as a whole. Its absence is not without influence, because it is then necessary to choose the food more carefully. Food rich in fat must be avoided. We merely wish to emphasize the fact that it does not follow because an animal is able to exist without a certain function, and even be kept in good health, that the function is perfectly dispensable. Thus we should make a serious error if we reasoned that because a man could live without a stomach that this organ occupies physiologically a subordinate position. We must accustom ourselves to have in mind the working together of all the organs, and never follow the functions of a single organ only under certain special conditions but under as many different conditions as possible, and especially under those which occur normally. Only in such cases are we able to form a proper judgment as to the relative value of the functions of a given organ.

Now that we have traced the transition between the digestion of the stomach and that of the intestines, we must turn our attention especially to the functions of the pancreatic juice. This contains, as we have stated repeatedly, three ferments, — trypsin, steapsin, and a ferment which has a diastatic action. While it has not yet been established positively that the last ferment is secreted originally in a zymogen condition, this is surely the case with the two other ferments, trypsin and steapsin. Trypsinogen is, according to the important observations of Pawlow, activated by a substance which occurs in the intestinal juices. Pawlow called this substance *enterokinase*. It is very likely that this substance itself belongs

to the group of ferments. It is possible to activate, by means of very small quantities of enterokinase, large amounts of trypsinogen. It is evidently to be considered as a secretion of the intestinal membrane. Intestinal juice, obtained through a fistula, may be added directly to the inactive pancreatic juice. A preparation of enterokinase may also be obtained by scraping off the superficial layers of the intestinal membrane and preparing an extract from these scrapings. The action of the enterokinase may be well shown by taking some pancreatic juice which has never been in contact with the walls of the intestine, so that the ferments contained in it are inactive,[1] and placing some fibrin in it which will not dissolve. Now if we add to another portion of the same juice a few drops of intestinal fluid, or of the extract prepared from the intestinal walls, it will be seen that a piece of fibrin in it dissolves at once. It is not yet perfectly clear how this enterokinase acts. It might be even assumed that there is not a true activating in this case, but that the enterokinase is itself a proteolytic ferment and begins the cleavage of albumin. We have already seen that pepsin attacks the albumin molecule in an entirely different place than does trypsin. It is possible that the enterokinase continues the work of the pepsin and gives up cleavage-products to trypsin which the latter is capable of acting upon. We might also assume that enterokinase attacks trypsinogen itself, modifying it in some way so that trypsin now has certain groups free whereby it can react with albumin, or its cleavage-products. One might be tempted even to assume that between trypsinogen and enterokinase a union takes place and that active trypsin is thereby formed. If this assumption were correct, then of course the enterokinase and trypsinogen must always be active in quite definite proportions. This does not appear to be the case, however, for it is possible by means of but very little enterokinase to activate a great deal of trypsinogen. We have here again a reciprocal effect of different organs. The pancreas sends out a zymogen, and the cells of the intestinal membrane form the activator. Pawlow and his student Sawitsch [2] have shown by very pretty experiments that the secretion of the intestine does not invariably contain enterokinase, whether the pancreatic juice reaches the intestine or not. If a canula be introduced in an intestinal fistula, then this mechanical irritation causes a secretion of intestinal juice. Only a small amount of enterokinase is present in this juice, which consists chiefly of water. In the course of a few hours the intestinal fluid, which is now scanty in amount, contains almost no enterokinase. If now a few cubic centimeters of pancreatic juice be introduced into the intestinal canal, then a juice flows

[1] It should be mentioned here that apparently the ferments in the pancreatic juice always contain a small amount of trypsin in the active form. Cf. B. P. Babkin: Ber. kaiserl. Militärärztl. Akad. St. Petersburg **11**, Nos. 2 and 3, 93 (1904).

[2] W. Sawitsch: Soc. méd. russes St. Petersburg (1900–01).

out which is rich in enterokinase. Boiled pancreatic juice, however, has no such effect. The secretion of the intestinal juice is, according to this, by no means such a simple process as has ordinarily been assumed. Its composition is determined by at least two factors which are largely independent of one another. The production of enterokinase, and the formation of the other constituents of the secretion produced by the intestinal membrane and its glands, are distinct processes. The intestinal juices have a favorable action upon the digestion of albumin, not only by reason of the enterokinase, but, according to many observations, also on account of the other ferments in the pancreatic juice. These juices have on the whole a quite similar effect to the bile.

In describing the secretion of the stomach, we saw that the amount and composition of the gastric juice are dependent upon various external influences, and that above all psychic influences play an important part. Is the secretion of the pancreatic juice similarly affected? The following observations give us some light with regard to this important question. In the case of herbivora in which the digestion is, so to speak, a continuous process, the secretion of the pancreatic juice takes place continually. In carnivora, it is possible to trace at once some connection between the introduction of food and the subsequent digestion.

Pawlow called attention, in the first place, to the following experiment: If a few drops of 0.5 per cent hydrochloric acid are introduced into the stomach of a dog having a pancreatic fistula from which only a few drops of pancreatic juice are flowing in a minute, there is then an increase in the secretion after a short time. If instead of acid a little lime-water is introduced into the dog's stomach, the contrary effect is obtained. Phosphoric, lactic, citric, and acetic acids each have the same effect as hydrochloric acid. The concentration of the acid, moreover, greatly influences the secretion, as the following experiment shows:[1] 250 cubic centimeters of HCl of the following concentrations were introduced into the stomach of a dog:

	0.5%	0.1%	0.05%
Cubic centimeters of pancreatic juice secreted in one hour	70.8	....	....
	79.5	25.7	....
	82.5	26.8	20.5
	89.4	32.5	....

The pancreatic gland reacts promptly with the acid, and even with concentrations which barely have an acid taste. Other irritants, such as pepper and mustard, are without influence. Acid, therefore, is to be regarded as exerting a specific effect upon the pancreas. Naturally the

[1] Pawlow: Vorlesungen etc., *op. cit.* p. 150.

gastric juice has the same effect as a pure acid solution of the corresponding concentrations. The following experiment is important: If soda or lime-water be introduced into the stomach of an animal in the midst of the process of digestion, there is a rapid diminution in the amount of secretion from the pancreas.

We have here a new link in the chain representing the mutual dependence of one organ upon another. The pancreas regulates its activity according to that of the stomach, and is governed chiefly by the acid produced in the latter. The next question that arises is how the hydrochloric acid of the stomach effects the stimulation of the pancreatic gland. There are two possibilities. It may be that the acid stimulates the peripheral end-apparatus of the centripetal nerves in the mucous membrane, or that it acts upon the nerve center of the secretory cells of the pancreas, or upon the cells themselves, after it has been taken up by the blood. The latter method of action is improbable for a number of reasons. Pawlow showed that the acid taken up by the blood could only have an indirect action; namely, by diminishing the alkalinity of the blood. Now, normally, the alkalinity of the blood is increased by the production of hydrochloric acid, and even in case of an absorption of hydrochloric acid, it remains higher than usual during the period of digestion. Direct experiment confirms this view, for, on the one hand, it is not possible to stimulate the secretion of the pancreatic gland by means of introducing hydrochloric acid into the rectum, while, on the other hand, the action of the hydrochloric acid is still felt even when its passage out of the stomach is prevented.[1]

Now how shall we explain the action of the hydrochloric acid? Pawlow brings out the following points: — Trypsin reacts best in an alkaline solution, but is still active in a neutral or even barely acid solution. As soon as the amount of acid becomes in any way considerable, the action of the trypsin is prevented. Now the pancreatic juice always contains an abundance of alkali by means of which the acid in the chyme is neutralized. The more acid the stomach produces, the more acid reaches the intestine with the chyme, and the more alkali is required to combat the injurious effect of the acid. The fact that the secretion of the pancreas is governed by that of the stomach tends to equalize the conditions. If the amount of pancreatic juice secreted were independent of the hydrochloric acid in the chyme, then it would often happen that the trypsin would be made inactive, and the activity of the pepsin, which under normal conditions is prevented by the neutralization of the acid it requires, would continue in the intestine. The whole arrangement may be traced in the cycle of common salt, somewhat as follows: — The cells of the stomach prepare hydrochloric acid from the sodium chloride in the blood. The

[1] L. Popielski: Inaug. Diss. St. Petersburg (1896); Zentr. Physiol. **10**, 405 (1896); Pflüger's Arch. **86**, 215 (1901), and Zentr. Physiol. **16**, 43 (1903).

more hydrochloric acid there is produced, the greater becomes the alkalinity of the blood. This excess of alkalinity is given up by the blood to the cells of the pancreas which employ it in the production of the pancreatic juice. With the pancreatic juice, this alkali, chiefly as sodium carbonate, flows into the intestines, and meets there the hydrochloric acid from the stomach. Here again common salt is formed which may enter into the circulation anew. At the same time, by means of such a mechanism the alkalinity of the blood varies only within narrow limits. We should not, however, imagine that the process takes place in such a simple form that the cells of the pancreas are immediately brought into activity by the increased alkalinity of the blood, which, in turn, is caused by the production of hydrochloric acid in the stomach. Plausible though such an assumption may be, it does not correspond with the results of experimental research. The production of acid does not have such a direct influence upon the activity of the cells in the pancreas. We must remember that hydrochloric acid introduced from without, also effects the production of the pancreatic juice. In the last case the alkalinity of the blood is diminished rather than increased. Although the above-described salt-cycle appears to be a very suitable arrangement, it does not, on the other hand, stand in direct connection with the action of the acid upon the function of the pancreatic gland. We must, on the contrary, conceive this to be due to some phenomenon brought about by the action of the acid upon the membrane.

We shall soon come to a very important observation of Bayliss and Starling which will shed considerable light upon the nature of the action of the hydrochloric acid.

It is interesting to find that even the composition of the pancreatic juice is adjusted to that of the acid in the chyme, for, as Walther [1] has showed, the amount of organic material in the former is regulated by the amount of acid in the latter. The juice produced by hydrochloric acid alone contains less organic material and more alkali than the normal. Here again we meet with the same conditions as in the secretion of the gastric and intestinal juices. Likewise the formation of the pancreatic juice is not that of a simple substance. It must not be thought that the cells of the pancreas always yield one and the same secretion. At one time the juice is rich in ferments, and at another time alkali predominates. For the present we do not know whether this is due to the fact that cells are influenced differently by various kinds of nervous stimulation, or whether particular cells are provided with quite definite functions. The fact that even the juice rich in alkali, which is produced by the action of acid alone, always contains ferments, makes it seem probable that the action of the individual cells is governed by the nature of the stimulation it receives, and that it is

[1] Inaug. Diss. St. Petersburg (1896).

hardly right to believe that certain cells produce the ferments while others merely give up salts.

At all events, in considering the work of digestion, we are constantly meeting with an extremely sensitive means for regulating the work of the cells. They do not always act in the same way, but adjust their action to the prevailing conditions. In considering the functions of the gland-cells we gain considerable insight into the activity of the cells of the animal organism in general. We are led to infer that even the individual cells of the body are to a considerable degree dependent upon one another. They adjust their work in the same way as the gland-cells, to the given conditions. To be sure, it is perfectly possible, and in fact most probable, that those cells of the body which are not directly connected with the work of the intestine, are much more regular in their activity than the cells of the intestine and the associated glands which are constantly meeting with new conditions. The intestine forms a solid barrier between the heterogeneous compounds in the food and the homogeneous building-material for the blood and tissues, the composition of which has been established by the entire development of the given animal species. The destructive activity of the digestive ferments, together with the syntheses taking place in the intestine, enables the cells of the body to work within certain limits always under the same conditions. At the same time, the greater demands which are now and then placed upon an organ, influence the cell work quite specifically.

The activity of the pancreas is not dependent upon the acid content alone of the food as it reaches the duodenum. It has been found that fats, likewise, have an effect. We have already seen that such food diminishes the amount of gastric juice secreted. The secretion of the pancreatic juice cannot then, as in the case of meats, be influenced by an increased secretion of hydrochloric acid. Fats, on the contrary, stimulate directly the secretion of pancreatic juice. This may be shown by means of a dog provided with both gastric and intestinal fistulas.[1] If after waiting until there is practically no gastric secretion, olive oil is allowed to flow into the stomach, then the slight amount of gastric juice secreted will have an alkaline reaction. At the same time there will be a marked increase in the amount of pancreatic juice. It is questionable whether the fats, and the soaps produced from them, have the same point of attack as the hydrochloric acid.[2]

It has proved very difficult to ascertain whether the secretion of the pancreas is influenced by the same chemical substances as that of the stomach. This could be answered satisfactorily only when there was no opportunity given for the hydrochloric acid itself to exert a stimulation. Under such

[1] N. Damaskin: Verhandl. Gesellsch. russ. Aerzte, St. Petersburg (1896).

[2] B. P. Babkine: Arch. des Sciences biol. **11**, No. 3 (1905).

conditions, it was found that even water is to be regarded as a direct stimulant of the pancreas. The extractive substances from beef, on the other hand, did not cause any stimulation. Quite recently the influence of alcohol upon the pancreatic secretion has been studied,[1] and in this case it was found that, while the amount of the secretion was augmented, the juice then had less digestive power. Alcohol also appears to have a direct action upon the ferments or their antecedents. If alcohol is added to pancreatic juice, then the digestive action of the latter upon starch and albumin is much lessened, while on the other hand the action upon the fat-splitting ferment is favorable.

It was highly important to establish the fact that the *psychic factor* also played a considerable part in the work of the pancreas. The vagus provides this organ as well as the stomach with secretory nerves. Furthermore, it is also claimed that the splanchnic sends fibres to the pancreas. It was extremely difficult to determine whether the secretion of the pancreatic gland was effected by a fictitious meal, and for the following reasons: We have seen that the secretion produced by the membrane of the stomach, and its glands, is greatly dependent upon psychic influences. A subsequent increase in the amount of pancreatic juice secreted may, therefore, take place on account of the increased acid production in the stomach; i.e., in this case the pancreas would be merely indirectly affected by the fictitious meal. Now we know that the secretion of the stomach does not take place at once, but only after a latent period of about four and one-half minutes. The pancreatic secretion similarly begins two or three minutes after it has become stimulated by acid. It was found that the augmented pancreatic secretion resulted within two or three minutes after the beginning of the fictitious meal, so that from this the conclusion may be drawn that the gland is directly influenced psychically. It is important that the pancreatic gland, in spite of its dependence upon the other organs, especially the stomach, still has a considerable amount of independence, so that even in the absence of stimulation from the stomach, it can perform its functions. Experience gained from day to day teaches us that if, for example, there is an insufficient amount of hydrochloric acid formed in the stomach, digestion is not prevented, but still progresses to a quite remarkable extent.

We must in addition consider a very important discovery for which we will have to thank two Englishmen, Bayliss and Starling.[2] They showed that by means of 4 per cent hydrochloric acid a substance could be extracted from the mucous membrane of the intestine which, when introduced into the circulation, increased the flow of pancreatic juice. They called this substance *secretin*. They have assumed that this substance is not present

[1] A. Gizelt: Zentr. Physiol. **19**, 769 (1906).

[2] **J.** Physiol. **30**, 61 (1903); Proc. Roy. Soc. **73**, 310 (1904).

as such in the intestinal membrane, but that its antecedent, *prosecretin*, is there, and becomes changed into secretin on being acted upon by acid; i.e., it may be set free in this way from some other compound. It might also be assumed, of course, that the prosecretin undergoes an atomic rearrangement in the molecule. Now how shall we regard the action of the secretin under normal conditions? We must remember that Pawlow found that the hydrochloric acid from the stomach stimulated the secretion of pancreatic juice. We have already shown how hydrochloric acid, directly or indirectly, by altering the alkalinity of the blood, can excite into activity the pancreas, and have left it open as to how the acid acts upon the intestinal membrane. The work of Bayliss and Starling may perhaps serve to explain how the hydrochloric acid can influence the pancreatic gland. Evidently it is constantly changing prosecretin into secretin. As quickly as the latter is formed, it is taken into the circulation, and now acts in some way upon the gland. It seems most probable from certain observations that secretin affects the blood-vessels in the pancreas and increases the circulation. This does not necessarily imply that there is not some specific action as well. This would seem quite likely from the fact that secretin stimulates only the pancreas to any extent. Now this fact gives to the hydrochloric acid of the stomach an entirely new significance. Not only does this aid us in our knowledge concerning the action of acid upon the intestinal membrane, but we obtain, at the same time, new prospects for further investigations concerning the cell-work of the glands and tissues. Even though we may be a long way from being able to understand the entire chain of processes, from the formation of the hydrochloric acid in the stomach to the production of the secretion on the part of the pancreas, and understand the phenomena only approximately, still we are justified in hoping from the work of Pawlow and of Bayliss and Starling that in following the paths now broken it will not be very long before one link after another will be added until finally the complete chain is forged. To be sure, there remain countless problems to be solved. We should like to know exactly what prosecretin is, and to what class of chemical compounds it belongs.[1] The fact that it is not a ferment is shown by its being unchanged by moderate heat. The intestine, therefore, concerns itself not only with the absorption and assimilation of the food, but takes part to a considerable extent in the digestion itself. The anatomical evolution of a unit from the intestine and its accessory

[1] Popielski, Zentr. Physiol. **19**, 801 (1906), has recently proved that unquestionably HCl also reflexively influences the secretion of the pancreas by its action upon the intestinal membrane. He believes that secretin belongs to the group of peptones. If this be true we have here a case of one of the products of digestion acting upon this pancreatic secretion. The discovery of Bayliss and Starling will of course only receive its full value when it is possible to isolate the secretin, and study by itself the action of HCl upon it.

glands, the liver and the pancreas, also to a certain extent corresponds to the physiological significance. The work of digestion is not entirely relegated to these glands, but the intestines help to a considerable extent.

From what we know concerning the work of the stomach, and the intestine with its accessory glands, we can readily understand at how many places the total work of these organs may be disturbed, and how many disturbances may result from the loss of a single function. Let us imagine, for example, that the stomach fails to secrete hydrochloric acid. First of all the food will not be utilized in the system to so good an advantage. To be sure, our knowledge of cookery enables us to overcome many such difficulties. If we were compelled to rely upon food in its original condition, then the effect of the loss of hydrochloric acid would be far more pronounced. Thus, for example, connective tissue is scarcely attacked at all by trypsin, while it is readily digested by means of pepsin in acid solutions. Thus the albumins present in such tissue would reach the intestine in an undigested condition. An increased secretion of trypsin would be required on account of the deficient preliminary digestion. The stimulation usually brought about by means of hydrochloric acid, however, would not take place. Thus one disturbance follows another. It is not safe to assume that in such cases the pancreas would entirely fail to undergo any stimulation. We have seen, on the contrary, that it is a fairly independent organ, and may be stimulated by fats and by water as well as by psychic events.

The knowledge of all these mutual relations with regard to the most varied functions of different organs at once explains the therapeutic measures that are taken in case of stomach trouble, whether it be on account of nervous or organic disease. Now we understand how the so-called *stomachics* have an effect, and why under some conditions hydrochloric acid itself is introduced into the stomach. On the other hand, it becomes clear to us how cautious we should be with the use of alkalies. They serve not only to neutralize the gastric secretion, but they also lessen the secretion of the pancreatic juice. We are now able to view in a clear light the functions of the intestine in the economy of the animal organism. It seems to us not at all impossible that a faulty function on the part of the intestine in any one of its various functions has an influence in much greater measure than is ordinarily assumed upon numerous pathological processes. Not the least cause of diseases of metabolism is an anomally in the complicated processes of the intestine. In the intestine the cleavage-products of proteins, the fats, and certain other compounds, are again welded together. A faulty synthesis, or a building-up in the wrong direction, must immediately have its effect upon the general metabolism, for the ferments in the cells are only adjusted to react with quite definite compounds. We make these suggestions merely to show what a

deep significance is to be ascribed to the intestine in the general metabolism of the organism.

We have up to this point merely considered the pancreatic juice as such, and, with the exception of its alkali content, have paid little attention to the secretion of its individual ferments. We have seen that trypsin and steapsin are secreted as zymogens, while for the diastase arguments have also been advanced, though probably wrongly, in favor of a zymogen condition. The knowledge of the fact that the two first-named ferments exist in two states, was of great importance for subsequent investigation, and above all it was very significant that trypsinogen was activated by a constituent of the intestinal fluid, namely enterokinase. In introducing pancreatic fistulæ, usually the entrance point of the principal duct from the pancreas into the duodenum is sought, and then the papillæ, together with the piece of intestinal membrane bearing it, is cut out from the alimentary canal and sewed into the wound in the body. If the pancreatic juice flowing through such a fistula be examined, it will be found that it is always active. This is due to the fact that the pancreatic juice thus obtained is always mixed with some secretion from the piece of intestine. If it be desired to obtain the pancreatic juice in an inactive condition, this little piece of intestinal membrane must be removed completely.[1] It has been found that even such juice, under certain conditions, may contain, besides the zymogens, active ferments as well. Thus we know that by the introduction of acid and of soaps into the intestine a juice more or less rich in active ferments results. Again, in the case of nourishment with a mixed diet, there is obtained a varying amount of active ferments, the quantity depending upon the nature of the food.[2] When meat is eaten, for example, the largest amount of zymogens is obtained, while the least amount results from a milk diet. Bread occupies an intermediate position.

Before the fact was known that the pancreatic ferments are, for the most part, given up in the form of zymogens, and that these are activated by the intestinal juices, it was considered as proven that each food caused the production of all three ferments, but that the fat-splitting ferment was present in largest amount. As Babkin has shown, this specialization does not take place. The three principal ferments of the pancreatic juice are, under physiological conditions, secreted practically evenly. If the value of the pancreatic juice obtained after eating a certain kind of food is based upon the amount of proteolytic ferment the juice contains, it is found that milk produces a secretion of greatest digestive power. The two other ferments, diastase and steapsin, are likewise present in considerable amount. The activity of these two ferments remains in this case

[1] B. P. Babkin: Ber. kaiserl. militärärztl. Akad. zu St. Petersburg **9**, Nos. 2 and 3, 93 (1904).

[2] Babkin: *ibid.* **11**, Nos. 2 and 3, p. 93 (1904).

about the same for several hours. After eating meat, the digestive power for albumin sinks very rapidly during the second hour, only to rise again considerably above its original value in the following hours. Diastase and steapsin behave similarly.

Again, the *amount* of juice depends upon the nature of the food. Bread produces the most secretion, then follows meat, while milk occupies the last place.

We must mention, in passing, the fact that it has been suggested that the spleen also is related to the secretion of the pancreas, and takes part in activating the trypsinogen. It has been observed that an extract made from the spleen, which has been removed during digestion, strengthens the action of the pancreatic juice. Pawlow, however, states that he could not find that the secretion produced from animals with the spleen missing had less digestive power than from those with the organ intact.[1]

Under normal conditions, a single foodstuff does not usually come by itself under the influence of the secretion of the pancreas, and of the walls of the intestine, but rather a mixture of foods. The relations are further complicated by reason of the fact that it is not these foods themselves, but rather their cleavage-products, which are acted upon. For the present, the effect of this heterogeneous mixture of products cannot be stated. We can merely assume from the work of Pawlow and his school, performed under uniform conditions, that there are a great many ways here in which the system adapts itself to the prevailing conditions. We have already mentioned the fact that the acid chyme from the stomach does not enter the duodenum in a continuous stream, but that the contents of the stomach leaves it intermittently in relatively small amounts. These portions are at once energetically digested. The products formed by digestion are constantly being absorbed. Even when a very large quantity of food is eaten, there is never a large amount of chyme in the intestine. The extent to which the food is utilized depends, as we shall see later on, largely upon its nature. Naturally the condition of the intestine also comes into consideration. In case of increased peristalsis, the absorption may be lessened. The unabsorbed residue, together with the secretions of bile, pancreas, intestinal membrane and its glands, compose the fæces, or stools. The absorption takes place throughout the entire small intestine, but is undoubtedly most energetic in the jejunum. We must mention in this connection erepsin, which, according to Cohnheim, acts like trypsin upon peptones, and assists in their absorption.

The chyme is carried on its way by peristalsis. The movements of the intestines are regulated by the central nervous system. Innervation is provided in part by the vagus and partly by the splanchnic nerves.

[1] Cf. Oskar Prym: Pflüger's Arch. **104**, 433 (1904).

The latter are said to contain inhibitory fibers. The innervation relations are, however, not perfectly understood.

We must now turn our attention to the absorption of the digested products. We approach this part of the subject with considerable hesitancy, for we must admit at the start that we are not yet able to give a full account of the nature of the absorption process. We can indeed affirm that undoubtedly physical forces come into play here, and that, for example, osmosis plays a part, as is obvious from the already-mentioned observations of Overton on the solubility of lipoids; and similarly we cannot doubt that the surface-tension is to be regarded as important here, in the sense suggested by Traube.[1] On the other hand, we are very well aware that none of the attempted explanations of absorption have of themselves brought the entire complicated process nearer to our comprehension. As soon as a single phenomenon in a single process is applied to the entire absorption, the explanation in all cases appears arbitrary.

We are not able in explaining absorption, to circumvent the conception of a specific action on the part of the cells. We can indeed believe that probably a purely physical explanation will account for an inter-epithelial absorption. The greater part of the products of digestion will, however, be taken up by the cells themselves, and these are undoubtedly very active in their work. We cannot imagine, for example, that the amino acids and polypeptides, which represent decomposition products of the proteins, penetrate into the cells purely on account of physical reasons without active coöperation on the part of the cells themselves. We must not forget that syntheses immediately follows the absorption; i.e., in other words, the activity of the cells then begins, and, indeed, as the relatively constant composition of the serum shows, in a quite definite direction. We have no reason for assuming that in the epithelium of the intestine and the cells of this organ, certain forces unknown to us are at work. If we were to make any such assumption, it would be entirely without empirical justification. Although, at present, we are denied an accurate insight into the nature of absorption, still on the other hand our knowledge of the work performed by the cells is constantly increasing. The intestinal

[1] It would not be difficult with the aid of the H. J. Hamburger's "Osmotischer Druck und Ionenlehre in den medizinischen Wissenschaften" (1902) to cite the different views held regarding intestinal absorption. On the other hand, it would be hard, without going into a detailed explanation of the laws and investigations of physical chemistry, to give a clear picture of the different hypotheses in this narrow field. We would refer the reader, therefore, to the above book and to the following literature: Rudolph Hoeber: Pflüger's Arch. **70**, 624 (1898); **74**, 246 (1899); **86**, 199 (1901); **94**, 337 (1903); O. Cohnheim: Z. Biol. **36**, 129 (1897); **38**, 443 (1899), and **39**, 167 (1900); also Z. physiol. Chem. **33**, 9 (1901); **35**, 396 and 416 (1902). J. Traube: Pflüger's Arch. **105**, 541 and 559 (1904). Cf. also Martin Heidenhain: Anatomische Hefte, **79–80**, **26**, 2–3 (1904). Published by Merkel and Bonnet.

absorption is to be regarded as a process which is no more complicated than the formation of a secretion. In the latter case cells take away certain substances from the blood, while in the former case other substances are taken from the digesting mixture. Just as the cells of the gland show a selective power, so also those of the intestine have the power of choosing their material. In considering the action of ferments we emphasized their specific action and suggested that this is due to the peculiar structure of the ferment molecule. We can apply the same reasoning to the cells themselves, and believe that they are specific in their entire construction, and similarly are merely able to take up substances having particular atomic groupings in the molecule. Indeed, we cannot abandon the thought that the cells of the intestine in a certain sense form a secretion from the substances obtained from the food which they give up on the other side of the intestinal wall. As the gland-cells take the raw material from the blood for the formation of their specific secretion, and then in a short time throw it off only to build up more of it, so here we can imagine that the cells in the intestine carry on their work in a similar manner, and acting together eventually give to the blood a homogeneous material. Certain residues are taken up by the lymph where they are carried first to the mesenteric glands, from thence to be gradually given up for further metabolism.

It would be absurd to consider absorption to be a result of an unknown force, merely because we are at present without insight into the process. It is not without interest in this connection to recall an example which at first glance appeared to show strikingly an actual intelligence on the part of unicellular organism, but which can be explained more simply. We refer to the observation of Cienkowski.[1] He studied the absorption of nourishment by the *Vampyrella Spirogyræ*. It is a microscopically-small, naked, reddish-colored cell. This simple being, in which not even a nucleus is discernible, seeks out, among all the various algæ that are at hand, always a certain especial kind, and leaves untouched all other varieties. When it has come in contact with the suitable kind of *Spirogyra*, it places itself firmly next to the cell-wall, dissolves it and sucks in the contents. We now know enough concerning the action of ferments, however, to show that the fact that this kind of *Vampyrella* feeds only upon special algæ is not so remarkable. It is quite certain that the cell-wall is dissolved by means of a ferment. The ferments are evidently capable of acting only upon a certain kind of alga. We mention this example at this place especially to show how we should look upon the active absorption of substances on the part of the cells. It may be merely

[1] Arch. mikroskop. Anat. **1**, 203 (1865). Cited by Bunge in his Lehrbuch der Physiologie des Menschen, Vol. II, p. 4 (1901).

pointed out, that the cells come into consideration according to the way that they are constructed, and that evidently chemical processes play an important part in the phenomena. In no case should it be implied that forces unknown to us come into play. It is self-evident that we should recognize clearly just how far we can go in accordance with observed facts and as to where the realms of pure speculation begin. Unquestionably we are at present far from understanding the action of the cells. As long as we do not understand the composition of albumin and especially that of the ferments, we cannot expect to receive much light upon the numerous problems which we meet with in studying the work of the cells.

The absorption of the individual foodstuffs, their further destiny in the tissues and eventual combustion, we have already considered in detail, so that we will now merely consider one other function of the intestine, namely, the formation of the fæces and their removal from the system. We have already found that the amount of excreta varies with the nourishment. The color of the fæces changes similarly. Where an abundance of meat is eaten the scybala are dark or grayish colored, while a diet largely of bread makes the color lighter. The bile-pigments have a good deal to do with the color of the fæces, although it is usually their transformation product, stercobilin, which is present. The fæces contain besides indigestible substances, the secretion of the intestines and of the accessory glands, and a certain amount of digestible matter which was not absorbed for some reason or other. We also meet with products of putrefaction such as skatole, indole, purine bases, lime and magnesia soaps and other substances. The fæces furthermore always contain inorganic salts, whether it be due to the fact that they were not absorbed from the food, or whether they were eliminated in the intestines.

The formation of the fæces takes place in the large intestine. Here the unabsorbed material passes, and becomes thickened by loss of water. Without doubt, in the case of the herbivora, the ferments continue their action in the large intestine, and utilize for the organism certain amounts of otherwise undigested material. In the carnivora, however, there is no digestion worth considering in the large intestine.

We have now mentioned all the functions of the digestive organs. We are well aware that we have failed to give a clear picture of the total work of digestion. Still, we are able to take up certain phases somewhat in detail. On the other hand, so many new vistas in this field have been opened up by the investigations of Pawlow and his school and of Bayliss and Starling, and so many new questions remain to be answered, that we no longer can have any doubt that the isolated discoveries obtained here and there will before long be welded together into an organic whole, so that little by little we shall win more and more from the vast field of the unknown.

LECTURE XXIII.

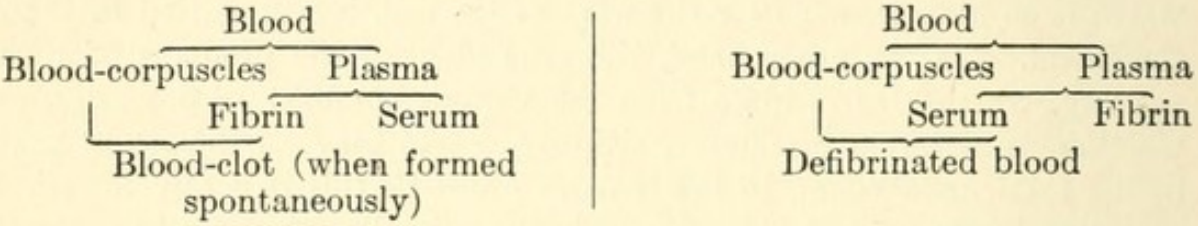
THE BLOOD.

Coagulation. Composition.

Blood is the intermediary in the general metabolism. It carries in part directly from the intestine, and in part indirectly by the aid of the lymphatics, the proper nourishment for each individual cell of the body. The oxygen, which is so indispensable for the work of the cell, is also carried to it by the blood. On the other hand, the cells give up the products of their activity, whether as residues from the various combustion processes, or whether as secretion products which are yet to play an important part in the total metabolism, to the blood. From all this it is obvious what a dominating position the blood holds in the animal organism. In contrast to the other tissues, it is a liquid which is kept in constant circulation by the action of the heart. The blood always contains numerous cells, especially the *red* and the *white blood-corpuscles*. We have already discussed the important part that the former play in external and internal respiration. Besides these form-elements there are *blood-plates*, the significance of which has never been explained satisfactorily. The cell elements of the blood lie suspended in a liquid rich in albumin, the *plasma*. They may be removed from the latter by a centrifugal machine. The clear yellowish plasma is then obtained above the deposited blood-corpuscles. This separation into these form-elements and the plasma can be effected, however, only under quite definite conditions. If we take blood from any blood-vessel (usually the carotid is chosen), and simply allow it to stand, it soon undergoes a peculiar transformation. There settles over the bottom of the dish containing it a firm coagulum which incloses the corpuscles. Above this so-called *blood-clot* there is a clear liquid which is very similar to the plasma, but is, notwithstanding, an entirely distinct substance, as we shall soon see. This liquid produced by the contraction of the blood-corpuscles, and whose volume subsequently increases considerably, is called the *serum*. If, instead of merely allowing the blood to stand, it is stirred vigorously with a wooden, or glass, stirring rod, immediately after taking it from the animal, a different coagulum is soon formed which is known as *fibrin*. In this case the blood-corpuscles remain for the most part suspended in the serum. This mixture of serum and blood-corpuscles is spoken of as *defibrinated blood*. The difference between the

two experiments lies merely in the fact that when there is a spontaneous coagulation of the blood, the fibrin holds the blood-corpuscles within its meshes and only squeezes out the serum, while by beating the blood the fibrin is separated from the blood-corpuscles.

The question that interests us first of all is as to the difference between the plasma and the serum. Whereas the normal blood, as it circulates through the blood-vessels, consists essentially of only two constituents, plasma and blood-corpuscles, clotted blood contains three, — namely, the blood-corpuscles, serum, and fibrin. In both conditions of the blood we find the corpuscles. They remain unchanged, or at least the red corpuscles do, as far as we know. The plasma, on the other hand, separates into two parts, serum and fibrin. The following scheme represents these relations:



Fibrin is unquestionably formed from the plasma. It was conceivable that it is present as such in the blood under normal conditions, being held in solution while the blood is circulating in the body and only caused to precipitate under definite conditions. On the other hand, it was also believed possible that fibrin is not present as such in the blood, i.e., the plasma, except in the form of a preliminary stage of its development. Careful investigation soon showed the latter view to be the correct one. It is perfectly plain that this peculiar and remarkable phenomenon of the clotting of blood which has attracted the attention of many investigators even in remote ages, affords to the animal an important means of protection against undue loss of blood, sufficient in most cases to cause the bleeding to cease.

The first thorough and systematic investigations, upon which our whole theory of blood coagulation is based, were made by the two scientists Buchanan [1] and Alexander Schmidt.[2] Each of these men, working independently, has explained the essential points concerning the clotting of blood. Buchanan made the important observation that hydrocele fluids

[1] Proc. Philosoph. Soc. Glasgow, **2**, 1844 (1845).

[2] Arch. Anat. Physiol. **1861, 1862**; Pflüger's Arch. **6**, 445 (1872); **9**, 354 (1874); **11**, 291 and 515 (1875); **13**, 103 (1876). For the literature on the subject, see P. Morawitz: Ergeb. Physiol. **4**, 307 (1905). Other comprehensive works on the clotting of blood to which we would call attention are, Alexander Schmidt: Die Lehre von den fermentativen Gerinnungserscheinungen (1876); Zur Blutlehre (1892) and Weitere Beiträge zur Blutlehre (1895); Arthus: Neuere Arbeiten zur Blutgerinnung (1899); E. Schwalbe: Beiträge zur Chemie und Morphologie der Koagulation des Blutes (1900); A. Schittenhelm: Zentr. Stoffwechs. u. Verdauungs Krankheiten, **6**, 143 (1905).

which of themselves do not clot, immediately coagulate if a little blood-serum, or a clot of blood, be added to them. Now blood-serum by itself does not clot; it is, in fact, formed by the clotting of blood. Thus the union of two liquids, either of which alone is not capable of forming a clot, produces coagulation. Buchanan concluded from this, and correctly, that two substances are necessary for the formation of blood-clot. He assumed one of these to be fibrin, while the other, probably originating from the white corpuscles, acted upon the fibrin and converted it into an insoluble form. Denis [1] attempted to isolate this "soluble fibrin." He first prevented coagulation by collecting the blood in one-sixth its volume of a saturated sodium sulphate solution. He then allowed the heavier blood-corpuscles to settle out, and precipitated, by the addition of common salt, an albuminous substance from the plasma which he had siphoned off. This substance dissolved in water, but coagulated after a short time; we will give to it the name of *fibrinogen*. It may be considered as the antecedent of fibrin. Buchanan recognized the fact that a second substance was probably necessary to change fibrinogen into fibrin. Our thanks are due to Alexander Schmidt, however, for showing that the coagulation process is due to a fermentation. He succeeded in isolating a substance from the blood-serum which was capable of causing the separation of a large quantity of fibrin. The substance becomes inactive after it has been heated to 100° C. Its optimum of activity lies at 37° C. This substance Schmidt designated as *fibrin ferment*. By its action upon fibrinogen, fibrin is formed. The circulating blood does not contain the fibrin ferment. It is formed, according to the experiments of Schmidt, by the disintegration of the white corpuscles. We must mention here that he himself did not regard the formation of the fibrin ferment as such a simple process. He did not assume the presence of an antecedent, but believed that fibrin was formed from two entirely distinct substances, a fibrinogenous substance and a fibrinoplastic one. Olof Hammarsten [2] disputed this view, and attributed the fermentative action to a conversion of fibrinogen into fibrin. Other investigation has shown that Hammarsten's theory is correct.

There is still another important point to mention. Alexander Schmidt pointed out that the formation of blood-clot also required the presence of neutral salts. According to his views, all soluble salts of the alkalies and alkaline earths reacted similarly. Hammarsten noticed, on the other

[1] Nouvelles études chimiques, physiologiques et médecines sur les substances albuminoids (1856), and Mémoire sur le sang (1859).

[2] Nova acta Reg. Soc. Scient. Upsala, Ser. 3, **10**, 1 (1875); Upsala läkareförenings förhandlingar, **11**, 1876; Pflüger's Arch. **17**, 413 (1878); **18**, 38 (1878); **19**, 563 (1879); **22**, 443 (1880); **30**, 437 (1883). See also Frédéricq: Bull. de l'acad. roy. Belgique, 2 série, **44**, 7 (1877).

hand, that calcium chloride exerts a particularly favorable action upon the rapidity of the coagulation. The necessity for the presence of lime-salts was proved clearly by Arthus, and Arthus and Pagès.[1] They showed that blood collected, as it flows from the animal's body, in a solution of alkali oxalate does not clot. If, however, a slight excess of lime-salts is added to this oxalate plasma, a clotting at once takes place. It is tempting to compare the clotting of blood with the coagulation of casein by rennin. The latter would correspond to the fibrin ferment. This ferment changes fibrinogen into fibrin, which may form an insoluble calcium salt, and is precipitated. This simple explanation of the clotting of blood has, however, been shown to be incorrect. The lime-salts must act in some other way.

In order to understand the clotting of blood, and the processes which take place in this connection, it is necessary to bear in mind the following points. In discussing the digestive ferments we were constantly confronted by the fact that the ferments as such are not given up by the cells, but in the form of their antecedents, to which in general we gave the name of zymogens. The transformation of these inactive substances into active ferments is brought about by various agents. How was it with trypsinogen? We remember that this was changed into trypsin by the so-called enterokinase which is given up by the epithelial cells of the intestinal membrane, and is contained in the intestinal juices. Again we remember that a substance called secretin has been obtained from the blood, which incites the gland-cells of the pancreas into greater activity. The secretin is likewise found in the intestinal membrane in a preliminary stage, which is activated by acid. We do not know how secretin influences the action of the pancreas, whether it acts directly upon the gland-cells or indirectly by increasing the blood-supply. At all events it is evident that the formation of ferments is a very complicated process, and even when the zymogen has been formed it does not at all signify that the fermentation will take place.

In accordance with this aspect, we must next find out whether the fibrin-ferment, sometimes called *thrombin*, possesses a zymogen stage in its development, and if so, how it is brought into activity. Further investigation has in fact shown that the fibrin-ferment does exist originally in an inactive form. We will call this simply the *zymogen* of the *fibrin-ferment*. This zymogen may be obtained in large amounts from the oxalate plasma and becomes active only after being treated with calcium chloride solution. In this way the fibrin-ferment is obtained. This ferment is capable of causing coagulation in the oxalate plasma, from which the calcium salts

[1] Arthus: Doctor's Thesis, Paris, 1890. Arthus and Pagès: Arch. Physiol. **22,** 739 (1890); Arthus: Compt. rend. soc. biol. **45,** 435 (1893); Arch. Physiol. **1896,** 47, and Compt. rend. soc. biol. **54,** 526 (1902).

have been precipitated. According to this, it is easy to assume that the calcium salts effect the activating of the zymogen. The plasma normally contains only the zymogen of the fibrin-ferment and not the ferment itself. In clotting blood, the ferment is made active under the influence of calcium salts, and is now exerting its action upon the fibrinogen. If, on the other hand, the calcium salts are removed by precipitation with oxalic acid before the coagulation has taken place, then the blood does not show the same tendency to form a clot, because the zymogen remains unchanged, and in this condition it is perfectly inactive. Further support for the view that the calcium salts are only active in this phase of the coagulation, and that they do not take part directly in the conversion of fibrinogen into fibrin, is shown by the work of Hammarsten. He pointed out that only those calcium compounds need be considered which are present in such a state that the calcium is precipitable by oxalic acid. The oxalate plasma contains calcium, in addition, which is evidently present in the form of complex organic compounds. It is possible now to convert fibrinogen into fibrin by the action of the fibrin-ferment in the absence of lime salts that can be thrown down by oxalate. This experiment is perfectly analogous to that with the oxalate plasma. Hammarsten was able also to show that the fibrin could not be regarded as a calcium compound. We do not know exactly how the calcium salts cause this conversion of zymogen into ferment. It is possible that it acts directly upon the zymogen, but it is likewise conceivable that the calcium salts have an indirect action in regulating the conditions. For the present, however, we shall consider that they themselves take part directly in the conversion of the zymogen into ferment, and that otherwise they have nothing whatever to do with the formation of the fibrin.

We shall at this place mention that quite recently it has been noticed that calcium salts exert an activating effect upon trypsinogen. If inactive pancreatic juice be treated with sodium fluoride it will remain in this condition, and is only activated by the addition of calcium salts. In such a case as this the calcium salt evidently does not act directly upon the zymogen. If the pancreatic juice be filtered through collodion, then it will no longer be activated by the addition of the calcium salt. Delezenne,[1] who performed this last experiment, believes that some substance is held back on filtering the pancreatic juice through collodion, which is capable of activating the trypsinogen. The calcium salts serve in some way to activate the unknown substance just as it is possible that enterokinase may have an antecedent. If we apply this observation, which to be sure has never been explained entirely satisfactorily, to the coagulation of the blood, then we should have to assume that the zymogen of the fibrin-ferment is activated by a substance which corresponds to enterokinase, and

[1] Compt. rend. soc. biol. No. 33 (1905).

that this activator is likewise present in the blood in an inactive condition, and is only changed into the active condition by the presence of calcium salts.[1]

We must recall one other fact that we met with in discussing the digestive ferments. We mentioned that the bile, and the intestinal juices in general, had the property of augmenting the action of the pancreatic juice. According to our present knowledge, this does not consist merely in changing zymogen into ferment. The above-mentioned secretions accelerate directly the fermentation process. Until we understand the nature of ferments better, there is naturally but little prospect of our being able to comprehend the accelerating effect. We can only mention the fact, and state in addition that there are other substances known which tend to retard the action of ferments without in any way injuring them directly.

We must also ascertain whether there are substances which tend to accelerate the action of the fibrin-ferment. It has indeed been long recognized that there are substances which accelerate the clotting of the blood. Even Buchanan noticed an acceleration produced by certain tissues. Rauschenbach[2] has proved beyond question that there are substances present in the cells of the tissues which aid in the formation of the clot. He found a particularly favorable action on the part of those tissues which were rich in nuclein substances. Foà and Pellacani[3] showed further that the injection of the juices from various tissues caused intravascular coagulation. This caused a tedious, unfruitful discussion as to whether the substances present in the cells of the tissues were to be considered as corresponding to the fibrin-ferment, or whether they merely augmented its action upon the plasma. The first assumption is a tempting one. In the first place, according to many observations the leucocytes are the antecedents of the fibrin-ferment. It would be of itself not inconceivable that other cells in the body should produce similar, or the same, products, especially as it is well known that after death a coagulation takes place in the cells which may be considered as perfectly analogous to the clotting of blood.

Certain observations, however, make it seem more probable that the coagulation action of the tissue extracts depends upon a quite different process than the direct introduction of fibrin-ferment. Above all, the work

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[1] It is probably true that the lime salts do not directly cause the activation of the fibrin-ferment zymogen, for it is not easy to see otherwise how the blood can contain the two substances side by side without their reacting together. The calcium salts are only brought into activity when the fibrin-ferment zymogen has been changed in some way.

[2] Ueber die Wechselwirkungen zwischen Protoplasma und Blutplasma. Dorpat, 1883.

[3] Arch. Science med. **7**, 113 (1883).

of Delezenne [1] should be mentioned in this connection. He showed that the blood of birds, reptiles, *Batrachia*, and fish coagulated but very slowly of itself. If for example the blood of a bird be carefully removed so that it does not come in contact at all with the tissues, it can be kept in vessels, out of contact with dust, for a long time, without forming a clot. By centrifugalizing such blood, plasma can be obtained which keeps until it putrefies without the formation of any clot. If, however, a little blood, or a little juice from the tissues, be added to such cell-free plasma, there is at once a formation of fibrin. If the blood clots of itself, then we notice that the clot is formed first at a place where there is present a considerable amount of leucocytes. This experiment cannot be carried out with the blood of mammals, for it coagulates too quickly. Evidently their leucocytes are less resistant than those of the above-mentioned classes of animals. The objection may be raised that in every case there is the possibility that active fibrin-ferment may be carried to the blood of plasma by the juices from the tissues, while the zymogen of this fibrin-ferment which is contained in the blood itself, for some reason is not changed into the active form. Morawitz [2] has proved, however, that this objection is not well founded. He showed that the juice from the tissues, even in the presence of calcium salts, was incapable of causing a fibrinogen solution to coagulate. If, however, the extract from the tissues be added to the blood itself, there is a marked acceleration of the coagulation provided that the lime-salts are present. In the absence of lime-salts, the extracts from the tissues are without action.

Alexander Schmidt distinguished between accelerating and retarding substances for the coagulating of the blood. He believed that these are present in the leucocytes, and in all other cells of the bodies. The former class of substances may be extracted with alcohol, while the latter cannot. We can imagine that the substances which accelerate the clotting of blood serve to activate the zymogen of the fibrin-ferment. Under normal conditions, i.e., while the blood is circulating through the blood-vessels, there is an equilibrium between these substances that accelerate and those that retard its coagulation, whereas when clotting takes place, this equilibrium is disturbed in favor of the former. Plausible though this hypothesis may be, it must be emphasized that it is not based upon a careful analysis of the separate processes.

If we draw a picture of blood-coagulation in accordance with what has been stated above, we may assume it to be well established that the blood, or, better, the plasma, contains a substance, fibrinogen, which is an antecedent of fibrin. This transformation of fibrinogen into fibrin is to be

[1] Compt. rend. soc. biol. **48**, 782; Compt. rend. **122**, 1281 (1896); Arch. Physiol. **1897**, 333; Compt. rend. soc. biol. **49**, 462, 489, and 507 (1897).

[2] Arch. klin. Med. **79**, 1 (1904); Hofmeister's Beitr. **4**, 381 (1903); **5**, 133 (1904).

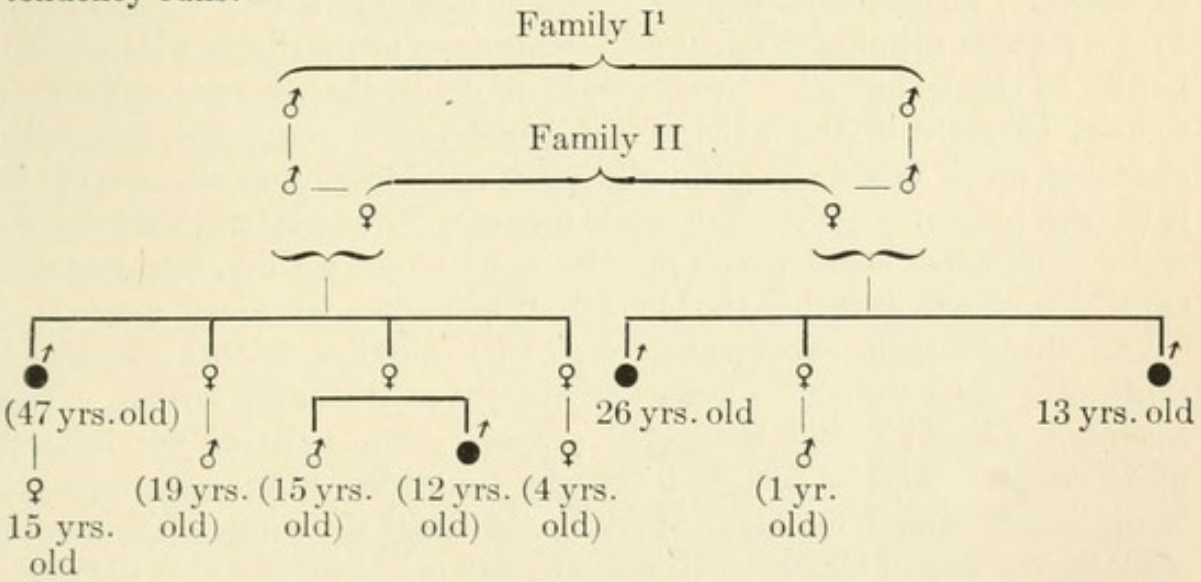
regarded as the result of fermentation. The fermentation is brought about by the fibrin-ferment which does not occur as such in the blood but is probably present in the plasma in an inactive condition, the zymogen of the fibrin-ferment. In order for the zymogen to become active, a second substance must act upon it. A true activation must take place. The exact nature of the activator of the zymogen is at present unknown. It is not impossible that calcium salts bring about this effect. Certain observations, however, make it appear improbable that the calcium salts take part directly in the formation of the ferment from its zymogen condition. Some facts indicate that a peculiar kinase is active, which is itself set free in some way so that it can act upon the zymogen. Apparently it is here that the calcium salts come into play. It may be mentioned, in addition, that two antecedents of the zymogen have been described in the literature. One of these, which has not been mentioned, is said to be activated by acids or alkalies. It has been found, namely, that the serum from clotted blood, which of itself contains but little active ferment, will become very active upon the addition of acid or alkali. There is, nevertheless, no ground for assuming that there are two kinds of zymogen present. We agree with Morawitz that the fibrin-ferment, for some reason or other, becomes inactive after the blood-clot has formed. It is simplest to assume that the fibrin-ferment unites with some other compound, whereby its active group is rendered inactive. It is equally plausible that by means of some intramolecular transformation, perhaps the formation of an anhydride, the inactive condition of the ferment is again obtained. The alkali or acid which is added to the serum would in the first instance set the ferment free, whereas, according to the latter view, the anhydride form would be lost. At all events, it is unnecessary to assume that the fibrin-ferment has two antecedents. It is evident from this attempted explanation, what a complicated process the clotting of blood really is, and how many different minor processes must be cleared up before we shall be able to understand the whole phenomenon of blood-coagulation.

The point that is of chief interest to us here, is as regards the nature of the fermentation. Does a hydrolysis take place, an oxidation, a reduction, or what? The first assumption only need be discussed. Formerly, it was believed that the fibrinogen took on water and formed fibrin together with a substance soluble in water, which was called *fibrin-globulin*. Recently every justification for such an assumption has been denied, and mainly because it is possible to prepare solutions of fibrinogen which will not split off fibrin-globulin either by clotting or coagulating by heat.[1] According to the more recent view, the formation of fibrin results from an intramolecular rearrangement of the atoms. There is at present no prospect of deciding this question definitely. Fibrinogen is an albuminous substance,

[1] Huiskamp: Z. physiol. Chem. **44**, 182 (1905).

of which we merely know that it is closely related to the globulins, and fibrin also belongs to the proteins. It is perfectly possible that fibrinogen consists of several molecules of fibrin which take up water and disintegrate, whereby eventually fibrin is formed, so that although fibrin is formed from fibrinogen, the coagulation is essentially a hydrolytic decomposition. There are no facts known at present which contradict such an assumption.

Under ordinary conditions when the body is wounded, the blood flows out from the blood-vessels past the tissues. Its clotting is thereby accelerated. We must here consider the anomalous behavior of the blood in *hemophilia*. This condition is especially characterized by the fact that the blood does not clot readily. Even from the slightest wounds there is a tendency to bleed freely. There may be even such a slight tendency for the blood to clot, that a wound resulting, for example, from the extraction of a tooth may cause the patient to bleed to death. Hemophilia has been of interest to biologists not only on account of this peculiar condition but also by a further peculiarity which holds almost invariably. This is the fact that it is hereditary, and, moreover, it is almost always the male members of the so-called *families with hemophilic blood*, who inherit this tendency. On the other hand, the female members of the families alone transmit the anomaly. The following tree shows how this tendency runs: —



The symbol ♂ indicates those male descendants which were normal, ♂ indicates those males having the tendency to bleed freely, and ♀ represents the female members of the family. We mention hemophilia at this place because this anomalous behavior, which is hardly to be regarded as a disease, inasmuch as the individuals are normal in every other respect, ought to be of assistance to us in the study of the coagulation of blood.

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[1] Abderhalden: Beiträge pathol. Anat. und allgem. Path. **35,** 213 (1903). See Stahel: Inaug. Dissert. 1903, Zurich, 1880. Hoessli: Inaug. Dissert. Basel, 1885. Sahli: Z. klin. Med. **56** (1905).

Here nature has performed for us a physiological experiment. Evidently some link in the chain of processes from those which start the coagulation, to those that complete it, is missing. First it was suggested that perhaps the calcium salts were not present. Direct experiment failed to confirm this. The behavior cannot result from a deficiency in fibrinogen, for the blood is found to yield just as much fibrin as normally. The remarkable thing is the time that it takes for the blood to clot. If a trace of defibrinated normal blood is added to the hemophilic blood, the coagulating is thereby accelerated.

It is evident that this abnormal blood contains all of the constituents that are required for forming the clot. It contains sufficient fibrinogen, sufficient calcium salts, and, as far as we are able to find out, enough of the zymogen which antecedes the fibrin-ferment; but, on the other hand, one obtains the impression that an insufficient amount of the agent is present, which changes the zymogen of the fibrin-ferment into the active state. We have designated this substance as a kinase, and suggested that it occupies a similar position to that of enterokinase. Now cases of hemophilia have been known in which all of the blood did not show this abnormal behavior, but in which it was restricted to the blood from certain parts of the body, especially from the blood-vessels in the mucous membrane. While injuries to the skin, for example, caused a normal bleeding, a very slight injury to the mucous membrane might cause enormous hemorrhages which could hardly be checked at all. Now there is no doubt that there is some anatomical anomaly in the walls of the blood-vessels concerned, for mere scratches which would not ordinarily cause any bleeding at all, now cause it to take place profusely. But while it is easy to understand that abnormally constructed blood-vessels may be injured more readily, this does not explain at all why it is that the blood from them does not coagulate.

The characteristic phenomenon with such bleeding is that the blood constantly oozes through a loose clot exactly as through a sponge. The coagulum formed is not connected properly with walls of the injured blood-vessels. At the best, it is merely suspended from walls without being closely bound to them. It does not fulfill its normal function of checking the flow of blood. This tends to lead one to suspect that the walls of the blood-vessels themselves have something to do with the clotting of the blood, and most probably in the sense that the injured cells give up something which accelerates the coagulation process. We must designate this substance as a kinase, and localize its function in the entire process to that of the preliminary stage; i.e., we must ascribe to it the property of activating the zymogen of the fibrin-ferment. Consequently a chemical anomaly is closely connected with this anatomical one. It is not impossible that further studies based upon these observations will eventually show what parts of the walls of the vessels take part in the secretion of the kinase.

Naturally it does not necessarily follow that all cases of hemophilia result from the same cause. Perhaps in different cases, different links in the whole chain of processes may be missing. We have introduced this discussion here not alone because it apparently sheds light upon the process of coagulation, a fact to which H. Sahli first called our attention, but because it seems to us as highly significant that such an extremely localized anomaly in the whole course of a fermentative action should prove to be hereditary.

We have up to this point failed to answer the question why the blood does not coagulate in the blood-vessels of the body under normal conditions. It is *a priori* not so easy to see why conditions could not prevail within the blood-vessels which would bring the fibrin-ferment into activity. On the other hand, the fact that the coagulation process depends upon the harmonious action of several distinct factors, presents several possibilities whereby the coagulation in the blood-vessels themselves may be prevented. It is merely necessary to prevent the bringing into activity of the fibrin-ferment. The following experiment[1] brings to our attention still another factor which we have not considered in the coagulation of blood. If blood is collected under vaseline or oil, it remains perfectly fluid for hours. It can be stirred with a greased rod without its coagulating. If, on the other hand, the precaution is not taken to grease the rod, coagulation at once takes place upon stirring. It is even possible to centrifugalize blood that has been collected in paraffined vessels, and thus obtain plasma, which similarly will not clot for a considerable length of time if it is kept in greased or paraffined vessels. On the other hand, if such blood is poured into an ordinary glass vessel, it will clot immediately. The contact of the blood with something that it can wet, seems to start the coagulation. As this paraffined plasma, after the calcium salts have been removed from it, does not coagulate when brought into contact with foreign bodies, it is out of the question to believe that it contains an active ferment. By the process of exclusion, we may conclude that the contact with a foreign body in some way accelerates the transformation of the zymogen into active ferment. One might assume from this that the reason the blood does not coagulate in the blood-vessels, with intact intima, is that the conditions are similar to that of the greased vessels outside of the body. In fact, we know that if the intima is in any way injured, intravascular coagulation readily sets in, which is called a *thrombus* when it merely stops up the blood-vessel which is injured, but is known as *embolism* when the coagulation influences other blood-vessels as well. The blood is continually wetting the intima of the blood-vessel walls. The

[1] Brücke: Virchow's Arch. **12**, 100 (1857). Freund: Wiener Med. Jahrbücher, **1888**, 259, and **1889**, 554. Bordet and Gengou: Ann. Institute Pasteur, **17**, 822 (1903); **18**, 1 (1904).

fact that coagulation does not take place cannot be due to an insufficient adhesion. The uninjured walls of the blood-vessels appear to exert a restraining influence upon the coagulation. It is indeed possible that they also produce a secretion of a negative catalytic nature.

We have mentioned that it is possible to prevent the clotting of blood outside of the body by collecting it in a solution of ammonium oxalate. Sodium fluoride acts similarly. The cause of the failure of coagulation to begin is to be considered as due to the precipitation of the lime-salts as oxalate or fluoride. The addition of neutral salts in sufficient concentration, or cooling the blood, also tends to prevent the formation of a clot. The influence of both measures is due to a restraining of the action of the ferment. Now we know of a number of substances which may be introduced into the circulation so that the blood which is subsequently removed will not coagulate. Commercial peptone is such a substance.[1] We may state in this connection that peptone acts differently in the case of different animal species, and in fact with one individual of a species it may prevent coagulation, in another merely retard the formation of coagulum, while in a third member of the same species it will be apparently without effect. This indicates that the influence of peptone upon the process of blood-coagulation is not so simple as is the case with oxalic acid for example. It is important also to find that it takes from ten to fifteen times as large a dose of peptone to prevent the coagulation in a test-tube as is required in the case of injection into the organism. It has never been found possible to localize in any way this action of the peptone, although apparently, in the light of recent investigations, the action is not due to the peptone itself, but to impurities present in commercial peptone. How this effect is produced is entirely unknown to us. It has been suggested that it causes the formation in the body of substances which tend to prevent the coagulation. It has been established that peptonized blood contains all the elements which are considered as necessary for the clotting. In spite of this fact the harmonious course of the entire chain of processes is in some way disturbed. We can indeed imagine that by the failure of some one of the substances which aid in the coagulating process, the clotting is prevented. The disturbance is at all events to be sought in the group of processes by which the activating of the zymogen of the fibrin-ferment is effected.

Other substances are known which act similarly to peptone. We will mention the serum of *Muræna* [2] and the extract of crabs' muscles and of

[1] Schmidt-Mülheim: Arch. Anat. Physiol. **180,** 33. Albertoni: Zentr. med. Wissensch. **1880,** No. 32. Fano: Arch. Anat. Physiol. **1881,** 277.

[2] Mosso: Ann. chim. farm. **8,** 198 (1888), and Arch. exper. Path. Pharm. **25,** 111 (1891). Delezenne: Arch. physiol. **646** (1897), and Compt. rend. soc. biol. **49,** 42 and 228 (1897). Heidenhain: Pflüger's Arch. **49,** 209.

snails. The characteristic of these substances which prevent clotting is, to repeat, that they evidently do not themselves directly affect the coagulation process, but excite the organism to the formation of products which tend to prevent the blood from forming a clot.

Besides these substances which exert a secondary effect upon the coagulation, we know of substances which directly prevent it. To these substances belongs *hirudin*, which, by reason of the extensive studies of Jakobj, has recently excited much interest. Hirudin is formed in the oral glands of leeches.[1] It is quite stable towards heat and is soluble in water. After injection into the organism it appears unchanged in the urine. It is not yet perfectly clear how hirudin acts. It is apparently able to neutralize a part of the fibrin ferment.[2] It is still an open question how we can best picture this process. It is usually assumed that the active ferment possesses groups which enable it to react with definite groups of other compounds. These groups impart to the ferment its specific nature. If now the ferment comes in contact with a substance which is capable of engaging these groups, i.e., combining with them for example, the ferment then becomes inactive. Substances like hirudin have been obtained from other blood-sucking animals, e.g., from the wood-tick (*Ixodes ricinus*) and from *Anchylostomum caninum*.

It is an old observation that the blood of animals which have died from snake-bite, often does not coagulate.[3] The poison from the cobra especially has been carefully studied. It is supposed to contain a substance which acts upon the kinase, i.e., the activator of the zymogen of the fibrin-ferment. It is clear that if the function of this activator is disturbed, a coagulation cannot take place.

Besides the substances which prevent the coagulation of blood, we know of others which accelerate it. In this respect we will recall the effect of calcium salts. Their internal application is said to favor the formation of blood-clot. Another substance which is used much more extensively for this purpose is gelatin.[4] It is altogether impossible to state why this property should be ascribed to gelatin, and if it really does exert the desired effect it is still more difficult to explain it. At all events, the statements concerning it that are to be found in the literature are very contradictory.

We have up to the present time intentionally disregarded a question of

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[1] Haycraft: Arch. exper. Path. Pharm. **18,** 209 (1884). Franz: *ibid.* **49,** 342 (1901). Andreas Bodong: *ibid.* **52,** 242 (1904).

[2] Fuld and Spiro: Hofmeister's Beitr. **5,** 171 (1904). P. Morawitz: Arch. klin. Med. **79,** 432 (1904).

[3] Fontana : On Poisons. London, 1787. Morawitz: Arch. klin. med. **80,** 340 (1905).

[4] Dastre and Floresco: Compt. rend. soc. biol. **48,** 243 and 358; Arch. physiol. **28,** 302.

deep significance, namely, that of the origin of fibrinogen. This is something of which we have no positive information. Apparently the liver plays an important part in its production. P. Nolf [1] found that after extirpation of the liver the fibrinogen content of the blood decreased rapidly. Experiments performed by M. Doyon, A. Morel, and N. Kareff [2] point in the same direction. They showed that after sub-acute poisoning of dogs with phosphorus oil, which causes a fatty degeneration of the liver, there is a decrease in the fibrinogen content of the blood plasma, which lessens the coagulation power of the blood. With a cock it was not found possible to cause a fatty degeneration of the liver by phosphorus poisoning, and it was likewise impossible in this case to cause fibrinogen to disappear from the plasma. It is entirely impossible to draw binding conclusions from these experiments, for both the extirpation of the liver and poisoning by phosphorus are attacks whose effect upon the action of the whole organism cannot be disregarded. It is possible that the liver not only influences the production of fibrinogen, but other phases of the coagulation process as well. We shall expect further experiments in this direction.

In the coagulation process we have become acquainted with a very essential *property* of the blood. We shall now turn to the individual constituents of defibrinated blood, the serum and blood-corpuscles. The former consists chiefly of two different albuminous substances, a globulin and an albumin. We shall not stop here to discuss the unedifying question as to whether these proteins are simple substances or not. This cannot be decided in the light of our present knowledge, and even if it is possible by fractional precipitation, or by "salting out," to effect a separation into simpler constituents, but little gain is made in our knowledge of these two proteins, for even these fractions cannot be characterized, by the means now at hand, nor upon the present basis of protein chemistry, as simple substances. Besides these proteins, we find varying amounts of fat in blood-serum. After a meal rich in fat, the amount present in the serum may become so large as to give it a milky appearance. Serum invariably contains cholesterol and lecithin, and in fact the former is, as we have already stated, largely present in the form of fatty-acid esters. A sugar, *d*-glucose, is also present in serum. The amount of the latter varies, but only within narrow limits.

We have already seen that the blood, besides providing nourishment, also serves to carry the end-products of metabolism away from the cells. For this reason we constantly meet with such products in the blood. It is certain that they belong for the most part to the plasma, or its serum. Their presence remained for a long time undiscovered, because from moment to moment but small amounts of such substances are present.

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[1] Bull. Acad. roy. Belgique, **1905**, 81.

[2] Compt. rend. **140**, 800 (1905).

As soon as they are formed, they are given up by the cells to the blood and immediately leave the body. Such substances are urea, uric acid, creatine, hippuric acid, and conjugated glucuronic acids, all of which we may in a sense regard as end-products of metabolism. Blood-serum is never perfectly colorless. It always has a yellow tint, the color being ascribed to a certain dyestuff, called lutein. Its chemical nature is wholly unexplained. Serum always contains inorganic constituents, and the amount appears to be very constant. It would be highly interesting to have definite knowledge concerning the distribution of the inorganic substances, and above all concerning the way they are combined in the blood, plasma, and serum. Unfortunately, there are no known methods for giving us such information. At present we are forced to rely upon the chemical examination of the ash, the results of which naturally have but a relative value. In this way we are able to ascertain what constituents are present in the ash, but we obtain absolutely no information as to how the phosphoric acid, for example, is combined in the blood or plasma. This phosphoric acid may arise from inorganic phosphates, or from organic phosphorus compounds, such as lecithin, nucleic acid, etc. The value of an ash analysis can be increased by attempting to determine in what different way the respective amounts of the constituents may have been combined. It would be, of course, likewise desirable to obtain by physico-chemical methods some idea as to the content of the blood and of the plasma in electrolytes and non-electrolytes. As the most important result of physico-chemical investigation of the blood, we will mention the highly interesting observations of Hoeber [1] that the concentration of the hydroxyl ions in blood-serum and in the blood is almost exactly the same as that of distilled water. Both liquids are from this point of view to be considered as neutral.

Blood always contains cells, namely, the red and white corpuscles. Whereas the latter are to be regarded as true cells, the former are, in man and mammals, not to be considered as perfect cell-structures. Only in the beginning of their development do they possess a nucleus, which they lose as soon as they become active in the blood. The red corpuscles of birds, reptiles, amphibia, and fishes do, however, contain nuclei. In spite of extensive investigations but little is known concerning the chemical construction of the red corpuscles. It is true that we know fairly well what components are present, but we do not understand how they are combined. The red corpuscles do not possess any true membrane. It has been assumed that they consist of stroma filled with liquid.[2] They are

[1] Pflüger's Arch. **81**, 522 (1900). Géza Farkas: Mathematikai és természettudományi értesítö, **21**, Vol. 1 (1902). P. Fraenkel: Pflüger's Arch. **96**, 601 (1903).

[2] H. J. Hamburger: Osmotischer Druck und Ionenlehre in den medizinischen Wissenschaften, Wiesbaden, 1902. Cf. Rollett: Pflüger's Arch. **82**, 199 (1900).

enveloped with a substance similar to fat which forms a semi-permeable wall. It is certain that the red blood-corpuscles, also called *erythrocytes*, do not take up all substances. The wall will not allow many salts to pass through, though it is easily penetrated by water. If the erythrocytes are placed in a solution of common salt, whose osmotic pressure corresponds exactly to that of blood-plasma, the blood-corpuscles will remain unchanged. Such a salt solution is said to be *isotonic*. Its concentration is different for different species of animals, and is called a "physiological salt solution." In the case of mammals such a solution contains 0.9 per cent of sodium chloride. If there is more salt present in the solution, it is said to be *hyperisotonic*, in which case the red corpuscles will give up water to the solution and shrink in size. Conversely in a *hypisotonic* salt solution (one containing less salt than an isotonic solution) the corpuscles take up water and swell. This swelling may take place to such an extent that the red corpuscles lose the characteristic pigment which passes into solution. In this case the blood undergoes a peculiar transformation. Whereas it was opaque before, it now becomes a clear, transparent, red-colored liquid. The blood is said to be "laked."[1] In the laked blood, the blood-corpuscles robbed of their hemoglobin, the so-called "shades," are found in which only stroma is present. The "shades" appear under the microscope as colorless structures, often retaining the form of the erythrocytes. The red corpuscles are not impenetrable to all substances. Urea, for example, is taken up by the blood-disks. If urea is added to blood it distributes itself equally between the blood-corpuscles and the plasma. Its solutions, therefore, exert no osmotic pressure upon the red corpuscles. The latter behave in urea solutions of all concentrations exactly as in distilled water. They give up their hemoglobin to the urea solution; this is not the case if the urea is added to an isotonic solution of common salt. We know of certain substances, such as ammonium chloride, for example, which behave differently from urea in the last case. The wall surrounding the red corpuscles is readily penetrated by this salt, and the hemoglobin goes into solution even if the ammonium chloride is added to an isotonic common salt solution. Ammonium chloride, therefore, has a poisonous action upon the blood-corpuscles. A great many experiments have been carried out with regard to the permeability of the red corpuscles. At present they do not give us much information concerning the behavior of the blood-corpuscles in the blood itself, and concerning the substances dissolved in the plasma. We are not justified in applying experiments performed under peculiar conditions to the blood itself as it exists in the living organism.

The exit of the pigment from the red corpuscles, a process which is

[1] Hans Koeppe: Pflüger's Arch. **103,** 140 (1904); *ibid.* **107,** 86, 183 (1905).

designated as *hemolysis*, can be brought about in quite a number of different ways, as, for example, by freezing the blood and then thawing it. Hemolysis is also effected by certain bacterial metabolic products, by those of the higher plants, and also those of animals. We shall subsequently take up this process more in detail.

The constituent of the red corpuscle which has been best studied as regards its functions, is the pigment of blood, *hemoglobin*, which we have already met with in the discussion of the respiratory exchange. Before discussing its chemical construction, we will consider the above-mentioned, cellular constituent of the blood, the white corpuscles, and briefly take up the composition of the blood as a whole, and its content of individual substances. The white corpuscles are fully endowed cells. They are not uniform, but occur in various shapes and sizes. It is extremely difficult to say much about the sphere of activity of these bodies, also called leucocytes. Their function has never been satisfactorily explained. They have frequently been designated as agents of transportation. It is very probable that they play an important part in this function of metabolism, and accomplish the exchange of substance between the cells of different organs. The number of leucocytes can increase extraordinarily under certain conditions. This phenomenon is most strikingly illustrated in cases of infection, in which case the seat of infection is, under normal conditions, surrounded very quickly by a cordon of leucocytes. They are by no means limited to the blood circulation. They can leave this and penetrate into the tissues. The white corpuscles play a quite different part in the blood from that of the red ones. They are not peculiar to the blood, but merely make use of it as a vehicle. They enter and leave it quite at will. They are to be considered as independent entities. This is evident from the fact that they are independent of the nervous system and can move themselves forward, autonomously like the amœbæ, by sending out pseudopodia. It is possible that the blood contains, besides leucocytes, which are only temporarily present, others which stand in more intimate relations to the blood. We are not at all sure whether we are to regard the white corpuscles as forming a physiological unit, or whether certain of them have special tasks to fulfill. Our experience with pathological processes makes it seem more probable that different tasks fall to leucocytes of different forms. The large accumulation of leucocytes during intestinal digestion remains absolutely unexplained. It is extremely probable that they in some way take part in the digestive process, being perhaps active in the assimilation of the food. The fact that they are able to take up substances directly, and transport them away, is shown, for example, by observations concerning the absorption of iron. It is not at all difficult, particularly after administration of inorganic iron salts, to find, by testing with ammonium sulphide, many white

corpuscles heavily laden with particles of iron, which they carry to the nearest lymphatic. Many discoveries show distinctly that the leucocytes endeavor to carry away foreign substances from the body. It may be regarded as positively established that they play an active part during infectious diseases in striving to make the injurious products of the metabolism of micro-organisms harmless, although it is going a little too far to assume that the leucocytes alone have this tendency. The leucocytes also serve to disintegrate dead tissue. In this direction, the solution of the masses of fibrin, which fill the bronchi and the finest bronchioles during pneumonia, is very interesting. There is in this case, as we have already indicated at another place, a regular digestive process that comes into play. The fibrin is decomposed into its constituents and these are resorbed.

We cannot say much concerning the structure of the white blood-corpuscles. They contain, as cells, all those constituents which we usually meet with in cells. There is not much use in mentioning these constituents, for we are not able at present to draw any conclusions from them, or from their union with the other building stones of the protoplasm and nucleus, concerning the participation of this or that substance in the exercise of definite functions. As soon as our investigations reach the cell, the enigma is too great.

In addition to the leucocytes we find the *blood-plates*, which we have already mentioned. These are colorless, gummy disks of a round form. They are said to possess all the characteristics of true cells, and to be also capable of active, amœboid movement. They undoubtedly participate in the clotting of blood. It is, however, still a disputed question as to the point in the entire coagulation process at which their activity begins.

Let us now return to the composition of the blood itself. We must at once state that the blood, in its natural state, is almost never utilized for quantitative analytical determinations. Almost all of the investigations in this direction have been made with defibrinated blood. In the first place, we are interested to know the relative amounts of blood-corpuscles and serum. This varies with different kinds of animals, and even in different animals of one and the same species. Moreover, the estimation of the number of blood-corpuscles, and the amount of the serum, cannot be made very exactly. It is an indirect determination. We will briefly mention here that method upon which the figures that we shall give below have been based. It is that of Hoppe-Seyler.[1] The blood-corpuscles may be separated from the serum by means of the centrifuge. By repeatedly stirring the blood with an isotonic salt solution, and renewed centri-

[1] Handbuch der physiol. und pathol. chem. Analyse. p. 272 (1883).

fugalizing, the serum lying between the separate blood-corpuscles is eventually removed. In these blood-corpuscles we can determine the sum of the hemoglobin and protein. If in addition the hemoglobin and albumin content of the total blood and the albumin content of the serum are determined, then from these values the relative amounts of serum and blood-corpuscles in the blood as a whole can be estimated. We may cite an example:[1]

One thousand grams of defibrinated beef-blood contain on an average 172.9 grams of hemoglobin plus albumin.

In the blood-corpuscles from 1000 grams of the same blood there were found 124.0 grams of hemoglobin plus albumin. 1000 grams serum contained 72.5 grams of albumin.

In the serum from 1000 grams of blood there was present, therefore, 172.9 − 124.0 = 48.9 grams of albumin.

Accordingly, we may compute the amount of serum in 1000 grams of defibrinated blood as follows:

$$\frac{48.9}{72.5} .100 = 67.45 \text{ per cent serum.}$$

$$100 - 67.45 = 32.55 \text{ per cent blood-corpuscles.}$$

After having established the relative amounts of serum and blood-corpuscles, then from an analysis of the blood as a whole, and of the serum alone, we can estimate how much of each substance is present in the blood-corpuscles.

G. von Bunge[2] has shown, by means of the following observation, that this method gives us, within certain limits, quite reliable results. The blood-corpuscles of pig's blood contain no soda. This enables us to compute the relative amounts of serum and blood corpuscles by merely determining the amount of soda in the blood, as a whole, and in the serum by itself.

Bunge found in 1000 grams of defibrinated pig's blood, 2.406 grams Na_2O
in " " " the serum . . . . . 4.272 grams Na_2O

Pig's blood therefore contains

$$\frac{2.406}{4.272} . 100 = 56.3 \text{ per cent serum.}$$

$$100 - 56.3 = 43.7 \text{ per cent blood-corpuscles.}$$

Now if the calculation was made with reference to the albumin content, as in the first case cited, he obtained the values: 56.6 per cent serum and 43.4 per cent blood-corpuscles.

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[1] Cf. Abderhalden: Z. physiol. Chem. **23**, 521 (1897); **25**, 67 (1898).

[2] Z. Biol. **12**, 191 (1876).

	Cow.	Bull.	Sheep I.	Sheep II.	Goat.	Horse I.	Horse II.	Pig.	Rabbit.	Dog I.	Dog II.	Cat.
I. 1000 PARTS BY WEIGHT OF BLOOD CONTAIN:												
Water	808.9	814.8	821.7	824.6	803.9	749.0	795.0	790.6	816.9	810.1	792.0	795.5
Total solids	191.1	185.2	178.3	175.5	196.1	251.0	205.0	209.4	183.1	190.0	208.0	204.5
Hemoglobin	103.1	106.4	92.9	102.8	112.6	166.9	125.8	142.2	123.5	133.4	145.6	143.2
Albumin	69.80	61.79	70.85	58.66	69.72	69.7	62.70	46.61	25.02	39.68	36.41	44.78
Sugar	0.7	0.68	0.732	0.708	0.829	0.526	0.900	0.686	1.026	1.09	0.72	0.851
Cholesterol	1.935	1.209	1.332	2.038	1.299	0.346	0.576	0.444	0.611	1.298	0.922	0.895
Lecithin	2.349	2.197	2.220	2.417	2.466	2.913	2.982	2.309	2.827	2.052	1.994	2.325
Fat	0.567	2.363	0.937	0.864	0.535	0.611	0.534	1.095	0.734	0.631	0.914	0.373
Fatty acids	...	0.495	0.488	0.490	0.395	...	0.387	0.475	0.507	0.759	0.684	0.280
Phosphoric acid as Nucleic acid	0.0267	0.0283	0.0285	0.0344	0.039	0.060	0.059	0.058	0.055	0.054	0.054	0.072
Soda	3.635	3.712	3.638	3.677	3.579	2.691	2.630	2.406	2.785	3.675	3.657	3.686
Potash	0.407	0.407	0.405	0.408	0.396	2.738	1.475	2.309	2.108	0.251	0.258	0.260
Ferric Oxide	0.544	0.562	0.492	0.545	0.547	0.828	0.592	0.696	0.615	0.641	0.706	0.694
Lime	0.069	0.064	0.070	0.069	0.066	0.051	0.054	0.068	0.072	0.062	0.049	0.053
Magnesia	0.0356	0.036	0.033	0.033	0.040	0.064	0.066	0.089	0.057	0.052	0.054	0.059
Chlorine	3.079	3.081	3.080	3.091	2.923	2.785	2.384	2.690	2.898	2.935	2.908	2.815
Phosphoric acid in total ash	0.404	0.392	0.412	0.391	0.397	1.120	1.126	1.007	0.986	0.809	0.812	0.830
Inorganic Phosphoric acid	0.171	0.174	0.190	0.145	0.142	0.806	0.807	0.749	0.685	0.576	0.583	0.555
II. 1000 PARTS BY WEIGHT OF SERUM CONTAIN:												
Water	913.6	913.4	917.4	916.8	907.7	902.1	915.1	917.6	925.6	924.0	923.0	926.9
Total solids	86.36	86.62	82.56	83.19	92.31	97.95	84.94	82.39	74.40	76.02	76.98	73.07
Albumin	72.5	69.73	67.50	68.40	78.07	84.24	70.82	67.74	53.57	60.14	61.12	58.60
Sugar	1.05	1.02	1.06	1.04	1.26	1.176	1.49	1.21	1.65	1.83	1.32	1.52
Cholesterol	1.238	0.901	0.879	1.309	1.070	0.298	0.521	0.409	0.547	0.709	0.658	0.600
Lecithin	1.675	1.869	1.709	1.599	1.727	1.720	1.746	1.426	1.760	1.699	1.755	1.716

Fat	0.926	3.542	1.352	1.262	0.624	1.300	0.834	1.956	1.193	1.051	1.642	0.788
Fatty acids	...	0.743	0.710	0.721	0.611	...	0.604	0.794	0.809	1.221	1.254	0.499
Phosphoric acid as Nucleic acid	0.0133	0.0134	0.0106	0.0161	0.018	0.020	0.015	0.0218	0.025	0.016	0.017	0.016
Soda	4.312	4.316	4.303	4.285	4.326	4.434	4.358	4.251	4.442	4.263	4.293	4.439
Potash	0.255	0.262	0.256	0.254	0.246	0.263	0.254	0.270	0.259	0.226	0.259	0.262
Ferric Oxide	...	...	...	...	...	...	...	...	...	...	...	...
Lime	0.119	0.111	0.117	0.131	0.121	0.111	0.111	0.122	0.116	0.113	0.111	0.110
Magnesia	0.045	0.042	0.041	0.041	0.041	0.045	0.046	0.041	0.046	0.040	0.046	0.043
Chlorine	3.69	3.686	3.711	3.697	3.691	3.726	3.655	3.627	3.883	4.023	4.138	4.170
Phosphoric acid in ash.	0.244	0.235	0.232	0.240	0.237	0.240	0.242	0.197	0.242	0.242	0.250	0.236
Inorg. Phosphoric acid.	0.085	0.062	0.073	0.085	0.070	0.071	0.076	0.052	0.064	0.080	0.082	0.071

III. 1000 PARTS BY WEIGHT OF BLOOD-CORPUSCLES CONTAIN:

Water	591.86	618.63	604.79	627.78	608.72	613.15	613.20	625.61	633.53	644.26	627.16	624.17
Total solids	408.14	381.39	395.23	372.24	391.30	386.84	386.82	374.38	366.48	355.75	372.85	375.82
Hemoglobin	316.74	318.27	303.29	322.05	324.02	315.08	316.31	326.82	331.90	327.52	328.81	329.95
Albumin	64.20	46.00	78.45	37.90	54.03	56.78	50.41	19.19	12.22	9.918	5.32	26.77
Sugar	...	...	...	...	...	...	...	...	...	...	...	...
Cholesterol	3.379	1.824	2.360	3.593	1.730	0.388	0.661	0.489	0.720	2.155	1.255	1.281
Lecithin	3.748	2.850	3.379	4.163	3.856	3.973	4.855	3.456	4.627	2.568	2.296	3.119
Fat	...	...	...	...	...	...	...	...	...	...	...	...
Fatty acids	...	...	...	...	...	...	0.0603	0.062	...	0.088	...	...
Phosphoric acid as Nucleic acid	0.0546	0.0580	0.069	0.0736	0.0806	0.095	0.125	0.1045	0.107	0.110	0.101	0.145
Soda	2.232	2.509	2.135	2.380	2.174	...	...	...	...	2.821	2.856	2.705
Potash	0.722	0.696	0.744	0.739	0.679	4.935	3.326	4.957	5.229	0.289	0.257	0.258
Ferric Oxide	1.671	1.681	1.606	1.707	1.575	1.563	1.488	1.599	1.652	1.573	1.594	1.599
Lime	...	...	...	...	...	...	...	...	...	...	...	...
Magnesia	0.0172	0.026	0.016	0.0187	0.0403	0.0809	0.098	0.150	0.077	0.071	0.065	0.0806
Chlorine	1.813	1.878	1.651	1.801	1.480	1.949	0.460	1.475	1.236	1.352	1.361	1.048
Phosphoric acid in ash.	0.735	0.705	0.822	0.714	0.699	1.901	2.466	2.058	2.244	1.635	1.519	1.605
Inorg. Phosphoric acid.	0.350	0.397	0.455	0.275	0.279	1.458	1.916	1.653	1.733	1.298	1.214	1.186

The results obtained in the analysis of the blood of different species of animals are given on pages 554 and 555,[1] now emphazing, however, that the analyses of the ash have only a relative value, but on the other hand such values may well serve, and in fact have served, as a foundation for further inquiry, although the methods employed are not yet such that the analytical results will prove fruitful in all directions.

From the table it is evident that the serum from various animal species is of much the same composition. There are, however, marked differences in the composition of the blood as a whole, and of the blood-corpuscles. It is interesting that the blood of related animals is very similar. This is apparent when we compare, for example, the relative amounts of the separate constituents of the blood-corpuscles of the carnivora with those of the ruminants. It is certainly not without significance that the blood of both these families contains considerable soda, while that of the horse, pig, and rabbit contains none at all. Certain constituents, as, for example, sugar, fat, and lime, are apparently wanting in the blood-corpuscles. It is rather questionable whether we can assume that the substances are entirely absent. The methods employed are not sensitive enough to make such a decision possible. To be sure, the fact that this result has been repeatedly obtained speaks in favor of its correctness. We may call attention to the fact that quite recently glucuronic acid has also been found in the blood-corpuscles.

We should mention the fact that the amount of blood contained in different animals has been estimated. A sample of blood was taken from the animal in question, after which it was bled to death, and the blood-vessels washed out with water, until the latter came out perfectly clear and colorless. The wash-water was mixed with the blood, collected, leaving out the first sample, and the total volume estimated. Then the first sample was diluted with water until it corresponded in shade with the other mixture. In this way it was easy to compute the amount of blood contained in the animal.

This method is not very accurate, and there are several sources of error. It is, in fact, impossible to remove all the blood from the body in such a way. In the case of dogs the weight of the blood amounts to from seven to nine per cent of the dog's weight, in rabbits the blood corresponds to from five to nine per cent, while in man it is only from one-sixteenth to one-thirtieth of the body-weight.

In human beings, a cubic millimeter of blood contains on an average 5,000,000 red corpuscles in the case of males, and 4,500,000 in females. There is usually one white corpuscle for every 350 to 500 red ones. Naturally these values vary according to the blood-vessel from which the

[1] E. Abderhalden: Z. physiol. Chem. **25**, 67 (1898).

sample is taken. We know, furthermore, that certain conditions greatly affect these values. Thus it is well known that when a large amount of water is passed, the blood may become thickened, while inanition has the same effect. Vasomotor influences also can cause changes in the composition of the blood throughout the entire organism. More and more it has become recognized that it is not always possible to draw conclusions concerning the behavior of the blood as a whole from the examination of a single sample. For experimental work it is naturally most satisfactory to withdraw all of the blood, but when this is not possible, it is always advisable to make several examinations of different samples, remembering at the same time that we are dealing with only relative values.

LECTURE XXIV.

BLOOD AND LYMPH.

In discussing the respiratory exchange, we called attention to the important part played by the red blood-corpuscles in this process; namely, their significance in the transportation of oxygen. We mentioned the important fact that it is not the entire red corpuscle which combines with the oxygen, but that this power is limited to the pigment contained in it, which is known as hemoglobin. This substance is not a simple compound. It consists of two components, which, according to their chemical nature, belong to two entirely distinct classes. One of these components, *globin*, is a protein. On account of the relatively large proportion of bases which it contains, and especially of histidine, it is classed with the histones. We have already mentioned that this classification is to be regarded as a temporary one. Globin contains the same constituents as are usually present in proteins.[1] The other component, which may be separated fairly easily from the globin, contains iron and is designated as *hemochromogen*. In the presence of oxygen the latter is readily oxidized to *hematin*. In spite of a great deal of careful investigation, our knowledge concerning the nature of the combination between globin and hemochromogen is still very incomplete. We merely know that about 4 per cent of hemochromogen can be obtained from hemoglobin.[2] It is still unsettled whether we are justified in assuming that one molecule of globin unites with one molecule of the iron-containing constituent, or whether several molecules of globin are in combination with a single molecule of hemochromogen. There is in fact no absolute proof at hand that even globin itself is a simple substance. We are emphasizing these uncertainties, which in part have been mentioned elsewhere, because hemoglobin has been usually chosen as a foundation for the calculation of the molecular weights of proteins.

When oxygen combines with hemoglobin, *oxyhemoglobin* is formed, and this compound crystallizes readily. From squirrels it crystallizes in six-sided plates of the hexagonal system, while that from other animal species crystallizes in needles, prisms, tetrahedrons or plates of the orthorhombic system. The solubility of the oxyhemoglobins from different species of animals is widely different. That from dogs, for example, is less soluble

[1] Abderhalden: Z. physiol. Chem. **37,** 484 (1903).

[2] F. N. Schulz: *ibid.* **24,** 449 (1898).

than that from cats.[1] More soluble, and for that reason more difficult to prepare, are the oxyhemoglobins from the blood of men, cattle, and pigs.[2] It has been attempted to draw conclusions concerning the uniformity or differences in the different kinds of oxyhemoglobin by studying their elementary compositions. We shall cite a few of such analyses in the table below, but will state again, that the elementary composition of such complicated compounds signifies scarcely anything at all. Even if it were possible to decompose hemoglobin quantitatively into simpler components, we would not be justified in assuming, if we obtained the same relative amounts of the various constituents, that the different kinds of hemoglobin were uniform. It may be that the various amino acids are arranged in a different order in the globin molecule, to say nothing of the various possibilities for the formation of isomers. We hold that it is extremely essential to emphasize the fact that the elementary analyses of proteins and their complicated cleavage-products should only be used with great caution as a basis for drawing conclusions, or for further investigations, and that the real value of each ultimate analysis is but very slight.

ELEMENTARY ANALYSIS OF OXYHEMOGLOBIN.

In per cents.

	C	H	N	S	O	Fe	P
Horse's blood	54.75	6.98	17.35	0.42	20.12	0.38	0.[3]
Dog's blood	54.57	7.22	16.38	0.57	20.43	0.34	0.[4]
Cat's blood	54.60	7.25	16.52	0.62	20.66	0.35	0.[6]
Pig's blood	54.17	7.38	16.23	0.66	21.37	0.43	0.[5]
Beef blood	54.42	7.18	17.45	0.48	20.07	0.40	0.[5]
Guinea pig's blood	54.12	7.36	16.78	0.58	20.68	0.48[7]	...
Squirrel's blood	54.09	7.39	16.09	0.59	21.44	0.4[7]	...[7]
Goose's blood	54.26	7.10	16.21	0.54	20.69	0.43	0.34[7]
Hen's blood	52.47	7.19	16.45	0.86	22.5	0.34	0.20[4]

We find from these analyses that the hemoglobin of mammals contains the elements carbon, hydrogen, nitrogen, sulphur, oxygen, and iron, while that of birds contains phosphorus in addition. It is very questionable whether the phosphorus content is due to a peculiarity of the oxyhemoglobin in birds or whether it is not rather due to an impurity. We remem-

[1] Abderhalden: Z. physiol. Chem. **24**, 545 (1898), and F. Krüger: Z. Biol. **26**, 469 (1890), and Z. physiol. Chem. **25**, 256 (1898).

[2] G. Hüfner: Beiträge zur Lehre vom Blutfarbstoff (1887).

[3] Abderhalden: Z. physiol. Chem. **37**, 484 (1903).

[4] A. Jaquet: Dissert. Basel, 1899, and Z. physiol. Chem. **12**, 285 (1888).

[5] J. C. Otto: *ibid.* **7**, 57 (1882).

[6] According to the author's analyses.

[7] Hoppe-Seyler: Med.-Chem. Untersuchungen, p. 366 (1868).

ber that the red blood-corpuscles of birds contain nuclei and considerable amounts of nuclein substances. It is perfectly possible that the presence of such an impurity accounts for the apparent phosphorus content in the hemoglobin of different species of birds, whose blood has been studied. This assumption appears more probable when we state that the oxyhemoglobin from birds has never been prepared in a satisfactory manner, nor purified to the extent accomplished with that from animals, and, moreover, if we examine the beautifully formed crystals under the microscope, we shall find that they may include within themselves considerable amounts of impurity. In the hemoglobin from horses, pigs, and cattle, two atoms of sulphur are present for each atom of iron, while in the blood of dogs, the iron is to the sulphur as 1 : 3. We may also mention the fact that the various oxyhemoglobins contain different amounts of water of crystallization. It is still an open question whether the oxyhemoglobin from one and the same species of animals is always identical. C. Bohr [1] holds that this is not the case. He believes he has proved that differences exist by determining the power of combining with oxygen in different fractions of crystals from a single kind of blood. Hüfner,[2] whose investigations in this field have been thorough and most carefully made, holds that such an assumption is not justifiable. It must be admitted that it is not easy to prove beyond all doubt that there is an actual difference in different oxyhemoglobins. There is always the possibility that the observed differences may arise from secondary changes which have taken place in the oxyhemoglobin that is under examination.

As regards the combination of the oxygen in hemoglobin, we have already seen that only the hemochromogen takes part in this, and that the iron is of much significance here.

The spectroscopic behavior of oxyhemoglobin is very characteristic. A dilute solution shows in the spectroscope two absorption bands in the yellow and green, between the Fraunhofer lines *D* and *E*. The band near the *D* line is narrower than that near the *E* line. Arterial blood gives the same absorption spectrum on account of the presence of oxyhemoglobin in it. We must also add that reduced oxyhemoglobin, the true hemoglobin, likewise shows characteristic absorption bands. A solution of hemoglobin, of not too great a concentration, shows a single broad band, not very sharply defined, lying between the *D* and *E* lines; in fact, this band extends a little beyond the *D* line into the red end of the spectrum. Venous blood shows such a spectrum, although, except in cases of suffocation, there is always some oxyhemoglobin present. The greater part of the oxyhemoglobin in such blood has, however, been reduced. Consequently venous blood does not have the bright red color of arterial blood. It is darker

[1] Zentr. Physiol. **4**, 249 (1890).

[2] Arch. Anat. Physiol. **1894**, 130

and has a more violet color. The shade of color naturally varies according to the ratio of the oxyhemoglobin to the hemoglobin. Hemoglobin is more readily soluble in water, and for this reason more difficult to prepare and maintain in a crystalline form. It may be obtained easily from oxyhemoglobin by the withdrawal of oxygen, and this may be accomplished by placing the oxyhemoglobin in vacuum, conducting an indifferent gas through its solution, or by the use of a reducing agent. Beautiful crystals of hemoglobin are also obtained by allowing a solution of oxyhemoglobin to stand for some time in a sealed glass tube.[1] The oxygen of the oxyhemoglobin is gradually consumed, and hemoglobin is formed by the reduction.

Carbon monoxide[2] may take the place of oxygen in the oxyhemoglobin molecule. It evidently is fastened at the same part of the molecule as the oxygen, for it replaces the latter by its action upon oxyhemoglobin, and makes it incapable of combining with oxygen except in the presence of a large excess of the latter gas. It is herein that the poisonous action of carbon monoxide lies. Carbon-monoxide-hemoglobin can be obtained in a crystalline form, its crystals being isomorphous with those of oxyhemoglobin. Its absorption spectrum is very similar. It also shows two absorption bands, which are, however, nearer the violet end of the spectrum. The action of reducing agents does not have the effect upon its spectrum that is obtained with oxyhemoglobin. The two absorption bands do not dissolve into one band, or at least not within a short time.

Hemoglobin is also capable of combining with nitric oxide,[3] NO, and this last gas is even capable of driving carbon monoxide out of its combination in the blood. Nitric-oxide-hemoglobin is also crystalline and very stable. It shows an absorption spectrum very similar to that of oxyhemoglobin except that the bands are paler. It is even less affected by reducing agents than is the carbon-monoxide compound.

The action of hydrogen sulphide[4] upon oxyhemoglobin gives rise first of all to the formation of hemoglobin. Then, little by little, a greenish-black coloration is formed, which reminds one of the appearance of a trace of ferrous sulphide. This green shade is due to the formation of sulphhemoglobin, which, however, has never been prepared in a pure state. It may be distinguished from hemoglobin by means of its spectral behavior.

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[1] G. Hüfner: Z. physiol. **4**, 382 (1880). If oxyhemoglobin is allowed to stand for two weeks in a quiet place at 40° C., crystals from 1 to 2 centimeters long can be obtained.

[2] G. Hüfner: Arch. Anat. Physiol. **1895**, 209, 213. Hüfner and Küster: *ibid.* **1904**, 387.

[3] L. Hermann: Arch. Anat. Physiol. **1865**, 469. Hüfner and Reinbold: **1904**, Suppl. II, 391.

[4] Hoppe-Seyler: Zentr. Med. Wissensch. **1863**, No. 28, p. 433. T. Araki: Z. physiol. chem. **14**, 405 (1890). E. Harnack: *Ibid.* **26**, 558, (1898-99).

It shows one absorption band in the green, and another in the orange-red, between the *C* and *D* lines. If hydrogen sulphide acts upon hemoglobin in the presence of oxygen, the hemoglobin is completely decomposed, so that the product formed, as well as its derivatives, no longer shows a characteristic absorption spectrum.

Quite different from the above compounds is *carbohemoglobin*, in which carbon dioxide is present, but combined at a different place in the molecule from that occupied by the oxygen in oxyhemoglobin. In fact, carbon dioxide and oxygen are taken up by hemoglobin quite independently of one another. The carbonic acid is evidently combined with globin, while oxygen combines with hemochromogen.

Methemoglobin[1] also occupies a unique position. It corresponds to oxyhemoglobin in its composition, and differs from it merely in holding the oxygen in a firmer state of combination. It may be formed from the latter on standing, or be prepared by the action of various agents, such as iodine, chlorates, permanganates, nitrites, nitrates, palladium hydride, pyrogallol, quinol, or ozone. The formation of methemoglobin has also been observed by the action of aniline, toluidine, acetanilide, acetophenetidine, and glycerol upon oxyhemoglobin. Methemoglobin may be formed even in the circulating blood, when it comes in contact with substances such as amyl nitrite, nitrobenzene and antifebrin.

The oxygen cannot be removed from methemoglobin by reducing the partial pressure of this gas. At present it is not known just how this transformation of oxyhemoglobin into methemoglobin is effected. It has been established positively that both compounds contain the same amounts of oxygen. Reducing agents tend to convert methemoglobin back into oxyhemoglobin. Methemoglobin crystallizes in brownish-red needles, prisms, and also in six-sided plates. It is most readily prepared by adding potassium ferricyanide solution to a solution of oxyhemoglobin and then, after cooling to 0° C., adding one-fourth its volume of alcohol. In acid solutions methemoglobin shows an absorption spectrum of a band in the orange-red between the *C* and *D* lines, and a second paler band in the blue between the *G* and *F* lines. Besides these absorption bands, the acid solutions show two other bands in the same place as the bands which characterize the spectrum of oxyhemoglobin. It seems probable that these last two bands are not characteristic of methemoglobin, but are due to the presence of some oxyhemoglobin as impurity. In alkaline solutions methemoglobin shows three lines, one on either side of the *D* line, and one near the *E* line. The spectrum of methemoglobins is, in fact, very similar to that of hematin.

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[1] Hoppe-Seyler: Zentr. med. Wissensch. **1863**, No. 28. G. Hüfner: Z. physiol. Chem. **8**, 366 (1884). Hüfner and Otto: *ibid.* **7**, 65 (1882–83). A. Jäderholm: Z. Biol. **16**, 1 (1880); **20**, 419 (1884). R. von Zeyneck: Arch. Anat. Physiol. **1899**, 460.

It is highly probable that hemoglobin not only occurs in the red blood-corpuscles, but in the muscles as well. The latter likewise contain a red pigment which cannot be washed out by way of the vascular system, and which, according to its entire behavior, is certainly closely related to hemoglobin. The relation of this pigment to hemoglobin has never been satisfactorily explained.

Hemoglobin is decomposed by gentle attack into its two components, globin and hemochromogen. The separation can be effected, for example, by adding a few drops of dilute acid to a hemoglobin solution which is free from salts. *Acid hemoglobin* is formed as an intermediate product. It shows an absorption spectrum similar to that of methemoglobin. By more energetic action of the acid, hemochromogen is split off, but only when the solution is kept out of contact with the air. In the presence of air, hematin is always formed; from the latter, by reduction, hemochromogen may be obtained. The digestive juices of the stomach and pancreas are also capable of effecting this separation of hemoglobin into its two constituents.

Hematin, whose reduction product, hemochromogen, plays such an important part in allowing the blood to combine with oxygen, has been carefully studied in recent years. Although its constitution has not been established positively, still we are now able to explain certain relations existing between it and other compounds of similar construction. The most important work in this field has been that of Nencki [1] and that of William Küster.[2] According to Küster, the empirical formula of hematin is $C_{34}H_{34}N_4FeO_5$. Nencki and Sieber assume its formula to be $C_{32}H_{32}N_4FeO_4$. The starting-point of these investigations was not usually hematin itself, but its hydrochloric acid ester, hemin, or Teichmann's crystals as it is sometimes called. Several different formulæ have been proposed for this ester.

It is questionable whether hemin is actually to be regarded as the hydrochloric acid ester of hematin. Nencki in his work stated that it was not formed by merely annexing the hydrochloric acid to the hematin molecule, but that there was a replacement of an OH group by chlorine:

$$\underset{\text{Hematin}}{C_{32}H_{32}N_4FeO_4} + HCl = \underset{\text{Hemin}}{C_{32}H_{31}ClN_4FeO_3} + H_2O.$$

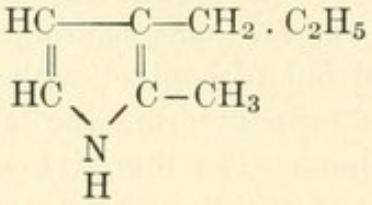
[1] Nencki and Sieber: Arch. exper. Path. Pharm. **24,** 430 (1888), and Monatsh. **9,** 115 (1888). Nencki and Rotschy: *ibid.* **10,** 568 (1889). Nencki and Zaleski: Z. physiol. Chem. **30,** 384 (1900); Ber. **34,** 997 (1901).

[2] Ber. **27,** 572 (1894); **29,** 821 (1896); **30,** 105 (1897); Z. physiol. Chem. **28,** 1 (1899); **29,** 185 (1900); Ber. **32,** 678 (1899); **33,** 3021 (1900); **35,** 1268, 2948 (1902); Ann. **315,** 174 (1900). Z. physiol. Chem. **44,** 391 (1905); Ann. **345,** 1 (1906).

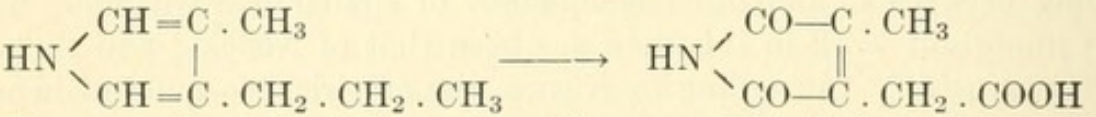
The iron in hematin may be removed easily by the action of acid. A product free from iron is thus obtained which is known as *hematoporphyrin* and is given the formula $C_{16}H_{18}N_2O_3$. Thus, for example, we may allow hydrobromic acid to act upon hematin:

$$\underset{\text{Hematin}}{C_{32}H_{32}N_4FeO_4} + 2\ H_2O + 2\ HBr = 2\ \underset{\text{Hematoporphyrin}}{C_{16}H_{18}N_2O_3} + FeBr_2 + H_2.$$

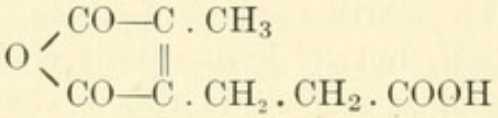
By reduction of hematoporphyrin, *mesoporphyrin* $C_{16}H_{18}N_2O_2$ is obtained, or if the reducing agent is more energetic, *hemopyrrole*, $C_8H_{13}N$, is formed. This last substance is methyl-propyl-pyrrole:



By oxidizing hematin, Küster obtained two crystalline acids, which he designated as *hematinic acids*. One of these is a dibasic acid with the empirical formula $C_8H_9NO_4$, while the other is to be regarded as the anhydride of a tribasic acid, $C_8H_8O_5$. The formation of these two hematinic acids is illustrated by the following schema:



Hemopyrrole or Methylpropylpyrrole — Dibasic hematinic acid



Partial anhydride of tribasic hematinic acid.

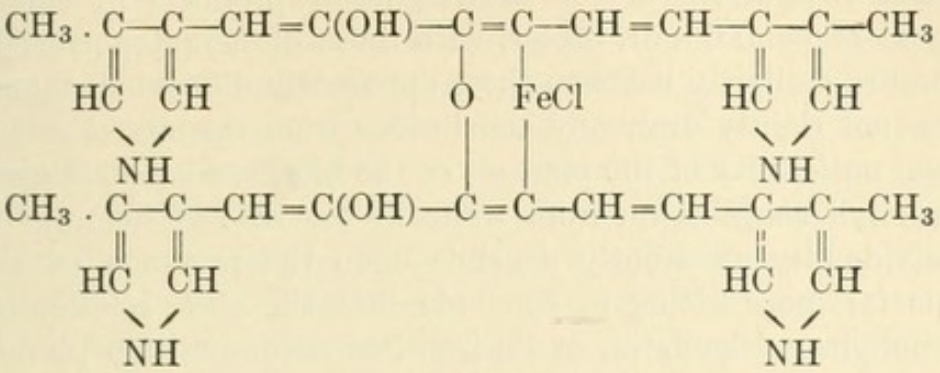
The last compound, by losing carbon dioxide, goes over into the anhydride of methyl-ethyl-maleic acid, $C_7H_8O_3$.

If we assume that hematin is a simple substance and not a mixture, and that its transformation into hematoporphyrin takes place quantitatively according to the equation,

$$C_{32}H_{32}N_4FeO_4 + 2\ H_2O - Fe = 2\ C_{16}H_{18}N_2O_3,$$

then it is very easy to go a step farther and assume that hematin is constructed of two symmetrical halves connected together by means of an atom of iron. Now, as we have seen above, hematin and hematoporphyrin, by undergoing an oxidation and hydrolysis, both give rise to the same acids and to the same extent. As these cleavage-products each contain eight atoms of carbon, it may be safely assumed that hematoporphyrin likewise is composed of two equal parts. As was mentioned,

the hematinic acids are to be regarded as the oxidation products of hemopyrrole. On this basis we can assign to hemin, the hydrochloric ester of hematin, the following structural formula:



The correctness of the above formula has not been established in all its details.[1] It should serve merely to give us an approximate picture of the structure of hematin. We will state in this connection that the question has been discussed often, whether the hematin of different species of animals, or even of animals in the same species, has a uniform composition, or whether we shall have to assume the existence of different hematins. This question arose from the fact that different observers claimed to isolate hemins of different compositions so that the hematins from which they were made must have been different. The most recent investigations, however, make it seem more probable that there is but one hematin.[2] The observed differences in the composition of hemin may be explained partly by the different ways in which the substance was prepared, and partly by the tendency that hemin has of crystallizing out together with a portion of the solvent.

From the experiments performed in the attempt to explain the constitution of hematin, interesting relations have been discovered between it and a color-principle, which for a long time has been assumed to exert quite similar biological functions. We refer to chlorophyll, the pigment of green plants; although it would be perhaps better to include under the name *chromophyll* all the different pigments of the vegetable kingdom which exert parallel functions. At present, however, chlorophyll, the green pigment, is the only one of such substances which has been studied exhaustively. It is hardly to be doubted that the other pigments in the vegetable kingdom which play the same part in plant economy as that of chlorophyll have quite similar compositions. We have already stated that we are not justified in regarding the function of chlorophyll as parallel to that of hemoglobin, or to hemochromogen. It appears,

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[1] Cf Lecture XVII, p. 396.

[2] William Küster: Z. physiol. Chem. **29**, 185 (1900); **40**, 391 (1904). K. A. H. Mörner: *ibid.* **41**, 542 (1904).

on the contrary, that chlorophyll participates in the metabolism of plants, and especially in the assimilation processes, in a way that finds no analogy in the case of the pigment of the blood. We may say, however, that a comparison of the disintegration products of chlorophyll with those of hematin, or, better, with those of hematoporphyrin, shows considerable similarity between these apparently unlike substances. And yet we cannot rightly draw any conclusions from this agreement as to the biological uniformity of the pigment of the blood and that of green leaves. Chlorophyll contains no iron, while in the case of hematin its functional individuality apparently depends upon the presence of this element. It is far more fitting to conclude that the close relationship between chlorophyll and hematin, or its iron-free decomposition product hematoporphyrin, is explained by the fact that hematin is formed from chlorophyll. Unfortunately, no one has been able to prove positively that chlorophyll is actually the mother-substance of hematin. Chlorophyll is unquestionably transformed to a considerable extent while in the alimentary canal;[1] apparently various decomposition products are formed. It is not impossible that the animal organism may make use of these products for the synthesis of hematin. We make this suggestion because again and again we are forced to admit that the animal organism is greatly dependent upon the synthetical work of the vegetable kingdom. To be sure, the animal cells are capable of effecting complicated synthesis, but they require for this purpose building material which has already been well worked over. The plant cells are capable of producing such material from the elements. Now hematin is a compound of highly complicated structure. It is then hardly probable that the animal organism, which in every other case makes use when possible of the building stones furnished by the vegetable kingdom for accomplishing its synthesis, leaves this material available for the construction of hemochromogen actually untouched, and instead effects the complicated synthesis of hematin from the very simplest material. Here unquestionably is a great gap in our present knowledge, — a gap which has resulted from the attempt to decide the question whether *inorganic* iron compounds or only *organic* ones can be utilized by the animal cells for the synthesis of hematin. We have already shown what a subordinate position is taken by the iron assimilation compared to the formation of the complicated hematin molecule. We cannot yet decide this question, but desire to leave the impression that it is by no means improbable that the herbivora obtain in the chlorophyll of their fodder the building material for that component of hematin which contains the iron; and that the carnivora also utilize the color-principle of the blood which is contained in their food for the formation of their own hemochromogen, perhaps, to be sure, only after the hematin in

[1] Cf. L. Marchlewski: Z. physiol. Chem. **41**, 33 (1904).

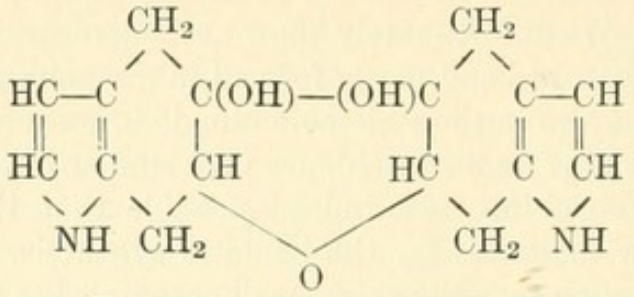
the food has undergone a more or less complete preliminary disintegration. The fact that large amounts of decomposition products of chlorophyll are found in the fæces of herbivora, does not in any way speak against the above assumption. We unfortunately know nothing regarding the extent to which hematin is destroyed and newly formed in the animal organism. In the yolk of eggs, and also in the vegetable kingdom, we meet with nuclein-like substances which, as we have said, are very similar in their elementary composition to hemoglobin. It is indeed possible that these substances are used as the raw materials for the hematin synthesis. We do not in any case need to assume that the animal cell accomplishes the complicated construction of hemochromogen from simple building material. We only wish to sound another warning against drawing any conclusions from the elementary composition of such complicated products. The formation of hemoglobin, and especially of hematin, in the animal organism is a process which remains absolutely unexplained. The question whether iron in an inorganic or organic condition is assimilated in order to take part in the synthesis, is far less interesting than that concerning the building material of the hematoporphyrin. In order to make our position perfectly clear, we will repeat again that we hold it to be perfectly possible that iron itself, or in the form of salts, may be utilized as such for combining the two hematoporphyrin molecules, i.e., in other words the iron is not necessarily an organic constituent of hematin or of hematoporphyrin, and perhaps it can take part in the synthesis only after it has been set free from any organic compounds which may contain it. A glance at the above tentative formula of hematin gives us some idea of the separate phases in its construction.

In the breaking down of chlorophyll, Schunck and Marchlewski [1] ran across a derivative which they called *phylloporphyrin*. It has the following empirical formula, $C_{16}H_{18}N_2O$. Hematoporphyrin corresponds to the formula, $C_{16}H_{18}N_2O_3$. According to this the two compounds differ from one another in the amount of oxygen which they contain. Marchlewski [2] showed that both of these substances are to be regarded as different oxidation products of one and the same mother-substance, by obtaining hemopyrrole, as well as the hematinic acids, from phylloporphyrin. Thereby the close relationship between phylloporphyrin and hematoporphyrin was established.

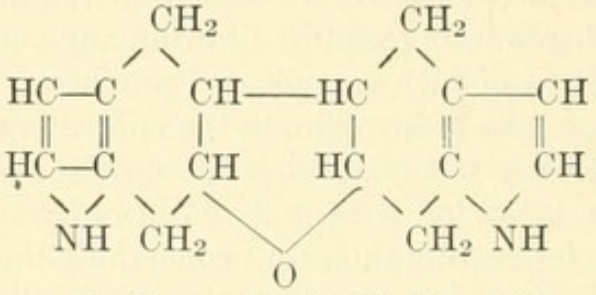
Nencki and Zaleski [3] attempted to transform hematoporphyrin directly into phylloporphyrin. They were able, however, to remove but one of the hydroxyl groups from the hematoporphyrin. They obtained the so-called *mesoporphyrin* which occupies an intermediate position between

[1] Ann. **278**, 329 (1894); **284**, 81 (1895); **288**, 209 (1895); **290**, 306 (1896).
[2] J. pr. Chem. **65**, 161 (1902).
[3] Ber. **34**, 997 (1901).

hemato- and phyllo-porphyrin. The following formulæ show the close relationship between these last two compounds:



Hematoporphyrin: $C_{16}H_{18}N_2O_3$.



Phylloporphyrin: $C_{16}H_{18}N_2O$.

Now that we have shown the relations of hematin to chlorophyll, we shall return to the pigment of the blood and describe the products formed from its disintegration. While we are not yet in a position to give a very satisfactory picture of the formation of hemoglobin, or hematin, we are better informed concerning its decomposition products. The red corpuscles are without doubt being constantly destroyed, and thereby hemoglobin is set free. This breaks down, according to our present knowledge, first into hematin and globin. The latter is probably further decomposed exactly as is usually the case with proteins. A substance which for a long time has been recognized as one of the decomposition products of hematin, is a pigment occurring in the bile which is known as bilirubin, and has the empirical formula $C_{16}H_{18}N_2O_3$ which corresponds to that of hematoporphyrin. That, as a matter of fact, these two compounds are very closely related to one another has been shown by Küster,[1] who obtained from bilirubin, by the same methods that were used with hematin, two hematinic acids which correspond to those obtained from hematin. Bilirubin and hematoporphyrin appear to be isomers. The transformation of hematin into the biliary pigment takes place in the cells of the liver. If the blood is injected under the skin of an animal, there will be noticed an elimination of iron at that very spot. The greater part of the blood's pigment, however, will reach the circulation and be carried to the liver. Here again the iron is first of all

[1] Z. physiol. Chem. **26**, 314 (1898); Ber. **32**, 677 (1899); *ibid.* **35**, 1268 (1902); Z. physiol. Chem. **47**, 294 (1906).

removed. The large deposits of iron in the liver indicate the extent to which this process has taken place. Bilirubin is not the only pigment present in the bile. Oxidation products are likewise present which represent different stages to which bilirubin has been oxidized. We need not mention their names here, partly because their relations to bilirubin are not very well known, and partly because it is hard to decide which of these colored substances are previously formed in the cell and which result from secondary processes. The best known of these substances is *biliverdin*, which may be readily prepared from bilirubin by allowing an alkaline solution of it to stand in contact with the air. Oxygen is absorbed, and the solution turns green. One of the tests for the biliary pigments, the so-called *Gmelin's test for bile-pigments*, is based upon the readiness with which bilirubin is oxidized. If a solution of bilirubin-alkali is cautiously covered in a test tube with a little nitric acid containing some nitrous acid, color rings appear at the contact surface of the two liquids and in the following order from top to bottom: — green, blue, violet, red, and reddish-yellow. These different colors are due to different stages resulting from the oxidation of bilirubin. Before considering the subsequent destiny of the bile-pigments, we must answer the question whether the liver is, under normal conditions, the only organ in which the bile-pigments are formed, or whether it is not perhaps merely the place where these pigments are eliminated.

Even Virchow[1] recognized the fact that peculiar transformations take place in blood extravasations. The protein component of the hemoglobin and the remaining constituents of the blood disappear as such, and there remain beautifully formed crystals of brick-red to ruby-red color. They are known as hematoidin. This substance contains no iron, and is considered by many to be identical with bilirubin. According to our present knowledge, nothing further can be said concerning this pigment than that it is closely related to hematin and to hematoporphyrin. Besides this pigment we will state that pigments containing iron have also been observed as being formed in the tissues, and that there has been a constant endeavor to trace a relationship between all the animal pigments, whether resulting from normal or from pathological processes, to the blood-pigment. The most pronounced characteristic that has been noted, however, in every case is as regards the presence or absence of iron. We would refer merely to what was said in considering the formation of hematoidin to show that the iron content in no way indicates the origin of the pigment. Our knowledge in this direction is still far too limited for us to predict much concerning the relations of the animal pigments to the other compounds found in the tissues.

[1] Virchow's Arch. **1**, 379 and 407 (1847).

The fact that under certain conditions a compound may be formed from the blood-pigment in the tissues which is very similar to the bile-pigment is proved by the discovery of hematoidin. Whether under normal conditions such products are formed in organs other than the liver remains undecided. In pigeons it has been found that after ligating the bile-duct, the bile-pigments are found at the end of five hours in the blood-serum. If at the same time the blood-vessels of the liver are also bound, the bile-pigments cannot be detected in the blood nor in the tissues after several hours.[1] Minkowski and Naunyn[2] arrived at the same result. They extirpated the liver from a goose, and exposed this goose together with a normal one to the action of arseniureted hydrogen. Whereas the latter showed within a short time a copious elimination of urine containing biliverdin, with the former only hemoglobin was found in the urine. Such experiments have not yet been carried out with mammals on account of the great difficulty in completely extirpating the liver from them. We may safely assume, that with them also the liver is the sole place where the bile-pigment is formed.

For quite a time this assumption was doubted. It had been observed that if for any reason the flow of bile to the intestines was prevented, bile-pigments would appear in the tissues. There is a yellowish coloration of the skin and of the mucous membrane. The complex of symptoms which are produced when there is such a stoppage in the flow of the bile, is known as icterus, or jaundice. Formerly there was a distinction made between icterus of the above-mentioned etiology, also designated as *hepatogenic icterus*, and *hematogenic icterus*. The reason for this was because it had been observed that when for any reason there was an increased destruction of blood-pigment, whether due to the action of poisons (arseniureted hydrogen, ether, chloroform, toluylene-diamine) or by infectious diseases, then bile-pigment passed into the urine even when the flow of bile into the intestines was unrestricted. It was easy to imagine from this that in such cases the blood-pigment was directly transformed while in the blood-vessels into bile-pigment. It is not necessary, however, to make any such assumption. The fact has been established that intravenous injection of bilirubin causes a considerable increase in the elimination of bile-pigment in the bile.[3] This observation indicates that the liver also can cause the elimination of bile-pigment, even when it is circulating in the blood-vessels. Now it is possible that when there is a greatly increased disintegration of the blood it may eventually contain so much hemoglobin, and finally so much

[1] Hans Stern: Arch. exper. Path. Pharm. **19,** 39 and 42 (1885).

[2] *Ibid.* **21,** 1 (7) (1886).

[3] J. F. Tarchanoff: Pflüger's Arch. **9,** 53 (1874). A. Vossius: Arch. exper. Path. Pharm. **11,** 427 (1879).

bile-pigment, that it becomes impossible for the liver to take away all of it.

Stadelmann[1] has shown it to be very probable that in spite of the apparent unhindered passage of bile into the intestine, nevertheless there may be a stoppage in the flow of the bile. The bile flows under a very slight pressure, so that the slightest obstruction will stop it, and thereby cause an absorption of bile by the lymphatics. This may result from a greatly increased secretion, or from the greater viscosity of the concentrated bile. At all events, according to these observations we are not justified in assuming that the transformation of hematin into bile-pigment takes place in any other organ than the liver.

We shall mention in addition that the restricted flow of the bile towards the intestines may lead to severe nervous disturbances. Cerebral effects result, causing delirium, convulsions, coma, and finally death. It has never been found possible to establish satisfactorily the cause of these phenomena. It is important to know that they almost invariably result from chronic obstructions to the flow of the bile. It has been assumed that the absorbed constituents, and their segregation in the tissues and blood, were the cause of these severe disturbances. There is no proof of this, however. We must not forget that where there is a chronic obstruction to the flow of the bile, it is certain that the metabolism of the whole liver must suffer as a result. The central position of the liver in the general metabolism has already been indicated. It is clear that if any one of its important functions is entirely abolished, this is likely to affect the entire organism. On the other hand, the objection may be raised that the liver can undergo all sorts of severe treatment without necessarily causing any disturbance in this direction. It may be said, however, that we are not able to draw conclusions solely on the basis of anatomical changes concerning the functional condition of a tissue. It is an open question as to which part is first and most seriously affected. A serious suppression of metabolism in the cells may take place without there being any indication of it in the external appearance of the cells or of the contents, and, on the other hand, there may be very great anatomical changes in a tissue without the functions being other than normal, as long as the cell complex or the constituents of the cells do not take part in a pathological process.

In the transformation of hematin into bilirubin, iron is split off. What becomes of it we do not know. Only a part is eliminated with the bile itself. It is possible that it is immediately utilized again, or that it chooses to be eliminated through the intestines.

The constituents of the bile, especially cholesterol and the bile-

[1] Arch. exper. Path. Pharm. **14**, 231 and 422 (1881); **15**, 337 (1882); **16**, 118 and 221 (1883).

pigments, often serve for the formation of concretions and calculi in human beings. It is not easy to say what causes the formation of these concretions, which often lead to such severe symptoms. Apparently the primary cause is usually a catarrh of the bile-ducts. Crystals of the above-mentioned substances are then, as a secondary effect, deposited upon the diseased mucous membrane. Naturally changes in the conditions of solubility and in the relations of concentration also play a part in their formation. The *pigment-stones* usually contain the calcium compound of bilirubin. Often mixed stones are found, although quite frequently we meet with stones of pure cholesterol which show, in a fractured surface, beautiful clusters of crystals.

The bile-pigments appear in the fæces to some extent as such. For the most part, they succumb to the putrefactive processes in the alimentary canal, especially those of the large intestine. There is a reduction of the bilirubin, and *urobilin* is formed. This last substance may be obtained directly by the reduction of hematin, or of hematoporphyrin.[1] It is also formed by oxidation, when hemopyrrole is allowed to stand in contact with the air.[2] Finally, it has been established that by feeding hemopyrrole to rabbits an elimination of urobilin results. Urobilin, like hematin, is supposed to contain four molecules of hemopyrrole and to have the following empirical formula: $C_{32}H_{40}O_7N_4$. It is not absolutely known whether urobilin is identical with the so-called *hydrobilirubin* which is obtained by the reduction of bilirubin. Urobilin is absorbed in the intestine and passes into the urine. It helps to give to urine its yellow color. Different urobilins have been described as present in urine, and on the other hand it has even been asserted that urobilin is not originally present as such in the urine, but is formed by the action of light. We need not stop to discuss this question, because at present it is all a matter of conjecture, and we are forced to base our assumptions in part upon some very doubtful observations. It is exactly the same with the question as to the origin of urobilin. No one doubts that it is formed in the intestines as a result of putrefaction, but it is questioned by many that all the urobilin in the urine arises from this source. Many assume that urobilin is formed outside of the intestines and in the tissues from bilirubin.

Closely related to urobilin is *urochrome*, the principal yellow pigment contained in urine. Its chemical nature is but little known. *Uroerythrin* also frequently occurs in urine, and is likewise of unknown composition. It is the red pigment which causes the color of the sediment in urine, and is also known as *Sedimentum lateritium*.

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[1] Nencki and Sieber: Arch. exper. Path. Pharm. **18**, 401 (1884).

[2] Nencki and Zaleski: Ber. **34**, 997 (1901).

Small amounts of hematoporphyrin are also present in urine. It is not without interest that the amount of this substance increases after certain kinds of poisoning, e.g. lead, trional, sulfonal, and in certain diseases of the liver. The urine then has the red color of Burgundy.

We have now mentioned what is known of the destiny of the blood-pigment in the animal organism, and have become acquainted at the same time with a new, important function of the liver-cells. We must admit that there are still many gaps which must be bridged over with regard to our knowledge of the disintegration of hematin, and there are a great many contradictory results obtained in the study of the final end-product, urobilin, which remain to be explained.

It might be expected that some insight into the construction of hemoglobin would be obtained if we were able to find out where it is formed. Up to the present time this has not been definitely settled. We are inclined to believe that hemoglobin is formed at the place where the red blood-corpuscles come into existence, but even the origin of these corpuscles is in doubt. Bone-marrow is chiefly considered in this connection; and in cases of an unusual new formation of the blood-corpuscles, the extraordinary activity of the cancellous tissue is apparent, even to the naked eye, by the marked red coloration. It has been asserted frequently that the spleen is also able to produce red blood-corpuscles, but this assertion has been contradicted. It has been attempted to decide this question by extirpating the spleen. The operation is withstood quite well by the organism. While some authors have claimed that there resulted a marked diminution in the number of red corpuscles, others were not able to find this the case. Finally, the spleen has been assigned a part in the destruction of the red corpuscles. Unquestionably this last assumption is not well founded. To be sure, the spleen usually contains iron. Experience also teaches us that the spleen is able to capture foreign substances from metabolism and to retain them, and on the other hand, by administering iron salts the iron content of the spleen is considerably increased within a short time, without there being any evidence of a destruction of red blood-corpuscles. At all events, the part played by the spleen, either in the formation or the destruction of red blood-corpuscles, is absolutely unexplained. The same is true regarding the function of the so-called *hemal-lymph glands* which are joined to the aorta and differ from the ordinary lymph-glands by the fact that the lymph-passages are either missing or incompletely developed. The capillaries from the arteries pass directly into the lymph sinus and into the blood-spaces representing the interfollicular lymphatics, and from thence the veins lead out directly. The hemal-lymph glands occupy anatomically an intermediate position between the ordinary lymph-

glands and the spleen. Their function has never been satisfactorily explained.[1]

We must now mention an important kind of cells which are intermediate between those of the blood and of the tissues. The blood does not communicate directly with the tissue-cells. It neither imparts nutritive material directly to them nor receives the products of metabolism directly from them. In this connection the kidneys occupy an exceptional position, for in them the blood-vessels of the *Glomeruli Malpighi* lie directly at the end of a uriniferous tube. The reason for this is clear: in this way the waste-products contained in the blood are given up to the urine as quickly as possible.

In the other tissues of the body, the exchange of material between the blood and tissue takes place through the lymph. First of all we must consider the formation of lymph. We may say at once that it has two sources of material, — the blood on the one hand, and the tissues on the other. Qualitatively it contains the same substances as the blood-plasma. Quantitatively, however, there is a difference between the two liquids. The formation of the lymph, which penetrates into all of the tissues, has been repeatedly referred to a purely physical process, as, for example, that of a filtration. We must admit that unquestionably, purely physical processes do come into play in the giving up of material by the blood and by the tissues to the lymph, and, on the other hand, in the passage of material from the lymph either into the blood or tissues.[2] We have a great many reasons, however, for not being willing to admit that our present knowledge concerning the formation of lymph and all its functions is in accordance with the assumption that only physical forces are concerned here.

In considering processes which are much easier to understand than the formation of lymph, we have constantly encountered phenomena which indicate the existence of a specific activity for every individual cell, whether it be that of secretion or of absorption. We must admit that it is far more difficult to study accurately each individual process when the relations are so complicated as they are in the animal organism. In every case there are a number of different processes which we at present cannot examine very closely. One process crosses another,

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[1] See J. Seemann: Die blutbildenden Organe, Ergeb. Physiol. (Asher and Spiro) Jg. **3**, p. 1 (1904).

[2] See Bayliss and Starling: J. Physiol. **16**, 159 (1894). W. Connstein: Virchow's Arch. **135**, 514 (1894); Pflüger's Arch. **59**, 350 (1899); **59**, 508 (1894); **60**, 291 (1894); **62**, 58, (1895); **63**, 587 (1896); H. J. Hamburger: Z. Biol. **30**, 143 (1893); Arch. Anat. Physiol. **1895**, 281; **1897**, 132. R. Heidenhain: Pfluger's Arch. **49**, 209 (1881); **56**, 632 (1894); **62**, 320 (1895). A. Ellinger: Ergeb. Physiol. (Asher and Spiro) Jg. 1 Abt. 1, p. 355 (1902).

and thus creates new conditions, so that one effect follows another at the most rapid rate imaginable. It would not be right for us to be satisfied with the bare explanation that the activity of the cell is of a specific nature. Of course further investigations must have free play here. It would be equally wrong to disregard the uncertainty which systematic explanatory experiments encounter. Just as it is incorrect to maintain that a chemical process prevails in the organism because it is possible to carry out certain processes in a test tube, so also we are not justified in assuming that we have established the fact that a purely physical process takes place because, under certain definite conditions, we can in this way find a possible cause of a certain phenomenon. It must be perfectly clear with each advance in the knowledge of the processes taking place in the animal organism just where the certainty exists and where it ceases. There is no doubt that the assumption of a specific secretion being produced by the individual cells has given rise to an uncomfortable feeling of insecurity in our entire representations of the life processes. An advance in the science is only possible when the gaps which still exist in our present information are circumscribed as sharply as possible. For this reason we shall not attempt to trace the formation of lymph and its metabolic exchanges between the blood and the tissue-cells in accordance to physico-chemical laws.

We should like first of all to abolish any idea that the lymph-stream is analogous to the blood-stream and carries the substances it contains from cell to cell. The lymph circulates far too slowly for it to be able to replenish the cells quickly enough with the material which they need in cases of active metabolism. We must rather assume that the lymph of a given organ enters into more intimate relations between the blood-vessels and the surrounding tissue. G. von Bunge[1] has called our attention to an observation which well characterizes these relations. The milk of animals whose young grow rapidly is very rich in lime. The milk of dogs contains from four to five grams of lime per liter. A bitch weighing 20 to 30 kilograms will secrete half a liter of milk in 24 hours which will contain from two to two and one-half grams of lime. A liter of plasma contains only about 0.2 gram of lime, or only $\frac{1}{10}$ to $\frac{1}{12}$ as much as the same volume of milk. If, however, the epithelial cells of the mammary glands must obtain their material for the milk requirements from the transudated plasma, then during the 24 hours, at least 10 liters of plasma must flow through the mammary glands. This is altogether out of the question, for only one or two liters of lymph flows through the entire body in the course of a day, and far less through the mammary glands. It

[1] Lehrbuch der Physiologie des Menschen, Vol. II, p. 289 (1901).

follows from this that in the walls of the blood-capillaries of the mammary glands, a liquid rich in lime is secreted, and that, therefore, the endothelial cells of the capillary walls exercise a selection, as is also the case with every cell of all living organisms.

It is also possible that we are dealing here with a purely physical process in which the gland-cells of the mammary gland constantly utilize calcium, i.e., combine with it, and thus cause a continuous diffusion of calcium. But here again we shall have to conjecture how this may take place. Even the end-products of metabolism are not carried away, at least not wholly, by a stream of lymph. In this case, likewise, these products can evidently leave the lymph and penetrate into the neighboring blood-capillaries and thus be eliminated more rapidly. The fact that lymph from different places, or even from a single lymphatic fistula at different times, has different compositions speaks in favor of such an assumption.

Lymph, as we have said, is qualitatively very similar to the plasma. It contains the same substances. Of especial interest is its content of fibrinogen and fibrin-ferment. The amount of each of these substances is, however, very slight. Lymph coagulates slowly, and not all at once. Serum-albumin and serum-globulin are the two principal proteins which have been found in it. Lymph also contains cellular elements, especially the leucocytes, in varying numbers. Unfortunately, we are not very well informed concerning the composition of the tissue-lymph and that of the lymphatics. Most of the analyses that have been made are of lymph obtained from the thoracic duct, which contains chyle. For this reason, we are able to say but little concerning the formation of lymph and its dependence upon definite conditions. On the other hand, the work of Asher [1] and his collaborators has served to explain the relations between the amount of lymph formed and the work of certain organs. The influence exerted by the work of an organ is best illustrated in the case of the salivary glands. If a strip of blotting paper moistened with vinegar is placed in the mouth of a dog, there is a copious secretion of saliva, and at the same time there is an increased flow of lymph from the lymphatics of the neck. This increased flow of lymph does not depend upon a corresponding increase in the rate of flow of the blood. In muscular work, likewise, it may be shown that there is an increased formation of lymph.

We must consider, here, an important discovery, namely, that there are certain substances which incite the lymph flow. These are known as *lymphagogues*, and are of two kinds. The lymphagogues of the first group are obtained from the extracts of crab-muscles, blood-leeches, anodons,

[1] Asher and Barbèra: Z. Biol. **36**, 154 (1897); **37**, 261 (1898); Asher and Gies: *ibid.* **40**, 180 (1900); Asher and Busch: *ibid.* **40**, 333 (1900); Asher and Barbèra: Zentr. Physiol. **11, 403** (1897).

from the liver and intestines of dogs, and from strawberry-extracts, etc. In this case, the increased flow of the lymph can also be attributed to the increased activity of an organ, the liver especially. On the other hand, it has been assumed that these substances act upon the endothelium of the capillaries in such a way that they are incited to increased activity. To the lymphagogues of the second group belong sugar, urea, common salt, etc. These cause an abundant lymph formation, whereby the plasma, as well as the lymph, becomes more dilute. In this case also a specific activity of the cells is presumed; here, that of the tissue cells.

It is not difficult to understand that the work of organs increases the production of lymph; for, on the one hand, the cells of the active organ require an increased supply of nutriment, and, on the other hand, they yield an increased amount of metabolic end-products.

One might be tempted to ask why the presence of the lymph and the liquid in the tissues is at all necessary, and why it would not be better to have a more direct interchange between the blood and the cells of the tissue. The utility of this arrangement is very clear; for, in the first place, it is evident that by the interposition of a liquid which penetrates into the smallest spaces between the tissues, it is made possible to effect a more delicate exchange of material; and, on the other hand, it prevents the cells from being over-supplied with nutriment at any one time; and, furthermore, by this means it is possible to keep the composition of the blood fairly constant, which would not be the case if the end-products of the metabolism in an active organ were given up to the blood all at once. The lymph also serves as a diluting medium. An observation of Asher supports this view. He showed that normal lymph contains products which, if directly introduced into the blood circulation, would give rise to disturbances. If, for example, some of their own lymph from the neck be injected into the internal carotid artery of dogs, changes are at once produced in the blood pressure.

The organism also has a means of protection in the so-called *lymphatic glands*, or *lymph-nodes*, which are situated in the course of a lymph-vessel. They have various functions. According to the way they are constructed, it is easy to imagine that they, to a certain extent, act as filters and keep back certain substances which are injurious to the body. It is also conceivable that they are able to combine with substances which are given up to the lymph in large amounts. They are constantly giving up leucocytes, so that they in this way take part in the general metabolism.

Closely related to the lymph are certain liquids which are secreted by the serous membranes, which are provided with an endothelium and fulfil, for the most part, purely mechanical functions. These liquids are called *transudates*. Under normal conditions there is but a small amount of these. They are deficient in formed elements. As regards the formation

of the transudates the question has been much discussed whether they result from a filtration from the blood-vessels or whether they represent an "active" secretion. Here the relations are somewhat similar to those of the lymph. The fact that transudates contain the same substances as plasma, and, with the exception of albumin, in about the same proportion, cannot be regarded as an absolute proof of a filtration having taken place. Under pathological conditions the amount of transudate may increase enormously. If this formation results from an inflammatory process, it is called an *exudate*. The exudates are richer in cellular elements. If the amount of the latter is greatly increased, we called the liquid *pus*.

Transudates are found normally in the sac of the pericardium, between the layers of the pleuræ, and in the peritoneum. To this class of liquid belongs the *cerebro-spinal fluid* and perhaps also the *aqueous humor*. A very similar liquid is found around the joints and in the *bursæ mucosæ* which is known as *synovia*. It contains a substance similar to mucin. The true transudates are composed, as we have said, of the same constituents as plasma, and it is noteworthy that they contain fibrinogen, but hardly to an extent sufficient to permit spontaneous coagulation.

If we examine more closely the relation between the lymph and the blood, we shall arrive at the conclusion that, to a certain extent, all the cell-elements of the animal tissues are either directly or indirectly moistened by these fluids. The tissues no longer appear to us as rigid structures, as it is customary to consider them from an anatomical point of view. There are no sharp lines drawn between the blood, lymph, and the body-cells. There is never any repose. A stream of blood continually flows towards the cells, and conversely, the cells by the aid of the lymph send their products to the blood, and thus all the different elements combine to form a physiological unit for each individual organ, or perhaps only for definite cell-groups. We can now understand what great difficulties are met with in the attempt to trace experimentally the course of a reaction in the animal organism. The lymph forms on the one hand an intimate means of communication between the blood and the tissue-cells, and on the other hand it serves as a barrier between them. Substances administered, whose participation in cell-metabolism we should like to be able to trace, may reach only as far as the lymph, and may be excluded from the metabolic processes of the cell itself. Whether transformations take place in the lymph, or whether the lymph modifies the products obtained from the blood and prepares them so as to meet the existing demands, is something of which we have no information. Here a great unexplored field lies before us.

LECTURE XXV.

THE ELIMINATION OF METABOLIC PRODUCTS FROM THE BODY.

We have already called attention to the important fact that the blood, and especially the plasma or the serum, has a remarkably constant composition. This holds true at least within certain limits, and in as far as we can detect by means of our faulty methods of examination. This relative constancy holds not only, as we have shown, with regard to the quantitative relations in which the substances are present, but also, as far as we are able to see, for the qualitative relations. Thus we feel justified in assuming, for example, that the amount of protein in the blood may indeed vary somewhat, but it does not change its nature in spite of the most varied forms of nourishment.[1] We intentionally speak of a *relative* constancy, because there can be no such thing as an *absolute* constancy in the composition of the blood, for from moment to moment, according as this or that organ comes into action, the blood is bound to receive quite different metabolic products. Such products are, however, present in such a state of dilution that we can detect them only in a large quantity of blood. Again, during the digestive process and when the absorption and assimilation is at its height, sometimes this substance and sometimes that one will circulate in the blood to an increased extent. As a rule, however, these differences are so slight that they cannot be detected by the present methods of analysis. They are usually concealed by experimental errors. At the same time it is perfectly evident that the composition of the blood is regulated to a remarkable extent and kept constant within narrow limits. There are many ways in which this regulation is effected, and they are not all of the same nature as is usually assumed. First of all, the blood is kept from being flooded with substances foreign to it, as they are contained in the food, by the activity of the intestine. Were it not for this, we could not understand, as we have repeatedly stated, why the composition of the serum should always remain qualitatively and quantitatively practically the same. By means of the activity of the digestive ferments, all of the complicated and widely different nutrient substances, which vary from day to day, are

[1] Abderhalden and Samuely: Z. physiol. Chem. **46**, 193 (1905). Emil Abderhalden: Zentr. Stoffwechsel- und Verdauungskrankheiten **5**, 647 (1904).

disintegrated; and from the resulting products the intestines, and perhaps the liver as well, forms substances suitable for the body. The intestine assumes by this function a characteristic position. It takes care that the tissue-cells always receive the same nutriment, and makes them absolutely independent of the nature of the food which is eaten. We cannot be far wrong in ascribing to the intestine in this sense an important part in the maintenance of the individuality of each species of animals.

A second mechanism for regulating the composition of the blood is found in the lymph. The exchange of material between the blood and tissue-cells takes place, as we have seen, through the lymph. The lymph receives substances from the blood which the cells of the tissues require, and on the other hand it gives up to the blood the waste-products which it receives from the tissue-cells. It is able to retain these last-mentioned substances for quite a time, only gradually giving them up to the blood, for further elimination from the body.

The chief organs for the elimination of the metabolic end-products, and of the substances which the organism cannot utilize, are the kidneys. They guarantee the maintenance, as far as possible, of the blood-uniformity. Under normal conditions they are perfectly adequate for this purpose. If it happens that the kidneys, for some reason or other, are not able to remove all of the foreign substances from the blood, then other organs attempt to act as their substitute. Most of all the different glands contained in the organism may be active in this sense, and even under normal conditions there is no doubt that small amounts of the waste-products are eliminated in this way. This is best shown by introducing into the body substances which are foreign to it. Thus, if we introduce potassium iodide into the intestines, some of it will soon appear in the saliva and in the sweat. If morphine is injected subcutaneously, a part of it is eliminated in the stomach. Urea is likewise found in sweat, particularly when the kidneys are not adequate to the demands placed upon them. The intestines also form an important organ for elimination, and normally. We have already seen that the alkaline earths and heavy metals are unquestionably largely eliminated directly in the intestines, and in fact chiefly through the rectum. The animal organism, furthermore, is able to combine many of these foreign substances together, whereby the blood and the tissues are prevented from being flooded with them. Such substances may then be eliminated gradually in the course of several weeks. We have also repeatedly called attention to the ability of the tissue-cells, and especially of the liver, to make many substances harmless by oxidizing or reducing them, and in some cases conjugating them with certain substances, such as glycocoll, glucuronic acid, sulphuric acid, or urea.

The importance of the other glands, large and small, as organs for the elimination of substances foreign to the organism and of the end-products of metabolism is insignificant compared to that of the kidneys. Their anatomical structure characterizes them for the exercise of their most important function. In the first place we notice the peculiar nature of the blood-vessels. The arteries form branches and side twigs; each of the afferent vessels terminates in a globular bunch of capillaries, the *glomerulus* or *Malpighian tuft*. The blood leaves the glomerulus through a so-called *efferent vessel*, or *Vas efferens*, which also breaks up into a close capillary plexus which surrounds the secreting tubes. From this network come venous radicals, which empty into the veins of the kidneys, and through which the blood, which has meanwhile been freed from the metabolic end-products and other waste-material, leaves the kidneys. The termination of the afferent vessels in the Malpighian tuft has awakened the most interest. It is worth mentioning that these vessels are considerably narrower than the corresponding efferents.

The Malpighian tuft is within the so-called Bowman-Müller capsule. This consists of a thin pouch consisting of epithelial cells, into which, as it were, the tuft has been pushed. It represents the beginning of a uriniferous tube. The latter are not simple drainage channels, but follow, first of all, a tortuous path, the *first convoluted tube*, or *tubulus contortus*. Then the tube narrows suddenly and describes a loop, reaching into the medulla, known as *Henle's loop*. The tube then turns back towards the cortex, forming an irregular convoluted tube, that of *Schweigger-Seidel*, which passes through a narrower arch into the *straight collecting tube*. Several tubes which have up to this point been entirely independent, empty into this collecting tube. It terminates, together with other similar tubes, at the surface of a papilla in the calyx of the kidney. We will state, moreover, that the epithelium of these different parts of a uriniferous tube is not uniform. We mention briefly these relations, in order to show that the process of forming a secretion by the kidneys is by no means a very simple process. There is some reason for the complicated construction of the kidneys. In considering the function of the kidneys, we must hold close to the anatomical relations, and attempt to explain the significance of the differently organized parts of the uriniferous tubes. Before taking up the question of the secretion of the urine, we will briefly mention, in connection with the above brief description of the construction of the uriniferous tubes, those researches which have been undertaken in the attempt to decide what the function of each different part of the tube is. Right at the start it may be stated, that, according to all we now know, the urine is not eliminated from the blood in the form in which it eventually reaches the calyx to be emptied into the bladder. There is probably an absorption of substances, partly of water and partly of other substances, while it is in

the tubes.[1] To be sure, there are a number of experiments which do not agree with such an assumption.[2] A reabsorption has been observed only under conditions which cannot be regarded as normal. The frog is a particularly suitable subject for such experiments. In this animal the kidneys receive their blood from two sources, the renal artery, and the renal portal vein. The first provides the Malpighian body with material, while the latter leads directly to the uriniferous tubule. If the renal artery is ligated, the secretion of urine stops completely. On the other hand, if the flow of blood through the renal portal vein is stopped, then urine continues to be secreted. If the uriniferous tubules have the function of taking up water and solid constituents from the products secreted by Bowman's capsules to give them up to the blood again, then it would be expected that after ligating the blood-vessels supplying the uriniferous tubule, that there would be an increased elimination of urine. This was, however, not the case. On the contrary, the amount of urine diminished. We must regard this question as unsettled. It is indeed conceivable that an absorption takes place only under certain conditions. We must at this place mention how extremely difficult it is to obtain a clear judgment when there is any meddling with the normal functions of the kidneys. We never know exactly what the primary cause of a phenomenon is, and what takes place only secondarily. Above all, we have to consider the important influence of the blood-supply upon the formation of the urine. It is, for example, perfectly possible that in the above case the diminished secretion of the urine may have been caused by an obstruction in the flow of the blood to the glomeruli. We shall come to this question again when we discuss the question of reabsorption.

In order to investigate the functions of the separate divisions of the uriniferous tube, substances have been introduced into the circulation which can be easily detected in microscopical preparations. Thus Heidenhain[3] found that after the injection of sodium sulphindigotate into the blood, it reappeared in the epithelial cells of the uriniferous tubules. He concluded from this discovery that these cells have the function of adding certain specific constituents of the urine to the secretion which it receives from the capsules of Bowman. This is not necessarily true, for the microscopical pictures might equally well have been caused by a reabsorption of the dye from the uriniferous tubule. Carmine has also been used for such experiments,[4] and the results of

[1] H. Ribbert: Virchow's Arch. **93,** 169 (1883). W. M. Sobieranski: Arch. exper. Path. Pharm. **35,** 144 (1895).

[2] A. Gurwitsch: Pflüger's Arch. **91,** 71 (1902). A. P. Beddard: J. Physiol. **28,** 20 (1901).

[3] R. Heidenhain: Arch. mikro. Anat. **10,** 1 (1874). Cf. Pflüger's Arch. **9,** 1 (1874).

[4] Cf. Adolf Schmidt: Pflüger's Arch. **48,** 34 (1891).

these experiments also are capable of more than one interpretation. Dreser[1] attempted to find out at which place acid-fuchsin was eliminated. He injected daily three or four cubic centimeters of acid-fuchsin into the dorsal lymph-sac of frogs. An hour or so afterwards there was no pigment present in the glomeruli nor in the convoluted tube, but only in the lumen of the central part of the tube. If the injection were repeated, the glomeruli remained colorless; but in the convoluted tubes, the red coloration gradually extended into the end of the epithelium which is toward the lumen. Experiments with alizarin-carmine showed that the coloration did not appear in the *Tubuli contorti*, but only in the distal parts of the tubes. Dresser concluded from these experiments and those with other dyes, that in the convoluted tubes secretion alone takes place, and no reabsorption. We see, therefore, that the decision as to the absorption capacity of the epithelium of the convoluted tubes is entirely dependent upon the interpretation of these microscopical pictures. As long as this problem cannot be decided positively, it is quite impossible to establish the function of the different parts of the uriniferous tubes. The same microscopical appearance may be regarded as resulting from an absorption or from an elimination. The entire question as to the special functions of the anatomically different parts of the uriniferous tubes remains absolutely unsettled by the above experiments.

Let us now see whether our knowledge of the functions of the kidneys is sufficient to give us a clear idea of the formation of urine. In the first place we must state that it has never been found possible to detect positively the presence of secretory nerves in the epithelium of the kidneys. All the nervous influences which have shown an effect upon the elimination of urine can be either directly or indirectly attributed to a change in the innervation of the blood-vessels. The amount of the blood circulating is closely related to the amount of urine secreted. This relation is almost self-evident, for the greater the amount of blood passing through the kidneys, the greater will be the opportunity for the cells of the kidney to form their secretion. The blood-pressure also is to be considered. The whole arrangement of the glomeruli is such that the blood must pass the Malpighian body with a relatively high pressure. In this respect the above-mentioned behavior of the efferent vessels is important, which are considerably narrower than the afferents. Ludwig[2] makes use of this fact for the foundation of his much-discussed theory of urine-elimination. He attempted to make his explanation as mechanical as possible, and assumed first of all that there was a filtration through the glomeruli at

[1] H. Dreser: Z. Biol. **21**, 41 (1885). Cf. P. Grützner: Pflüger's Arch. **24**, 441 (1881). M. Nussbaum: *ibid.* **16**, 139 (1878); **17**, 580 (1879).

[2] Ludwig: Wagner's Handwörterbuch der Physiol. **2**, 629 (1844); Wiener med. Wochenschr. **14**, No. 13, 14 (1864); Sitzber. Kais. Akad. Wissensch. **48** (1863).

the beginning of the uriniferous tubes. According to this, the urine would show a very similar composition to that of the plasma of the blood. This, however, is not the case. Ludwig furthermore assumed that there was a re-absorption in the uriniferous tubes, both of water and of dissolved substances. Very soon, however, important objections were raised against this theory of a filtration of all of the constituents of urine. Above all, it was pointed out that while it was comprehensible that the proteins in the blood could not pass through the vascular endothelium, on the other hand it would be expected that certain other substances, for example sugar, which is always present in the plasma, would filter through into the urine. This is, however, not the case under normal conditions, and the same is true of certain other substances. To account for this it has been suggested that perhaps the sugar may not circulate as such in the blood, but that it is combined with some other compound which will not filter through the medium. There is no support for any such assumption. The quantitative composition of the urine also speaks against any such simple filtration process taking place in the formation of urine. The plasma contains about 0.05 per cent of urea. We would then expect the urine to contain about the same amount of this substance, whereas in fact about 2 per cent is usually present. If we are to retain the idea of a pure filtration, then we must necessarily assume that the urine is concentrated in the uriniferous tubes to about $\frac{1}{40}$ of its original volume. It has, furthermore, been found that if the supply of blood to the kidneys is entirely stopped, that the secretion of urine ceases; but, on the other hand, when the blood is allowed to flow again through the kidneys, it is about 45 minutes before the secretion of urine begins. If we believe that the endothelium of the glomerulus and that of Bowman's capsule is a mere filtering membrane, it is hard to account for the long cessation of its function.

Especially quite recently, more and more facts have become known, which indicate that the kidneys act analogously to other glands during the formation of their secretion. For one thing, it has been observed that an increased secretion causes a rise of temperature, and that when there is more work done by the kidneys, there is more oxygen consumed and more carbonic acid produced.[1] Our present knowledge teaches us that we cannot be far wrong in assuming that practically the whole function of the kidneys is analogous to that of the other glands and that no simple filtration[2] can take place; both the epithelium of the glomerulus, as well as that of the uriniferous tube, probably produce an actual secretion. We know of various substances which increase the secretion

[1] J. Bancroft and T. G. Brodie: J. Physiol. **32**, 18 (1904).

[2] Cf. Torald Sollmann: Ann. J. Physiol. **13**, 241 (1905). W. Filehne and J. Biberfeld: Pflüger's Arch. **111**, 1 (1906).

of urine, just as we have met with such substances in the study of other glands. To be sure, it has been shown that a great many of these substances are capable of exerting only indirectly an influence upon the kidney-cells, by affecting the flow of the blood through the kidneys on the way to the vascular innervation.[1] For other substances, the result cannot be traced to this cause.

In order to prevent misunderstandings, we will at once state that purely physical processes undoubtedly do play an important part here as in the case of all absorption and secretion processes in the animal organism. It is perfectly possible that a pure filtration process may act in conjunction with other processes in the formation of urine. At the same time it would be wrong to assume that the existence of a filtration process is proved. We can only infer that it takes place, and are at once compelled to make the auxiliary hypothesis of the reabsorption by the epithelium of the uriniferous tubes. Another possibility seems to us as far more probable, namely, that the Malpighian tuft is not the sole place where the constituents of the urine are secreted. We have already seen that the efferent vessels, after emerging from the tuft, again break up into a capillary network, which surrounds the secreting tubes in the cortex. This behavior of the blood-vessels must surely be of some significance in the formation of the urine. It is possible that a back-absorption takes place here, but it is also conceivable that the epithelium of the uriniferous tube is only able to withdraw definite substances from the blood, concentrates them, and finally sends them on at different periods towards the lumen of the tubes. First of all, we must remember that the Bowman's capsule possesses numerous finely branching nerve-fibers, which originate in the vasomotor nerves. The blood-capillaries, also, are abundantly supplied with nervous plexuses. Furthermore, it has been found that nervous branches exist which supply the uriniferous tubes; and in fact we see separate plexuses of non-medullated fibres, arising especially from the *Tubuli contorti*. Such nerve-endings have also been observed for the epithelium of the Bowman's capsule, for the straight tube and also for the collecting tubes. The fact that the vessels of the kidneys, and especially those of the capillaries of the cortex have such an extensive innervation, suffices to account for the sensitiveness of the renal vessels to all sorts of different influences. More and more it has become evident that a great number of those substances to which has been ascribed a specific action upon the parenchyma of the kidneys, only influence the formation of urine by accelerating or retarding the flow of the blood. The intimate dependence of the secretion of the kidneys on the blood-supply may have been the chief reason for assigning to the kidneys a position different from that of the other organs of the body.

[1] Cf. O. Loewi: Arch. exper. Path. Pharm. **53**, 15, 33, and 49 (1905).

We have repeatedly learned that other glands are to a certain extent independent of the blood-supply. Thus the pancreas is constantly forming its secretion, but gives it up only after certain kinds of stimulation. It is otherwise with the kidneys. Their activity is different, because their function has an entirely different significance from that of all the other glands. The kidneys must be very delicately adjusted to the composition of the blood. They are obliged to work very rapidly in all cases, and are not obliged in every case to follow stimulations which are communicated to them reflexively by the nervous system. The chemical nature of the blood invariably has an influence. Now, is this because the vasomotor nerves are directly influenced by the composition of the blood, so that, for example, an enlargement of the vessel or restriction of it will be effected, or because certain components of the urine act directly upon the epithelium of the uriniferous tubes? We hold that the last assumption is very probable, and imagine that certain specific substances are captured by this epithelium, which are concentrated and then given up again. Only in some such way as this are we able to account for the relatively high concentration of the urea. There are quite a number of different observations, which indicate such a specific function of the kidney epithelium. A few examples will be cited. The elimination of uric acid has been studied most closely, and its presence is easy to detect.[1] If, for example, a solution of uric acid in piperazine is injected subcutaneously into rabbits, there takes place first of all a considerable diuresis. In from twenty minutes to an hour uric acid may be detected in the tubes of the medulla. The glomeruli and Bowman's capsules are perfectly free from deposits of uric acid; but, on the other hand, the epithelium of the convoluted tubes contains granules of uric acid, and chiefly in the end of the tubes facing the lumen. Anten[2] obtained corresponding results in the kidneys of dogs. He cut out the kidneys from the general circulation of a live dog, and then passed a solution of freshly-precipitated silver chloride in ammonia through the organs, in order to precipitate the uric acid present as silver urate. The kernels of the silver salt were found chiefly in the cells of the convoluted tubes, and particularly at the basal part of the cells. The epithelium of the ascending tube of Henle's loop also showed isolated accumulations, but this was not the case with the descending part of the loop. One might naturally be inclined to object that the appearance noted might result from a reabsorption just as well as from a secretion of the uric acid. There are, however, so many observations[3] of

[1] Cf. Sauer: Arch. mikros. Anat. **53,** 218 (1899). W. Ebstein and A. Nicolaier: Experimentelle Erzeugung von Harnsteinen, Wiesbaden, 1891, and Virchow's Arch. **143,** 337 (1896). O. Minkowski: Arch. exper. Path. Pharm. **41,** 375 and 410 (1898).

[2] Henri Anten: Arch. internat. de pharmacodynamie et de thérapie, **8,** 455 (1901).

[3] Cf. Courmont et André: J. physiol. et pathol. général, **7,** 255 (1905).

this nature made under quite different conditions that we may well assume that an actual secretion of uric acid takes place, and its separation from the blood is probably the result of a selective action of the epithelium of the above-mentioned portions of the uriniferous tube. We will mention in addition that Höber and Königsberg [1] have proved that the epithelium of the uriniferous tube is not only able to take up colors which are soluble in lipoids, but also those which are insoluble in lipoids. Unfortunately, it has up to the present not been found possible to localize the secretion of urea and other substances in the same way as in the case of uric acid.

We do not in any way intend to suggest that the secretion of the urine is a perfectly simple and uniform process. Unquestionably a great number of different processes are taking place side by side, which mutually assist one another. On one side constituents of the urine are given up through the Malpighian bodies to the capsules of Bowman, and evidently at this place the greater part of the water is sent out, while the epithelium of the uriniferous tube is constantly removing definite constituents from the blood, accumulating them and then giving them up again inward to the lumen of the tube. If we consider in addition that many observations indicate that there is more or less absorption in the uriniferous tube of some of the substances which have previously been secreted, of water especially, then we shall begin to understand that diuretics can find a number of different points of attack, and cause, in a number of different ways, disturbances in the secretion of the urine.

We must come back once more to the fact that under normal conditions there is no sugar in the urine. As we have said, it has been suggested that this was because the glucose did not circulate as such in the blood, but combined in some way with colloidal substances, although direct experiments [2] have shown that this representation is in no way justifiable. Sugar is present as such in the blood. Evidently the vascular endothelia of the kidneys are adjusted to a certain definite sugar content of the blood. When more than this is present the sugar passes over into the urine. Now it seems probable that certain substances cause sugar to pass into the urine even when there is no glucohemia. Such, for example, is phloridzin, which, according to many observers, acts directly upon the kidneys, or indeed at first upon the vascular endothelia.[3] Recently Underhill and Closson [4] have stated that those forms of glucosuria, which appear when common salt is introduced into the circulation, are in some cases to be regarded as due to a direct influence

[1] Höber and Königsberg: Pflüger's Arch. **108,** 323 (1905).

[2] Leon Asher and R. Rosenfeld: Zentr. Physiol. **19,** 449 (1905). See Lecture II, p. 30.

[3] See Lecture V, p. 81.

[4] Am. J. Physiol. **15,** 321 (1906).

upon the kidneys. They found that a great deal depended upon the place in which the salt solution was introduced. If it was an artery of the brain, glucohemia ensued, and consequently glucosuria, but no polyuria.[1] When, however, they injected the salt solution into one of the veins of the body, polyuria soon resulted, and at the same time an elimination of sugar took place, but, instead of the amount of sugar present in the blood increasing, it decreased. This case of glucosuria, therefore, must arise from another cause, and may be attributed to an action upon the endothelia of the kidneys.

If we consider that the kidneys have the function of removing all abnormal substances from the blood, and any excess of normal ones, then it is self-evident that definite statements cannot be made concerning the composition of the urine. It is dependent first of all upon the nature of the nourishment and upon the intensity of the metabolism of the cells. There is nothing uniform concerning the amount of urine eliminated in a day, nor concerning its reaction or other behavior. The individual products which are eliminated in urine we have already discussed, and, in each separate case, traced the product to its source. The end-products of metabolism are always eliminated with a greater or less quantity of salts. These originate, to be sure, partly from decomposition and partly from destruction of tissue, but for the most part they may be traced to the food itself. The amount of water in urine does not depend entirely upon the amount that is drunk, but is materially affected by the amount that is utilized in the organism. We shall soon see that by the evaporation of water from the surface of the body, the animal organism has a very efficient means of regulating its temperature. We may expect that one and the same individual, under conditions remaining constant and a diet which is qualitatively and quantitatively the same each day, would eliminate a urine which would show a constant composition within narrow limits. It is remarkable that but few exact and complete analyses of urine have ever been made. Usually the composition of the food that is eaten is entirely disregarded. It is clear that such analyses are useless for drawing any conclusions or for future inquiry. The great gap in our knowledge is thus made more apparent, for we would unquestionably be able to draw certain conclusions as to the metabolism of the cells if there were exact analyses which took into consideration all the substances present in urine, and such investigations would be of great help in the case of pathological processes. Quite recently Otto Folin[2] has undertaken the analysis of urine from a single individual during several days in which the diet remained the same. We regret that we cannot give all the values he obtained in his work, but we have to be

[1] See Lecture V, p. 81.

[2] Am. J. Physiol. **13**, No. 1, 45 and 66 (1905).

satisfied here with certain parts of it which are shown in the following summary. The nourishment consisted of 500 c.c. milk, 300 c.c. cream with a fat-content of 18 to 22 per cent, 450 grams egg, 200 grams Horlick's Malted Milk, 20 grams sugar, 6 grams salt. This mixture contained about two liters of water, and in addition 900 c.c. were drunk. In this food there was contained 119 grams albumin, about 148 grams fat, and 225 grams carbohydrate. The mixture was shown to have a constant composition by the determination of the chlorine from day to day (about 6.1 grams), of the sulphuric acid (about 3.7 grams), of the phosphoric acid (about 5.8 grams), and of the nitrogen (about 19.0 grams).

ANALYSIS OF THE URINE OF A NORMAL INDIVIDUAL.

Body Weight.	Date.	Volume of Urine.	Total Nitrogen.	Nitrogen as Urea.	Nitrogen as Ammonia.	Nitrogen as Creatinine.	Nitrogen as Uric Acid.	Nitrogen in Other Compounds.
kg.		c.c.						
70.8	Sept. 21	1520	15.9	13.7	0.64	0.61	0.08	0.81
	Sept. 22	1530	16.6	14.5	0.72	0.58	0.10	0.80
	Sept. 23	1460	16.6	14.4	0.73	0.56	0.11	0.83
	Sept. 24	1430	16.5	14.2	0.75	0.52	0.12	0.90
70.1	Sept. 25	1380	16.6	14.5	0.86	0.54	0.11	0.85

Each 100 grams of the total nitrogen were distributed as follows:

Date.	Urea.	Ammonia.	Urea and Ammonia.	Creatinine.	Uric Acid.	Nitrogen in Other Compounds.
September 21	85.9	4.1	90.0	3.8	0.5	5.7
September 22	86.9	4.3	91.2	3.6	0.6	4.6
September 23	86.5	4.4	90.9	3.4	0.7	5.0
September 24	86.1	4.5	90.6	3.2	0.7	5.5
September 25	85.7	5.2	90.9	3.3	0.7	5.1

Date.	Total Sulphur Determined as Sulphate.	Inorganic Sulphuric Acid. (S_1)	Ether-sulphuric Acid. (S_2)	Neutral Sulphur Determined as Sulphate. (S_3)
September 21	3.31	2.85	0.25	0.21
September 22	...	3.00	0.25	...
September 23	3.35	2.89	0.28	0.18
September 24	3.20	2.73	0.24	0.13
September 25	3.25	2.92	0.21	0.12

Date.	In Per cent of the Total Sulphur.			Acidity in c.c. of $\frac{N}{10}$ Solution.		
	S_1	S_2	S_3	Total.	Inorganic.	Organic.
September 21	86.1	7.6	6.3	589	219	370
September 22	...	...	...	630	299	331
September 23	86.3	8.3	5.4	625	432	193
September 24	85.3	7.5	4.1	617	...	...
September 25	89.8	6.5	3.7	646	276	370

Date.	Total Phosphate as P_2O_5	Chlorine in Grams.	Indican (Fehling's Solution = 100).
September 21	3.98	6.3	140
September 22	4.16	5.7	150
September 23	3.84	5.8	140
September 24	3.68	5.7	140
September 25	3.85	5.2	130

It is evident from these values that the urine during these five days showed a very constant composition. It would be very interesting to carry out such experiments with a uniform diet, and especially with the same kind and amount of albumin, also on a large scale during pathological conditions. In such a way we should obtain an insight into the cell-metabolism under different conditions. At the same time it is not true that we can draw very far-reaching conclusions in all cases as to the cell-metabolism from the composition of the urine. We must always remember how little we know concerning cell-metabolism and of the dependence of one organ upon another. It is indeed possible that there is an exchange of material in such a way that the decomposition-products from one organ are utilized by another. Thus there may be a considerable destruction of tissue of certain specific composition, which would not show any indication in such an analysis, because there might not be any of the products from the destruction of this tissue, in the urine. The kidney may be an economizing organ of the animal. It is perfectly possible that the constituents which it receives that are useful in the organism are in some way transformed and given back to the circulation. We will recall the fact that the kidneys are capable of effecting syntheses. Their cells conjugate benzoic acid with glycocoll. Is there any reason for believing that this is the only synthesis which the kidneys are capable of effecting? We will also mention the fact that the animal organism is exceedingly economical with its

supply of phosphoric acid. In increased diuresis the amount of urea and of common salt in the urine increases considerably, but the amount of phosphoric acid present remains remarkably constant.

The reaction of the urine depends, as we have already said, upon the nature of the food. The urine of herbivora is neutral or alkaline, while that of the carnivora is acid as a rule. The direct connection which this has with the food may be indicated theoretically by comparing the ash of plants with that of animals. That it is not any particular difference in the metabolism taking place in different classes of animals which causes the different reaction of the urine, may be shown by feeding vegetables to the carnivora. The urine then becomes neutral or alkaline. Conversely, herbivora may be forced to become carnivora by starvation. The animal is then obliged to live upon its own tissue, and the urine then has an acid reaction. Alkaline urine, especially in herbivora, is usually turbid on account of the precipitation of alkaline earth salts. The urine of a normal man with a mixed diet shows an acid reaction. The acid reaction is caused by the fact that during metabolism acid products are formed by the combustion of neutral substances such as albumin and lecithin for example. The sulphur contained in albumin is largely converted into sulphuric acid, the phosphorus of lecithin and of the nucleic acids is oxidized to phosphoric acid. Furthermore, organic acids, as, for example, hippuric acid, uric acid, oxalic acid, and aromatic oxyacids, are also formed. The organism, moreover, possesses ways and means for keeping the acidity within certain limits. For one thing, the acid formed may be neutralized by means of alkali carbonate; and if there is not enough of this present, then the ammonia which is set free by the decomposition of proteins comes into play.

It is perhaps well here to make a few general observations concerning the conception of acidity. An acid may be defined from two standpoints.[1] The chemist understands by an acid a substance whose hydrogen atom, or atoms, may be replaced by metals. When the metal enters the molecule the acid character is neutralized. Thus the acidity of a solution may be estimated by measuring the amount of alkali which is necessary to replace all of the acid hydrogen. Our discussion of the acidity of the urine was from this point of view. The physico-chemist, on the other hand, defines an acid as a chemical compound which when dissolved in water is dissociated, yielding positively charged hydrogen atoms (H^+). According to the degree of dissociation, we characterize an acid as strong or weak. A weak acid, for example, is one which at a given concentration is less dissociated than a strong acid. The difference between these two points of view may be perhaps best illustrated by an

[1] Cf. R. Hoeber: Hofmeister's Beiträge, **3**, 525 (1903).

example. Suppose we have a solution of $\frac{1}{32}$ normal hydrochloric acid, and one of acetic acid which is also $\frac{1}{32}$ normal. From the purely chemical standpoint these solutions are of the same strength, because we shall have to use as much alkali to neutralize a liter of the acetic acid as would be necessary for a liter of the hydrochloric acid. On the other hand, from the point of view taken by the physico-chemist, the acidity of the $\frac{1}{32}$ normal hydrochloric acid is about 40 times as great as that of the $\frac{1}{32}$ normal acetic acid. Thus the hydrochloric acid of the above concentration is about 97 per cent dissociated, while the acetic acid is only dissociated to an extent of 2.4 per cent. Now in our ordinary chemical methods we neutralize all of the hydrogen of the acid, because there is always a fraction of the whole molecule that is dissociated, the value of the fraction increasing with the dilution; and as fast as some of the ions are neutralized more of the molecule dissociates, so that eventually not only the hydrogen ions originally present, what we may designate as the *active hydrogen ions*, but also those which were originally undissociated, the *potential hydrogen ions*, are neutralized. The physico-chemist in his determination of the acidity takes into consideration only the former kind of hydrogen. From his point of view the urine is usually neutral. There seems to be no definite relations between the acidity determined by the titration of urine and the so-called *ion-acidity*. It is desirable that in all cases both values should be known.

In general, not much is known concerning the way in which the different substances proved to be present in urine are combined there. The analysis of the ash as such teaches us but little. It gives us considerable information in tracing the course of the non-volatile material, but in this case the intestinal elimination must not be disregarded. The fact that the inorganic substances are, at least to some extent, eliminated by the intestines, complicates our understanding of the general metabolism, and especially because of the fact that in every case it is impossible to decide what part of the constituents of the ash of the fæces is to be regarded as unabsorbed material and what part was eliminated from the intestinal walls after absorption had taken place. The value of mineral substances for the whole organism and their absolute indispensability have been repeatedly mentioned, and we are convinced that exact investigations concerning metabolism on as broad a basis as possible, taking into consideration the inorganic material introduced and that eliminated, will give us considerable information as to the nature of cell-metabolism.

We must also consider a phenomenon which is frequently met with in human urine. Fresh urine is usually clear and shows no sediment. After the urine has stood for some time a sediment often forms, sometimes as a reddish, crystalline powder, sometimes as a reddish-gray precipitate, resembling brick-dust. The latter is called *Sedimentum lateritium*. It

dissolves completely on heating, and appears again on cooling. On standing for some time, crystals often appear in the sediment, which will not dissolve on heating the urine. These crystals are free uric acid, while the sediment was monosodium urate. The precipitation of the latter is, partly at least, due to the cooling of the urine, for it is much more soluble in hot water than in cold. As the crystals form, the acidity of the urine decreases. The question arises whether the change in the reaction of the urine has any connection with the deposition of the urate precipitate. We have at a previous place mentioned the insolubility of uric acid;[1] it requires at 18° C., 39,000 parts of water to dissolve one part of the acid. The values given in the literature are often widely different from the above value which is taken from the work of His. This is partly explained by the fact that it is usually disregarded that glass, especially common glass, contains alkali, which it gives up to water which is in contact with it, and this affects the analysis. His, again, has called attention to the great tendency of uric acid to form supersaturated solutions. The acid urate of sodium, also called the mono-urate, is far more soluble in water than uric acid itself. Now this urate is deposited frequently in urine, and of other cases free uric acid is found in the sediment, and, in fact, so much in it that it is hard for us to believe that it was present as such in the urine. Camerer [2] compared the solution of uric acid in the urine with the following experiments. He mixed a saturated solution of acid urate of sodium, which reacted alkaline toward litmus, with a solution of acid phosphate of sodium. The mixed solution showed an acid reaction and was perfectly clear at 37° C. On cooling the mixture, the reaction toward litmus changed. The solution became alkaline and uric acid was precipitated. A chemical decomposition had taken place. From the acid phosphate of sodium (NaH_2PO_4), and at the cost of the sodium in the monosodium urate, disodium phosphate (Na_2HPO_4) had been formed, and uric acid had been set free; which, on account of its insolubility, was precipitated. On heating the solution, the reverse process took place, and the reaction of the urine became acid. In the urine there is always more or less alkali phosphate present, which may have the same effect as in the above test-tube experiment. Naturally, according to this explanation, it must be assumed that the uric acid is originally present as the monosodium salt. There is no question that part of the uric acid is actually present in this form; but, on the other hand, it is certain that a part of the uric acid is present, combined in some other way. This is evident from the fact that from a simple solution of alkali urate, the whole of the uric acid may be precipitated by the addition of acid, while this is not the case with urine. A part of the uric acid remains in solution after the urine has

[1] Cf. T. Paul: Pharm. Ztg. 1900, also Lecture XIII, p. 298.

[2] Deut. med. Wochschr. **17**, No. 10, p. 356 (1896).

been acidified. Now urea is a good solvent for uric acid. It is an open question whether we are justified in assuming with Rudel [1] that there is a chemical combination between the urea and the uric acid in this case, and it is equally uncertain whether the urea alone affects the solubility of the uric acid, or whether there are other compounds present in urine which have the same action.

We have already said that the epithelia of the blood-vessels and of the uriniferous tubes can only cause the elimination of those substances which do not belong to the plasma, or which are present in more than the normal amount. Thus the kidneys, for example, are very sensitive to an increase in the sugar-content of the blood. Albumin does not pass through the kidneys when they are acting normally, except when albumins foreign to the body evade the alimentary canal, and get into the circulation. It is a well-known fact that under pathological conditions, in diseases of the kidneys, albumin passes from the blood-capillaries, and enters through the epithelium of the uriniferous tubes. The presence of albumin in the urine is a symptom which may arise from a number of different processes. There is no question that a study of the nature of the eliminated albumin could be used as a basis for further inquiry. To be sure, in many cases there is a mere appearance of serum-albumin, but we can well imagine that in other cases the tissue-cells for some reason or another produce an albumin, and give it up to the plasma, of a nature which in its entire construction is foreign to the plasma, and that it is accordingly eliminated by the kidneys. This is not the place to discuss at any length such questions, which are closely related to the pathology of metabolism.

As we have already mentioned, the organism can under certain conditions eliminate the constituents of the urine through other glands, especially through the skin. Thus in many cases of uræmia, urea may be secreted in such quantities by the sweat-glands, that crystals deposit upon the skin. By uræmia we understand a very serious complex of symptoms, occurring when the kidneys to a greater or less extent have ceased to exercise their functions. The organism seeks by every means in its power to get rid of the constituents of urine which are circulating in the blood. If it does not succeed in accomplishing this, symptoms appear which are similar to intoxication. The attempt has often been made to trace the cause of the disease to some constituent of the urine, and in this connection urea has been principally considered. On the other hand, there are quite a number of observations which indicate that the urine itself exerts a poisonous action. Thus if human urine is injected into the veins of a rabbit, it will produce acute poisoning, which will result in the death of the animal. The urine from different animals shows different degrees of poisonous properties.

[1] Arch. exper. Path. Pharm. **30**, 469 (1892). Cf. T. J. Zerner: Wiener klin. Wochschr. **6**, No. 15, p. 272 (1893). A. Ritter: Z. Biol. **35**, 155 (1897).

From the material at hand it is hard to decide as to the significance of the phenomenon, and there is no indication of what substances in the urine exert this poisonous action. In the case of uræmia we have no reason for attributing any one substance as causing the whole complex of symptoms. It is self-evident that all sorts of different substances may come into play here, and furthermore it must never be forgotten that an abnormal composition of the plasma will immediately have an effect upon all the processes of metabolism in the cells, and result in the production of incompletely formed products, or of those which are built up in an unsuitable way. Here, as in all physiological and pathological processes, an organ should not be considered by itself, but we must trace the damages which start from it in a continuous line from organ to organ, from tissue to tissue, and finally from cell to cell.

The animal organism is also normally eliminating substances constantly through the skin. We find in mammals essentially two kinds of glands in the skin, the sweat-glands and the sebaceous glands. The former eliminate a secretion which consists almost entirely of water. The amount of sweat secreted in the course of a day varies tremendously, and is dependent upon certain conditions, and especially upon the demands for a regulation of the body temperature. In the evaporation of water from the surface of the body the animal organism finds its most important means for preventing the body from being overheated. A large amount of heat is required to transform water from the liquid to the gaseous state. This causes the body to be cooled. It is interesting to find that the activity of the sweat-glands is influenced by the central nervous system. A secretion may be produced directly by nervous stimulation.

The sebaceous glands have a different and more local function. Corresponding to this fact, their secretion has a quite different composition. In a fresh condition it is an oily, semi-liquid mass, which, on standing in the air, solidifies on the surface of the skin to a greasy tallow. It contains fat, albumin and cholesterol. Its most important function is to lubricate the skin. We will here mention again that a modified sebaceous gland, the oil-bag of birds, contains octadecyl alcohol $C_{18}H_{38}O$,[1] and finally that the mammary glands likewise may be considered as related to the sebaceous glands.

[1] Röhmann: Hofmeister's Beitr, **5**, 110 (1904).

LECTURE XXVI.

THE RELATIONS OF THE ORGANS TO ONE ANOTHER.

At the close of the last lecture we referred briefly to the mammary glands. These glands exercise their function only under certain definite conditions. The period of lactation does not begin until about the time the secretion is required by the suckling. Long before the birth, however, external changes may be noticed showing that the glands, then at rest, are developing in such a way that they will be able to meet the demands that are to be laid upon them. We have here an interesting example of the relation of widely different organs to one another. The function of the mammary glands is dependent directly upon the generative apparatus of the female. There must be an intimate connection between the two organs. Just what this is, we cannot tell. It is generally assumed that nervous influences cause the coincidence in the development of the pregnant uterus and that of the mammary glands. It is perfectly conceivable that this assumption is correct, although in recent years it has been shown that many apparently reflex nervous processes may be traced to chemical reactions. We would recall in this connection the influence of the hydrochloric acid from the stomach upon the secretion of the pancreas. The secretion of the latter is accelerated as soon as the hydrochloric acid enters the intestine. The simplest assumption was that the hydrochloric acid irritated the end-apparatus of the nerves in the intestinal membrane, and thus reflexively stimulated the pancreas into increased activity. It has been shown, however, that the mucous membrane of the intestine contains an antecedent, the prosecretin, which is set free by the hydrochloric acid, and as secretin is carried by the blood-passages into the pancreas. According to this, the alimentary tract, or at least that portion which produces the prosecretin, falls into line with those organs which are said to produce *internal secretions*. It is not right to give a particular position to an organ shown to produce internal secretions. There is no reason why an organ which gives up its secretion directly to the blood should be considered as essentially different from the ordinary glands which send out their secretion through ducts. Numerous observations in physiology and in pathology compel us to assume that all the organs are in some way related to one another. We must not be satisfied by merely saying that this connection is made by means of the nerves. It is far more probable that the separate cells of the body not only give up the metabolic end-products to the lymph and blood, but secretions as well. This view seems in accordance with

the entire anatomical construction of the animal tissue and with our ideas of metabolism.

It would be, in fact, hard to understand why the separate tissues should be so much differentiated if the essential part of metabolic processes consisted merely in enabling the cells already formed to retain their constituents and in furnishing them merely with sufficient heat units for the exercise of their functions. If even the digestive process, which *a priori* appears so simple, requires such a fullness of chemical processes, utilizes so many organs, and reacts so sensitively to the different conditions which prevail, we may conclude at once that the cell-metabolism certainly cannot proceed along altogether simple lines, but that here also secretions from certain groups of cells must be of considerable significance. We hold that it is not entirely impossible that every individual cell of the body takes part in secretory work, and thus has in some way a favorable effect upon the general metabolism. Perhaps this point of view may give us some idea of the reason why organisms constantly require a certain amount of albumin. Unquestionably the proteins occupy a quite different position in metabolism from that taken by the nitrogen-free foodstuffs. We can well imagine that they are required chiefly for the formation of secretions. We do not overlook by any means the fact that the large requirement of albumin is even then only partly explained unless one is ready to assume that in the formation of the secretions a large number of cells are disintegrated and therefore must be built up anew.[1] It seems to us very important to state that there is no essential difference between the glands with an excretory duct and those in which there is no duct. It is especially doubtful whether we are justified in assuming that only cells arranged in the shape of a gland are active in the formation of secretions. Many facts indicate that the contrary is true. Moreover, there are many intermediate stages between glands with ducts and those with none. The mucous membrane of the intestine secretes intestinal juice, enterokinase externally and secretin internally. The pancreas secretes externally the digestive juice, and also probably secretes substances internally which take part in the metabolism of carbohydrates. Again, the liver undoubtedly has several secretory functions. On the one hand, it yields the bile which, in its formation and the method of giving it up, corresponds to an external secretion. Now we know, for example, that the liver is constantly storing away sugar and assimilating it as glycogen in order that, at the right moment, fermentation may cause the reverse process to take place, — i.e. sugar be given up to the blood. Certainly this is an internal secretion just as much as the formation of any other substance under the influence of the cells. To be sure, in this case we know just what this secretion is,

[1] Cf. Lecture XI, p. 221 *et seq.*

namely, *d*-glucose. The cells of the liver take part in its formation, inasmuch as they furnish the ferment which hydrolyzes glycogen. We have already indicated that we must not regard these fermentation processes as simple in their nature. Wherever it has been possible to follow a fermentation closely, it has been found invariably that it consists of a whole chain of separate processes. The ferment itself does not originally exist as such, but in a precursory stage, which is changed to the active condition by the aid of a product obtained from other cells. We cannot be wrong in assuming that such relations take part in the breaking down of glycogen.

We have mentioned these processes particularly because they seem to be the most suitable for demonstrating how the different cells of the body work together. It is certain that greater clearness would prevail with regard to pathological processes if such relations were kept more in the foreground in each instance and the diseased organ itself not so much regarded as representing the whole "case." If we study all of the complicated processes which take part in a single fermentation from the beginning to the end, we shall realize at how many different places disturbances in the normal course of metabolism may arise.

Let us now return to the functions of the mammary glands. These may very likely be excited into lactation by means of a secretion produced by the pregnant uterus, or its accessories. The transformations taking place in the dormant mammary gland from the beginning of its preparatory period to the time when it enters into the full exercise of its functions, are profound. There is an extensive formation of new cells. The cells of these glands, which, like all other cells of the body, receive their nutriment from the blood, suddenly make new demands upon it. They abstract a great deal of material from the blood, which they transform considerably. They form casein from the albumins of serum, and lactose from *d*-glucose. Again the salts are removed in definite amounts and quite independently of the ratio in which they are present in the blood. We have previously gone into these details. How the cells of the mammary glands accomplish these changes is not known. We do not know of any intermediate stages between the serum-albumins and casein, nor between grape-sugar and milk-sugar. We can merely imagine that maltose is formed in the production of the latter. Before much was known concerning the composition of the various different proteins, the transformation of serum-albumin into casein did not appear to be a very complicated process. To-day we are already compelled to assume that before casein can be formed, the serum-albumin must be decomposed to a considerable extent, after which a synthesis is effected. The cells of the mammary gland do not in principle assume any extraordinary position. Their chemism is merely an individual and a specific

one, and consequently the products which they form are typical, just the same as the salivary glands yield one specific secretion and the pancreas another. There are not any essential differences between these processes. We should be making a sad mistake if we were to consider the function of the cells of the mammary glands by itself. We are able to understand it only when we trace its phases from a general standpoint. The cells of the mammary glands take up from the blood, or more directly from the lymph, certain substances which evidently must be transformed completely, and to a certain extent assimilated, in order to form the product which it will subsequently give up. Recent investigations indicate that even the actual process of secretion is not different from that of other glands, for here also, after the secretion has been given up by the cells, a residue of protoplasm and nucleus remains, and a new formation of the same secretion again ensues.

It is not alone the mammary glands that are dependent upon the sexual apparatus. More and more we become cognizant of the fact that the different parts of the latter stand in a number of different relations to other organs, though we are not able to discover the nature of the active principle any more definitely. We know at present merely of isolated facts which we cannot explain satisfactorily. The different female sexual organs are, in the first place, related to one another. As an example of this, we need only cite the influence of the ovaries, when they are exerting their normal function, upon the uterus, and especially upon the mucous membrane of the uterus at the time of menstruation. Here again we frequently find this attributed to nervous excitement, although there is no definite proof that such is the case. The experiments of Halban [1] have shown that the ovaries can exercise their function when there is no connection with the nervous system. He showed that if he extirpated the ovaries from young guinea pigs and inserted them at another part of the body, the development of the external genitals took place exactly as if the ovaries had remained in their original position. On the other hand, in immature animals in which the ovaries had been completely removed from the body, there was a halt in the development of the external organs of generation. It is also well known that when the function of the ovaries ceases, whether due to their ablation in a mature condition, or to the fact that they have reached the end of the period of their activity, changes take place in the uterus corresponding to a retrogression.

Similarly the male organs of generation stand in relation to one another. This is already evident from the way the cells of the testes work together with those of the prostate gland, although this may be explained as a result of a common stimulation. There are observations according to which the prostate atrophies after the removal of the testes.

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[1] Monatschr. f. Geburtshilfe u. Gynäkol, **12**, 496 (1900).

The relations of the sexual organs to the entire organism are very interesting. By numerous experiments on men and animals, it has become well recognized that extirpation, the so-called *castration*, before sexual ripening has taken place, tends to prevent the formation of the secondary sexual character. This is well illustrated in cocks, which, as we all know, when fully developed sexually may be recognized by their wattles and combs. These remain undeveloped, or at least are but scanty, if the testes are removed before the sexual development is complete. It is interesting also to find that a secondary sexual character develops if the extirpated testes are transplanted after their removal from the fowl.[1]

One of the most prominent results of the removal of the sexual glands is an abnormal growth of the bones. In castrates it is frequently found that especially the tibia and the femur are prolonged. The cause of this has been traced to a faulty ossification of the epiphysis cartilage such that there is no limit placed upon the growth of the bone. Apparently castration affects the general metabolism. The great tendency of castrates towards obesity is well known. It has never been positively established whether this results primarily from the loss of the sexual glands or whether it is a secondary effect.

Although there is undoubtedly a connection between the sexual organs and the other organs of the body, still at present we are unable to identify any definite product of their secretion as the active principle. We know of glands, however, which are ductless, but do not give rise to such secretions. We refer to the suprarenal bodies and the thyroid gland.

The extirpation of the two suprarenal capsules has been made by Brown-Sequard,[2] whom we have to thank for many investigations in this line of research. He found that their removal caused death in a short time. He was able to keep the animal alive, if a part of one of the capsules was allowed to remain. The animal soon lost in weight and showed a peculiar behavior. It was lazy, and if compelled to work soon became very tired. One of the pronounced effects was that the blood-pressure fell immediately after the operation. The fact that the blood from such animals had toxic properties, and when injected into normal animals led to similar symptoms, as in the animal which had undergone the operation, gave rise to the assumption that the suprarenal capsules served to destroy those products formed by metabolism which are injurious. According to this view, the suprarenal bodies serve merely as a means of protection. There is no conclusive proof of the correctness of this assumption. It is clear that the extirpation of these capsules may influence metabolism in such a way that when they are removed from the body some product circulates through the body in an abnormal condition, and the toxic properties

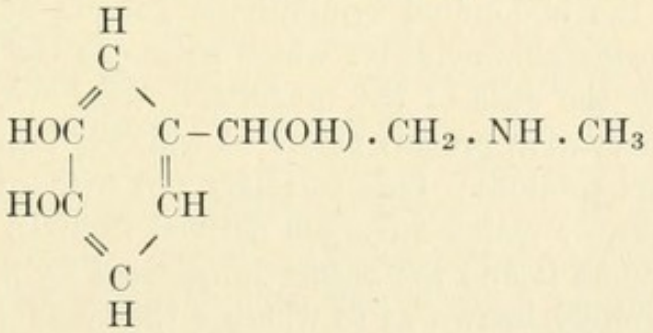
[1] Foges: Pflüger's Arch. **93,** 39 (1903).

[2] Compt. rend. **43,** 422 and 542 (1856); *ibid.* **45,** 1036 (1857).

of the blood may be due to this fact. It is perfectly unjustifiable to assume that substances appearing after the extirpation of an organ are in any way to be regarded as normal products, and that they are simply not removed because a certain organ is missing. It is possible that such an assumption does represent the truth, but at the same time it is equally probable that the injurious substances are formed because the organ is not present.

It has only quite recently been shown that the suprarenal bodies do actually give up to the blood a specific substance. It has been found possible to isolate this substance and crystallize it. Oliver and Schäfer[1] had observed that extracts of the suprarenal capsules when injected into the veins gave rise to a marked increase in the blood-pressure. These investigators traced this increase of pressure to a strong contraction of the blood-vessels, and also to the fact that the suprarenal extract had an action upon the heart. Long before this, in 1856, Vulpian[2] had his hands upon this active principle. He found that the suprarenal bodies contained a substance, the so-called *chromogen substance*, which turned dark on exposure to the air, and gave with ferric chloride solution a green coloration. It is only recently, however, that it was found possible to prepare this active principle in a pure state. Its discovery was not alone of physiological interest, but at the same time a remedy was added to our store, which met with a favorable reception such as is but seldom accorded to a new preparation. It is used especially to prevent bleeding in surgical operations. The composition of the substance corresponds to the formula $C_9H_{13}NO_3$.[3]

According to E. Friedmann[4] it has the following structure:



[1] J. Physiol. 16 (1894); **17**, IX (1894–95).

[2] Compt. rend. **43**, 663 (1856).

[3] Cf. von Fürth: Z. physiol. Chem. **24**, 142 (1898); **26**, 15 (1898–99); **29**, 105 (1900); Hofmeister's Beiträge, **1**, 243 (1901); Sitzber. kais. Akad. Wissensch. in Wien. Math.-natur. Klasse 112, Abt. 3 (March 5, 1903). Abel and Crawford: Johns Hopkins Hosp. Bull., No. 76 (1897). J. Abel: *ibid.* No. 90–91 (1898); Am. J. Physiol. March, 1899; Z. physiol. Chem. **28**, 318 (1899); Johns Hopkins, Bull., No. 120 (March, 1901); No. 128 (Nov. 1901); No. 130, 131 (Feb.–March, 1902); Am. J. Physiol. **8**, 2 (1903); Ber. **36**, 1839 (1903). J. Takamine: Am. J. Pharm. **73** (1901). H. Pauly: Ber. **36**, 2944 (1903). Abderhalden and Bergell: *ibid.* **37**, 2022 (1904).

[4] Hofmeister's Beiträge, **8**, 94 and 118 (1906).

Several names have been assigned to the substance, but that of *adrenalin* seems most suitable. By fusing it with caustic potash, protocatechuic acid is obtained. By the action of mineral acids, methylamine is split off. Among the other cleavage-products, pyrrole, methyl indole, and pyridine have also been observed. We are not much better informed concerning the formation of adrenalin than we are concerning its constitution. We do not know its source, although it is possibly derived from the proteins and their disintegration products.

As little as 0.0013 milligram of adrenalin will cause a noticeable increase of the blood-pressure when introduced into the circulation. At the same time it strengthens the heart-action. The peripheral vessels become strongly contracted. Mucous membranes appear nearly white on account of the almost complete absence of blood in them. Adrenalin also acts upon the *dilatator pupillæ*, *retractor membrana nictitans*, and the smooth muscles of the eyelids.[1] These muscles contract when under its influence. The movements of the stomach, the gall-bladder, and the urinary-bladder on the other hand are possibly restricted.[2]

How shall we imagine that the suprarenal capsules act in the economy of the animal organism? Evidently they are constantly giving up adrenalin to the blood either as adrenalin or perhaps in some kind of combination such that all the organs innervated by the *Sympatheticus* are affected by it. It is certainly interesting in this connection that neither their development nor anatomical structure indicates that these capsules are homogeneous organs. There are two distinct parts of the capsule, one of which is derived from a collection of mesenchymatous cells in the vicinity of the *inferior vena cava* and gives rise to the *cortex*, while the other originates from the abdominal sympathetic ganglia and forms the *medulla*. There is considerable evidence which points to the fact that adrenalin is produced by the cells of the medulla. We have, therefore, in a wide sense an innervation process before us which is brought about by the aid of a chemical product. It is certainly not without significance that the *Sympatheticus*, or rather an organ derived from it, produces a substance which acts upon it and the organs innervated by it. At present there is nothing definitely known as to whether the sympathetic nerve influences this internal secretion, but there is some evidence which indicates that such is the case.

We have already mentioned that there has been ascribed to the suprarenal bodies an action in combating poisons. Although we do not doubt in any way that these organs may have other functions than that of producing an internal secretion, still there is nothing positively known as to

[1] M. Lewandowski: Zentr. Physiol. **12**, 599 (1898).

[2] Boruttau: Pflüger's Arch. **78**, 97 (1899).

the nature of such functions. It may be mentioned that large doses of adrenalin produce glucosuria. It results from a glucohemia the cause of which is still obscure.

We may also mention that a disease is known to pathology which is related to the suprarenal glands. It is named after its discoverer, being known as Addison's disease.[1] The most important symptoms are as follows: There is an external pigmentation upon the skin. The mucous membrane also shows dark-colored patches, and there is a marked anæmia and extreme asthenia. Great muscular weakness characterizes the whole course of the disease. It is shown not alone in the inability of the muscles to perform work, but by the fact that they quickly become tired. We remember that these same symptoms were observed in animals with ablated suprarenals. All of the other symptoms in Addison's disease are of a secondary nature, and are due especially to the marked anæmia. A post-mortem examination shows that the suprarenal bodies are more or less destroyed by tumors, and usually as a result of tuberculosis. We must not fail to mention, however, that cases of the disease are known, in which the suprarenal capsules remain anatomically " normal." We have repeatedly laid stress upon the fact that the anatomical appearance of an organ does not always indicate whether the organ is exerting its normal function or not. Only when it has been found possible to show that the complex of symptoms of *Morbus Addisonii* can exist without there being any change in the *functions* of the suprarenal bodies, shall we be justified in questioning the connection of the above-mentioned disease with the suprarenal bodies. There has been a great deal of discussion as to the origin and significance of the bronze-colored patches upon the skin. The nature of the deposited pigment is not known. It is possible that it has some relation to the formation of the secretion by the suprarenals, and that it may perhaps represent an antecedent of adrenalin, which is deposited because in that condition it is of no benefit to the system. On the other hand, we must guard against putting the secretion of adrenalin too much in the foreground, simply because it is the only secretion of the glands of which we now know. It is pretty certain that the suprarenals have other functions, and it is perfectly possible that when these functions disappear the results are more serious than when the known secretion alone is wanting. Our knowledge is still far too limited for us to attempt to discuss the relations of the individual symptoms of the disease to the suprarenal bodies. Above all, the cause of the extreme lassitude of the patients remains unexplained.

Another very important organ is the *thyroid gland*. Its functions were investigated constantly for years, without its being possible to ascertain

[1] T. Addison : On the Constitutional and Local Effects of Disease of the Suprarenal Capsules. London, 1855.

what they were, and the attempts to isolate the active principle contained in the organ were fruitless. Here again we have observations from the fields of physiology and of pathology at our disposal. We shall begin with the latter. The thyroid gland frequently shows signs of degeneration, and this apparently takes place in a definite manner, and is most common in definite localities. These are the so-called *goitre regions;* i.e., regions in which there is frequently a cystic deformity producing an enlargement of the thyroid. The nature of the soil, and especially of the drinking-water, has been assumed to be the cause of the disease, without any one being able to show this conclusively. Frequently other severe derangements accompany the deformity of the gland. The mental development of those afflicted with the disease is slight. Such individuals are known as *cretins*. There is a derangement of their entire metabolism. Scholz [1] in particular, who studied a case very carefully, showed this to be true of the metabolism of albumin and salts. We are justified in assuming, as we shall soon see, that the thyroid gland has not ceased entirely to exercise its function. It seems highly probable that we shall realize more closely the part taken by the thyroid gland in metabolism, if we assume that it has different functions. We can well imagine that in cretinism it is possible for the thyroid to retain certain of its functions while others are missing.

An idea as to the complete function of the thyroid gland is probably to be obtained best by studying an organism after the complete ablation of the organ. The operation has been performed upon man as well as upon animals; in the first case, however, only at a time when we were not yet ready to study the whole consequence of the interference. To-day it is well recognized that the organ is quite essential, so that care is taken not to remove it completely. We may say, in this connection, that a small portion of the gland left in the body is usually sufficient for the retaining of all the functions of the organ. This fact should be well borne in mind, for it gives us the key to the cause of many contradictions which are to be found in the literature. The thyroid gland itself is an unpaired organ. Anatomically it is usually homogeneous. Embryologically it arises from clefts or bronchial arches at the lower part of the epithelium of the pharynx. Often, however, we find near the main gland isolated fragments of similar material in the surrounding tissue, and frequently these are quite far from the main gland. In the ablation of the latter, they may assume its functions, so that the typical results of the operation are not felt. Entirely distinct from the thyroid gland are the *parathyroid glands*. They are paired, and originate from the last pair of clefts of the pharyngeal epithelium. We shall find, later on, that their position relatively to

[1] Z. exper. Path. u. Therap. **2**, 271 (1905).

the main thyroid gland varies in different animals. Their significance has only very recently been realized.

If the thyroid gland is completely removed from the body, or its functions fail for any reason, peculiar changes result. They were first observed and described by William Gull [1] in 1874. The most prominent symptom is a thickening of the skin. It appears, on account of the increased amount of mucin in the subcutaneous connective tissue, as an edematous swelling. For this reason Ord [2] gave to the disease the name, *myxedema*. Subsequently the swelling goes down, and the skin then appears more atrophied. The secretion of the glands in the skin ceases, and the latter becomes hard and dry. Metabolism is disturbed, and so is the temperature of the body, and the mechanism for regulating the body-temperature. The most striking disturbances are those of the muscular and nervous systems. They are of various kinds. Sometimes there is evidence of increased sensitiveness, while in other cases the change is entirely in the other direction. The various effects, which also change as the disease progresses, may be due to the more or less complete failure of the functions of the gland.

J. L. Reverdin, A. Reverdin,[3] and afterwards Theodor Kocher,[4] had considerable opportunity to study the effects of the total extirpation of the organ in man. They found on the whole the same symptoms as in myxedema. Kocher embraced the whole complex of symptoms under the name of *Cachexia strumipriva*. It is not a simple disease. In general the same characteristics are manifest. In individuals which have not attained full growth, extirpation of the organ causes a tardy development of the length of the bones. We find here reminiscences of cretinism. It is specially noteworthy that individuals quickly lose the ability to reason. Finally idiocy may result.

After J. L. Reverdin had published his first results, physiologists recalled the experiments of Moritz Schiff [5] made in 1859, with regard to the total extirpation of the thyroid gland in animals. Schiff showed that dogs did not long survive the operation. They died within from 4 to 27 days. There is to-day no doubt prevailing as to the correctness of his observations, although the cause of the different behavior of various classes of animals has been much disputed. With dogs and cats death results quickly and usually in a convulsive attack (tetany). Muscu-

[1] Trans. Clin. Soc. London, 1874.

[2] On Myxœdema. Medic-chirurgical Transactions, Second Series, **43**, 57 (1878).

[3] J. L. Reverdin: Revue médicale de la Suisse romande, 2ième année, 539 (1882), and 3ième année, p. 47 (1883). J. L. Reverdin and Aug. Reverdin: *ibid.* 3ième année, No. 4, pp. 169, 233, 309, and 686 (1883).

[4] Arch. Clin. Chir. **29**, 254 (1883).

[5] Arch. exper. Path. Pharm. **18**, 25 (1884).

lar tremors first appear, which gradually pass into clonic spasms, finally resulting in tetanus. The muscular tremors are not of peripheral origin, for they disappear on section of the peripheral nerves. Apparently the thyroid gland in some way influences the higher nerve centers. It is evident, however, that the lower nerve centers are also affected, for the tremors continue after the removal of the cortical brain area concerned with the movement of the part. In the case of herbivora, the ruminants, rodents, and monkeys, tetany does not as a rule take place. Instead, cachexia becomes a prominent symptom. This contrast of symptoms in the two classes of animals, which was made more puzzling by reason of the fact that with the herbivora sometimes tetany appears and sometimes does not, has recently been offered an explanation. We have already mentioned the presence of the parathyroid glands. In the carnivora these are included in the main gland, whereas in the herbivora they are separated from it. For this reason the parathyroid glands are always removed from carnivora in cases of complete ablation of the thyroid gland, whereas in the herbivora this is rarely the case. In fact, it has usually been found that tetany in herbivora results when these parathyroid glands are removed.[1] According to this discovery, it would seem that the parathyroid glands and the main gland have different functions. It seems highly desirable that clinical observations should receive renewed study with regard to this point.

It might be objected with regard to the experiments in the ablation of the thyroid gland that the operation itself may be such a severe one that other injuries can produce some, at least, of the observed symptom complex. This objection, however, has been successfully refuted by means of a great many experiments. In the first place, the entire operation may be performed, except that the gland is allowed to remain in place, without any of the symptoms occurring. Again, if a part of the organ is allowed to remain in the body, the symptoms do not appear; and, finally, if a part of the organ is transplanted to another part of the body, the whole operation may then be carried out without fatal results. Such experiments were performed by Schiff and have been repeated by Eiselsberg[2] in a particularly convincing manner. The latter extirpated half of the thyroid gland from a cat and grafted it in the wound between the abdominal fascia and the peritoneum. Then, after this had been accomplished, the other half of the organ was carefully removed. The animal was kept under observation for two months without its showing any

[1] Cf. E. Gley: Compt. rend. soc. biol. Paris (9), 841 (1891). Vassale et Generali: Arch. ital. biol. **25**, 459 (1896); **26**, 61 (1896). Biedl: Innere Sekretion, Berlin, 1904. MacCallum: Zentr. allg. Path. u. path. Anat. **16**, No. 10 (1905).

[2] A. Freiherr von Eiselsberg, Wiener klin. Wochschr. **5**, 81 (1892).

indication of a cessation in the functions of the gland. Then the grafted piece of thyroid, which showed normal gland-tissue, was removed. The very next day tetany resulted, and the animal died at the end of the third day.

It is also important to learn that it is possible to prevent the severe disturbances resulting from the ablation of the organ, by injecting thyroid juice into a vein or under the skin, and even by feeding it, or raw thyroid, directly. In fact, it is even possible to improve the condition of the patient who has already begun to feel the effects of the operation. It is seldom that a therapeutic conception can be demonstrated so clearly and so strikingly as in the treatment of *Cachexia strumipriva*, and true myxedema, by means of thyroid preparations. The swelling of the skin goes down, and the mental faculties are noticeably improved. In a short time the habits of the patient are so changed that almost nothing remains to indicate the original severe disease.

As soon as the action of the thyroid gland became understood, attempts were made to isolate the active principle; but, up to the present time, such attempts have been in vain. It was indeed believed, for a short time, that the goal had been reached when E. Baumann,[1] after making the important discovery that the thyroid glands of many animals contain iodine, succeeded in isolating an amorphous substance, the so-called iodo-thyrin (or thyro-iodine). To-day the relation of this substance to the organ is still very vague. It contains phosphorus and about nine per cent of iodine. Now there is no doubt that iodine itself has an effect upon the thyroid gland, and, in fact, even when it is administered, not in the form of an organic compound, but as free iodine. Often an existing swelling of the gland subsides. It is still an open question how the iodine acts, but we are aware that it has a favorable effect upon various other filtration processes and facilitates, for example, the absorption of exudates. To be sure, iodine apparently has a quite specific action upon the thyroid gland. The fact, however, that iodine may be absent from a normal organ, makes it seem doubtful whether one is on the right road in assuming that iodo-thyrin is the active principle of the gland. It seems that possibly too much attention has been paid to the iodine constituent. It also must not be forgotten that we have no guarantee for assuming that iodo-thyrin is itself a simple substance. It is more probably a mixture of several different products. At all events, it is certain that the gland itself is more active than iodo-thyrin, and so are all the preparations which contain as many glandular constituents as possible.

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[1] E. Baumann: Z. physiol. Chem. **21**, 319 (1895–96). Baumann and Roos: *ibid.* **21**, 481 (1895–96). E. Baumann: *ibid.* **22**, 1 (1896–97). E. Roos: *ibid.* **22**, 16 (1896–97); **25**, 1 and 242 (1898). A. Oswald: *ibid.* **23**, 265 (1897).

We are still very far from being able to trace the functions of the thyroid gland to definite chemical processes. We merely understand the effect of its extirpation, and know, furthermore, that it stands in some relation to the sexual organs. It has been observed that at the time of menstruation, during pregnancy and lactation, there is frequently a swelling of the gland. To be sure, processes may be involved here which have nothing whatever to do with the cell-functions of this organ, but may be caused by vascular influences. Again, the observation that in cretins the sexual organs frequently remain undeveloped, does not necessarily prove that there is a direct relation between the thyroid gland and the sexual organs. It is certainly not remarkable that in the general metabolic disturbance even the sexual organs, which as a rule require a constant supply of material as they in a certain sense are constantly growing, will likewise suffer to a marked extent. At present it is impossible for us to distinguish here between primary and secondary phenomena.

That the thyroid gland yields a secretion, cannot be doubted. This is evident alone from its histological structure. Apparently the follicular cells produce the specific secretion. It then passes through openings in the follicular walls into the lymph, and is then given up to the blood.[1] We will merely mention the fact that Oswald isolated from the secretion of the follicles (the so-called colloid) two proteins, the so-called *thyreoglobulin* and a nucleoproteid. The former alone contains iodine.[2]

Our limited knowledge concerning the chemical processes taking place in the thyroid gland makes it impossible for us to in any way give a precise description of the nature of the function of this very important organ. Everything is hypothetical. According to the *autotoxication theory*, it is the purpose of the thyroid gland to remove, or render innocuous, one or more toxic substances which would otherwise accumulate in the blood. There is no ground for this assumption, but it is perfectly conceivable that the organ can secrete substances which are capable of combining with other products. It is possible that the iodine content of the thyroid gland serves such a purpose and perhaps indicates the presence of easily replaceable substances. It is, however, also very probable that the thyroid gland secretes substances that take part in the general metabolism and regulate chiefly the transformations which albumin undergoes. It is not at all difficult to formulate assumptions in this direction, particularly after repeatedly meeting with facts which show that in order to accomplish fermentation, a number of different body-cells act together. One cell yields an activator of the ferment, and another the ferment itself. It is perfectly conceivable that the thyroid gland is active in this sense, and

[1] Hürthle: Pflüger's Arch. **56**, 1 (1894).

[2] Z. physiol. Chem. **27**, 14 (1899). Hofmeister's Beiträge, **2**, 545 (1902). Z. physiol. Chem. **32**, 123 (1901).

that it perhaps secretes a kinase which is for the good of all the body-cells. But all this is speculation, and drawing inferences from analogy without any real foundation. We must not fail to repeat here that the functions of the thyroid gland are not all of the same kind. It may also serve to start certain processes.

We must now consider an action of the thyroid gland bearing a certain analogy to a disease, which, to a certain extent, is the exact opposite to *Cachexia strumipriva*. We refer to *Basedow's disease*. If too much thyroid gland is administered, there results an abnormal destruction of albumin. The elimination of nitrogen in the urine increases considerably. Furthermore, there is an apparent intoxication, with increased pulse frequency, polyphagia, polydipsia and polyuria. In Basedow's disease similar symptoms appear, especially the increased destruction of albumin. This disease has been traced to an increased activity of the follicular epithelium of the thyroid gland. Quite recently the parathyroids have also been held to be partly responsible. There are many observations which indicate such a connection,[1] but it has been by no means positively established.

The *hypophysis*, or *pituitary gland*, is always mentioned in connection with the thyroid. It is a compound organ. The anterior lobe is glandular and resembles somewhat the thyroid body, while the posterior portion consists chiefly of fibrous tissue. Between the two lobes there is a hollow space rich in vessels and lined with ciliated epithelium. The function of this body, which was once considered to be a rudimentary organ, is still unknown to us. In cases of myxedema there has frequently been hypertrophy of the pituitary gland, while extirpation of the thyroid tends to produce the same effect. In cases of hypertrophy and enlargement of the pituitary body peculiar symptoms often develop, especially an abnormal growth of the bones at the end of the extremities, the phalanges of the fingers and toes, although the softer parts, as the hands, feet, lips, tongue, and nose, are also affected. It is quite natural to compare this increased development with the retarded growth which takes place after the cessation of the functions of the thyroid gland. Yet we do not positively know that there is a direct connection with this disease, known as *acromegaly*, and the changes in the functions of the pituitary gland. Experiments carried out to determine the functions of this organ, by studying the effects of its removal, have not led to conclusive results. Pituitary extracts have also been administered and found to cause an increased elimination of nitrogen.[2] We are not justified in drawing any conclusion from this observation as to any existing analogy with the thyroid

[1] L. Humphry: The Parathyroid Glands in Grave's Disease. Lancet, 11 (1905).

[2] T. Malcolm: J. Physiol. **30**, 270 (1904). Thompson and Johnston: *ibid*, **33**, 189 (1905).

gland. The increased elimination of nitrogen may be caused in many ways. We shall not be in a position to decide such questions until it has been found possible to isolate the active principles from each of the organs.

We now turn to two other organs for which a specific function has been suggested. These are the spleen and the thymus. The latter is a temporary organ, being a true organ in the case of man only during infancy. After the child is two or three years old it no longer develops, but slowly and steadily atrophies, and has nearly disappeared by the fifteenth year, though traces of it remain in old age. It can be completely extirpated without causing death. It is, therefore, not to be classed with the organs that are essential to life. Its ablation is said to result in disturbances in the general health and in metabolism; but from the data at hand, it is not possible for us to obtain a very clear conception of the functions of the thymus gland. Similarly, its anatomical construction is not instructive.[1]

We are almost as much at sea concerning the significance of the spleen in the economy of the animal organism. All sorts of different functions have been ascribed to it. It has been said to influence the activity of the pancreas, an assumption which is not well founded. It has also been assumed that it plays a part in the production and destruction of the red corpuscles, and furthermore that it is able to remove and store up waste material from the blood and lymph. This much is certain, however: the spleen can be extirpated completely without any severe consequences. It would be of course unjustifiable to conclude from this that the spleen is an organ of subordinate importance. Everything depends upon the conditions under which the functions of an organ are tested. It is perfectly possible that under certain conditions the absence of the spleen might make itself felt. It may be mentioned, in this connection, that great importance has been ascribed to the spleen in combating disease germs. In the case of infections, the spleen sends out a great number of leucocytes. On the other hand, there are certain indications of the fact that the spleen on account of its anatomical construction is called upon to regulate the composition of the blood so that the cellular elements are kept in such a condition that they are capable of exercising their functions. Abnormal red and white blood-corpuscles are held back and destroyed. It is possible that the proteolytic ferment found in spleen, which has an action upon fibrin, may be active in the breaking down of these discarded elements. On the other hand, the high iron content of the spleen, to which our attention has been called repeatedly, is not necessarily to be regarded as

[1] A. Friedleben: Die Physiologie der Thymusdrüse (1858). Cf. J. Aug. Hammar: Pflüger's Arch. **110**, 337 (1905). Rudolf Fischl: Z. exper. Path. Therap. **1**, 388 (1904).

absolute proof of the destruction of blood-corpuscles, for it collects cellular decomposition products from other organs.[1] Many observations make it seem probable that the spleen is related in some way to bone-marrow. These two substances can mutually aid one another.

In discussing the individual organs we have lost sight of the chief constituents of the entire organism, namely the muscles, nerves, and the connective-tissue group. Of the latter, especially bone, cartilage, and true connective-tissue, we scarcely assume any intimate relations with the other organs, although undoubtedly such relations do exist. We are accustomed to consider them merely as mechanically-acting structures, and for this reason but little attempt has been made to ascertain what metabolic changes take place within this group of tissues. As a rule, investigators have been content with the study of their chemical composition without attempting to determine positively the extent to which they take part in the general metabolism. Now this connective-tissue group of substances takes part quite extensively in building up the organism, and its functions are not always the same. This is particularly true of connective-tissue itself, which, according to its histological construction, is related to different groups. For one thing it forms the fundamental support of the body-cells, which, to a certain extent, are embedded in it. From it the finer and coarser network, in which the lymph passes until it reaches the individual cells, is formed. It is very questionable whether one is justified in assuming that the cells of connective-tissue play a passive rôle here, or whether it is not more probable that they take active participation in the exchange of material between the blood and the lymph, and between the latter and the remaining cells of the body. Its adjustment to purely mechanical requirements is evident from the construction of the individual tissue; for example, in the segregation of elastic fibers. A particularly differentiated tissue, and one which also belongs in this group, is the fatty tissue; the importance of which we have already considered.

As regards the physiological functions of cartilage, but little is known beyond its purely mechanical properties. It is, however, not to be doubted that in it, as well as in body tissue, there is a constant occurrence of metabolism. Growth never really ceases, for new cells are constantly being formed. Numerous observations prove to us the dependence of the metabolism in these organs upon the requirements placed upon them. Their development ceases if for any reason there are no demands placed upon them, and in such cases they retrogress even if they are already well developed. The influence of the thyroid gland and other organs upon growth, we have already mentioned. Very active metabolic processes unquestionably take place in cartilage and bony tissue of the growing

[1] Blumenreich and Jacoby: Z. Hygiene u. Infectionskrankh., **29**, 419 (1898). G. Jawein: Virchow's Arch. **161**, 461 (1900).

organism. These two tissues are intimately related to one another. The latter can act for the former within certain limits. We find here quite extensive assimilation processes, and at the same time there is a considerable wearing away. It is seldom that we obtain such a deep insight into the transformations of tissue as in the case of the new formation of bones. To be sure our knowledge in this direction is almost wholly morphological. There has been but little attempt to study this interesting process from a physico-chemical standpoint. Even the fully-developed bone retains, especially as regards its periosteum and medulla, its embryonal character. From these, new bone material may be formed continually. Thus fractures are healed. Even under normal conditions, however, there is continuously taking place a fusion and new formation of bone-substance. Occasionally we notice the appearance of peculiar cells, the so-called *osteoclasts*, which cause the dissolution of bony tissue at the place where they appear, while on the other hand we meet with the so-called *perforating fibers*, which also destroy bones. In pathological conditions often the real disappearance of bony tissue is preceded by decalcification as a primary process. It is interesting to trace the course of all these processes, in order to ascertain how, in each separate case, the breaking down of the bony substance is effected, what agents are active in the process, and why it is necessary that this fusing together of bone and new formation of such tissue are constantly taking place. We must for the present allow all these and similar questions to remain unanswered. We mention these relations especially because we are indisputably justified in assuming that if in a tissue to which we are accustomed to assign a very specific function, and in which we would scarcely expect *a priori* that important metabolic processes would take place, there is a constant exchange of material, so much more will this be true of all the other tissues which are intimately connected with active metabolism and upon which great demands are placed, and that they will likewise participate in an active interchange of cell-material.

Such an assumption appears to be particularly justifiable for those organs whose activity, within certain limits, is a continuous one, as is true especially of muscular and nervous tissue. The latter, as we well know, is never at rest. Impulses are constantly passing along the nerve fibers, partly towards the central nervous system, and partly from this to the peripheral organs. The nerves are very intimately connected with the muscular tissue. This is evident even from the entire development of the organs, for they early enter into relations with one another. We know also that if the innervation ceases, retrogression soon results, bringing on atrophy, and, in fact, this is evidently due in part to the inactivity of the muscles. Unquestionably, the nerves have to some extent a direct influence upon the metabolic processes in the cells of the muscles themselves,

perhaps in the same way as we found in the formation and breaking down of glycogen in the liver that there was an indeterminable dependence upon the nervous system. Unfortunately, in comparison with the countless observations of pure physiology concerning the functions of the entire nervous system, we have nothing from the physiological-chemical standpoint. In fact, aside from the knowledge of a few constituents of the nerve substance, we know very little indeed concerning the metabolism taking place in nervous tissue. We will give here the results of the analysis of the gray and white substances of the brain.[1]

	White Substance.	Gray Substance.
Water	695.35	769.97
Total solids	304.65	230.03
Protagon	25.11	10.80
Insoluble albumin and connective-tissue	50.02	60.79
Cholesterol, free	18.19	6.30
Cholesterol, combined	26.96	17.51
Nuclein	2.94	1.99
Neurokeratin	18.93	10.43
Mineral matter	5.23	5.62

The most striking value — and this holds also for the composition of the peripheral nervous system — is the high phosphorus content of nervous tissue. Phosphorus is evidently found in very different states of combination, at one time as nuclein, again in the form of a substance which is known as protagon,[2] and at other times as lecithin. The latter occurs partly free, and partly results from the hydrolysis of different products, which have been isolated from nervous tissue, and have been designated by different names; thus in the decomposition of protagon among the fatty acids and other cleavage-products, the so-called *cerebrin* has been found. Of the substances which have been isolated directly from the brain, cerebron has been best studied, and its constitution established by Thierfelder.[3] He obtained from it, by hydrolysis, cerebronic acid, sphingosine, and galactose in the following proportions: cerebronic acid 48.13 per cent, sphingosine 34.46 per cent, galactose 21.77 per cent.

Cerebron has the empirical formula $C_{48}H_{93}NO_9$. Its hydrolysis takes place in accordance with the equation:

$$\underbrace{C_{48}H_{93}NO_9}_{\text{Cerebron}} + 2\,H_2O = \underbrace{C_{25}H_{50}O_3}_{\text{Cerebronic acid}} + \underbrace{C_{17}H_{35}NO_2}_{\text{Sphingosine}} + \underbrace{C_6H_{12}O_6}_{\text{Galactose}}$$

[1] F. Baumstark: Z. physiol. Chem. **9**, 145 (1885).

[2] Liebreich: Annal. **134**, 29 (1865).

[3] H. Thierfelder and Emil Wörner: Z. physiol. Chem. **30**, 542 (1900). H. Thierfelder: *ibid.* **43**, 21 (1904), and **44**, 366 (1905).

A number of other products containing nitrogen, and, for the most part, phosphorus as well, have been isolated from the brain and described under different names. We have no means of deciding at present anything regarding the nature of these substances as to whether they are simple substance or mixtures, and for that reason will not stop even to enumerate them.[1]

Nervous tissue always contains cholesterol, fat, and albumin, and, in fact, besides nucleoproteids and nucleoalbumins, globulin and albumin. The framework of the tissue is formed by neurokeratin.

If we compare the amounts of the separate constituents present in 100 grams of organic dry substance, we note especially the high albumin content of the nervous tissue. Thus Chevalier[2] analyzed the sciatic nerve of man (the central organs have a similar composition), and obtained the following values:

Protein	36.8 per cent
Lecithin	33.57 per cent
Cholesterol	12.22 per cent
Cerebrin	11.30 per cent
Neurokeratin	3.07 per cent
Other organic substances	4. per cent

It is a striking fact that gray brain matter contains much more water than white brain matter. The former contains about 77 per cent of water, while the latter has but 70 per cent. The proteins occur chiefly in the gray matter, and amount to more than one-half of its dry substance.

The metabolism of nervous tissue is almost entirely unknown to us. Whereas in the case of muscles we are able to detect purely external changes (shortening) during their activity, and at the same time can detect the liberation of heat and consumption of glycogen, this is impossible in the case of nervous tissue. In the ganglion cells alone have histological pictures been described which apparently indicate changes. The nature of these changes is, however, very obscure. We merely know that nervous

[1] As regards the chemical constituents of brain, see J. L. W. Thudichum: Die chemische Konstitution des Gehirns des Menschen und der Tiere. Tübingen, 1901. Although the investigations of Thudichum are in certain respects valuable as a basis for further physiological-chemical investigation, still it must be said that none of the many compounds described by him was proved to be a simple substance, nor was there given any proof concerning the chemical combination of the substance. There is an almost unexplored field for investigation here. The article by W. B. Halliburton, Die Biochemie der peripheren Nerven. Ergeb. Physiol. (Asher and Spiro) Jg. **4**, p. 23 (1905), is a valuable summary of the work that has been done.

[2] Z. physiol. Chem. **10**, 97 (1886).

tissue requires oxygen for the performance of its functions. This may be shown very prettily by means of methylene blue. As we have seen, this dyestuff is deprived of oxygen by the tissues, and is thereby transformed into the colorless reduction product. If the colorless organ is subsequently exposed to the air for some time, gradually the blue color of methylene blue reappears. In the case of narcotized animals in which the brain has been made inactive, the brain substance remains blue, so that evidently under these conditions the tissue does not require oxygen.[1] The abundance with which the nerve-centers are supplied with blood-vessels is an indication of their high oxygen requirement, and in fact anæmia in these places causes severe disturbances, eventually leading to the loss of function.

It might be thought that some idea concerning the metabolism of nervous tissue might be gained by seeking the end-products. As a matter of fact, in the cerebro-spinal fluid, which in a sense may be regarded as the lymph of the brain, choline, a decomposition product of lecithin, has been found. This, however, does not give us much information. We do not know whether choline is to be regarded as a true metabolic end-product, or whether it is not more probably produced by the destruction of nerve-tissue, and is far from being concerned in true metabolism. Its detection has usually been an indirect one and not quantitative. The fact that an increased amount of choline appears in degenerative processes indicates that its formation corresponds to a destruction of nervous tissue, so that it is not to be considered as a normal metabolic product. We do not wish to place any great weight upon this discovery of the presence of choline, and would rather take the attitude that at present we know nothing whatever concerning the metabolism in nervous tissue. It is also hardly to be expected that there will be much progress in this direction for a long time. There is no foundation for research. Even our knowledge of the composition of nervous tissue is extremely faulty. We have only to remember that, with few exceptions, we know but little concerning the metabolism of those cells of the body which are much more readily accessible. We recognize only the total results of metabolism, and are, as a rule, ignorant as regards the part taken by the separate organs. We shall soon come back to the fact that it is impossible to detect any effect of intense mental effort in metabolism experiments.

We might perhaps expect that pathology would tell us something concerning the metabolism in nervous tissue. We know that there exist the so-called *functional* nervous diseases, i.e., diseases which, according to the general assumption, are not caused by any anatomical change of the

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[1] C. A. Herter and A. N. Richards: Am. J. Physiol. **12**, 207 (1904). Cf. H. von Bayer: Z. allg. Physiol. **2**, 169 (1902). Fröhlich: *ibid.* **3**, 131 (1903). Fröhlich and Tait: *ibid.* **4**, 105 (1904). K. H. Baas: Pflüger's Arch. **103**, 276 (1904).

nerve-tissue. We observe all sorts of different symptoms, one of the most characteristic being the readiness with which the patient becomes fatigued. One gets the impression that the nerve-centers have but a limited supply of material at hand, or that the combustible material is consumed rapidly without a sufficient regulation. This, however, is merely supposition and rests upon no foundation. In the case of organic nervous diseases, the symptoms of which are characteristic according to the nerves and nerve-centers that are affected, we find degeneration. The nerve-cells and their processes are destroyed. We may say that the experiment has been tried, to trace some relation between the selection which certain poisons, e.g. lead and "syphilis poison," show toward the various nerve-paths and the activity of the metabolic processes in special regions. Those nerve-paths and nerve-centers are assumed to show soonest the action of the poison which are most affected because they have the most work to do. As interesting as this hypothesis of Edinger[1] may be, we must state that there is absolutely no direct proof possible at present. As long as the physiological course of metabolism remains so obscure, it is very difficult indeed for us to get any clear idea from pathological deviations. We can, it is true, imagine that cells which are in constant activity and are constantly being used up and reconstructed, will feel the effects of a given poison much more rapidly than those which are fairly stable. This, however, does not necessarily imply that there is an exhaustion in the sense meant by Edinger, but rather it may be that there is an effect upon the more active supply of new material and increased consumption which is necessary for the exercise of the enlarged function. We know that there are certain cells, e.g., of infusoria, algæ, etc., which have a particular power of attracting certain metals, even when the latter are present in extremely slight amount. They take up these substances even from very dilute solutions and store them up. This may very easily cause the destruction of the cells, evidently in part, at least, on account of the fact that these substances combine with the constituents of the cell in such a way that they prevent the further exercise of function by the cell-protoplasm, and to some extent destroy the cell. Similarly it is conceivable that the interposition of the above-mentioned poisons between the separate constituents of the protoplasm of nerve-centers, disturbs the normal construction of the cells, and, therefore, its functions. There is not necessarily any affinity existing between the nerve-cell as a whole, and the poison in question, for it is perfectly possible that in the breaking down and building up of the constituents of protoplasm, the products resulting combine with the poison and consequently place a limit upon

[1] Eine neue Theorie uber die Ursachen einigen Nervenkrankheiten insbesondere der Neuritis und Tabes, Leipsic, 1899, und Deut. med. Wochsch. Nos. 45, 49, 52 (1904); Nos. 1 and 4 (1905).

the normal composition of the cell-protoplasm. We mention these ideas merely to show that we are able to get along perfectly well without the conception of exhaustion. On the other hand, we must remember that the various nerve-cells are not necessarily all of uniform nature. It is perfectly possible that the different groups of nerve-cells, which serve for the exercise of definite functions, are differently constituted chemically and possess a perfectly distinct metabolism, and that the key to the cause of the different diseases of the system is to be sought in this fact. The familiar diseases of the nervous system which are always, within quite narrow limits, localized along definite paths are of quite particular interest in many directions. Here the close connection between nervous and muscular tissue becomes very evident.[1]

We have repeatedly spoken of the direct and indirect influence of the nervous system upon all the organs of the body. It is quite impossible to define this more closely, i.e., we cannot in any way explain the nature of the conveyance of sensation. We merely know that the nerve-fibers are merely outgrowths of the nerve-cells with which they form a unit. The former are in contact with the organs usually by means of a characteristic end-apparatus. It does not seem at all impossible that physiological chemistry, together with physical chemistry, will eventually throw light upon this process. It can hardly be doubted that definite changes, whether in the end-apparatus or in the nerve-cells, give rise to definite functional expressions of the nervous tissue.

Before we take up the relations of the musculature to the other organs, we will consider briefly a reaction which is common to both muscle and nervous tissue, namely the so-called *heat-rigor.*[2] If a muscle is gradually heated, it loses at a definite temperature its power of being stimulated, and contracts. The cause of this heat-rigor is the coagulation of the albumin, and it may be shown that this does not take place all at once, but in stages. Different albumins present in the muscle coagulate at different temperatures. The nerves behave in exactly the same way. They also show a heat-rigor. The lowest temperature at which one of the albumins coagulates, corresponds to the time that the nerve ceases to be capable of response to stimulation. Here, as in the case of muscles, there is a contraction.

As is well known, the albumins in muscles coagulate after death. Rigor mortis results, and this does not attack all the different muscles at one time. Its appearance after death also occurs at different intervals of time. The cause of death-rigor has been much studied. It is certain

[1] Cf. Robert Bing: Deut. Arch. klin. Med. **85**, 199 (1905); and Deut. Z. Nervenheilk. **26**, 163 (1904); and Ueber angeborene Muskeldefekte. Inaug.-Dissert. Basel (1902).

[2] Brodie and Richardson: Philos. Trans. London, Series B, **191**, 127 (1899). Vernon: J. Physiol. **24**, 239 (1899). O. von Fürth: Z. physiol. Chem. **31**, 338 (1900).

that it results from the coagulation of the protein in muscle, and in fact it is believed to be the protein known as myogen which takes part especially in the process. This is supposed to pass over first into soluble myogen-fibrin, which is subsequently transformed into the coagulated modification. The other protein, myosin, also takes part in the formation of the clot.[1] Although the assumption that death-rigor results from a coagulation of the protein has been generally accepted, on the other hand the manner in which the coagulation takes place has been variously explained. Considerable attention has always been paid to the fact that an acid reaction appears. This is apparently brought about by the formation of lactic acid, and by the resulting transformation of a part of the diphosphate contained in muscle to monophosphate. Now it has been observed that acid, and especially lactic acid, accelerates the coagulation process. The early appearance of death-rigor after previous active muscular contraction, as, for example, in tetanus, has been attributed to the action of the lactic acid, which is in such cases present in larger amount than usual. Furthermore, it has been assumed that a ferment assists in causing the coagulation. The final relaxation, which takes place after an indefinite length of time, is also not perfectly understood. Acids have been supposed to take part in this process also, while, on the other hand, it has also been assumed that autolytic processes come into play.

In certain directions we are well informed concerning the processes of metabolism which take place in muscles during the exercise of their functions. We have already discussed these points. We also know that the liver is apparently in direct relation to the muscles, for, by means of its glycogen store, it satisfies their nutritional requirements. It is not necessary that the relation between the liver and muscles should be a direct one. It may be brought about by means of the blood. This has, as we know, a sugar content which is very constant within narrow limits. In case the sugar in the blood is used up by the muscles, it receives a new supply from the liver, the cells of which in such cases at once break down glycogen into sugar. There must also, without doubt, be relations between the general metabolism of the cells and that of the muscles. If, for example, it is desired to fatten with albumin, this can be accomplished well only when there is muscular effort at the time the abundance of albumin is fed. This may be explained on the assumption that under such conditions albumin is assimilated to a greater extent than usual, although it is also possible that the other cells of the body are in some way stimulated to take up more albumin.

This finishes all that we have to say here with regard to the relations of the individual organs to one another. They are, to be sure, much more

[1] O. von Fürth: Arch. exper. Path. Phar. **36**, 231 (1895).

diversified than we have here represented, and are of great importance in the study of the processes which take place in the animal organism. It is one of our most important tasks to follow these problems farther. It is only when we shall have acquired as thorough a knowledge as possible in this direction, that we shall be in a position to draw a clear picture of the cell-metabolism and the functions of the organs. The dependence of the organs upon one another is, as a rule, too little emphasized.

LECTURE XXVII.

GENERAL METABOLISM.

I.

We have so far considered for each foodstuff the way it is absorbed, assimilated, and finally eliminated from the animal organism, and attempted, above all else, to follow the intimate processes of metabolism in the tissues and especially in the cells. The study of these processes separately has to-day become the particular field of the physiological chemist. We should err greatly, however, if we were to regard the chemical decompositions in the animal organism solely from the standpoint of the individual foodstuff. We should obtain an entirely false impression of the general metabolism, and should be unable to answer some of the most important questions. We have up to this time studied metabolism, as it were, from a more or less qualitative standpoint. There remains the quantitative side to be considered; i.e., we must compare the total income and the total outgo. We have already touched upon this problem in discussing the transformation of the different organic foodstuffs into one another, and in considering their mutual replacement according to their calorific value.

It is particularly important for the study of metabolism that in the economy of the animal organism the law of the conservation of matter and of energy holds absolutely. This fact forms the basis of all experiments in metabolism. We may determine, by studying as accurately as possible the income and outgo, the part played by each individual foodstuff in the general metabolism. Only by comparing the income with the outgo are we able to form judgment as regards the condition of the system. In this way alone is it possible to determine whether the animal experimented upon increases its balance of nutrition, is in nutritive equilibrium, or whether there is a deficit such that the organism is compelled to draw upon its reserve stores or to consume its own tissue in order to maintain the functions of its organs. The study of the body-weight alone can never take the place of this important method of examination. An increase or loss in weight may arise from a number of different causes. Such deviations, for example, may be brought about merely by a retention or an elimination of considerable water. The varied natures of the problems concerning metabolism have led to different methods of investigation. In some cases it is sufficient to follow the course of a single food-

stuff, while at other times it is necessary, in order to draw a clear picture, to measure the total intake and outgo. A simple problem, for example, is to determine whether a definite substance acts as an albumin sparer. Here, in most cases, it is sufficient to estimate the amount of protein in the food without introducing any serious error, by merely determining the amount of nitrogen contained therein. The amount of nitrogen in the urine and in the fæces then shows clearly whether the animal experimented upon is in nitrogen equilibrium or not. If the animal is once found to be in such equilibrium, i.e. eliminates the same quantity of nitrogen that it receives in the food, then by feeding the given food we can easily determine whether the elimination of nitrogen is increased, diminished, or remains the same. The experiment in this case is so simple, because we know that nitrogen is given up by the kidneys and not by the lungs or skin. Such an experiment, however, is not perfectly satisfactory. A number of questions always arise concerning such results. We are by no means justified in assuming that the appearance of nitrogen in the urine is a sign that there has been a total consumption of the albumin. We know that in all cases only a part of the carbon appears combined with nitrogen in urine. The rest of the carbon chains from the cleavage-products of proteins are broken down in a different manner. These chains may remain in the organism long after all of the nitrogen has been eliminated, and take part in metabolism in a way which is not yet clear to us. At all events, in an exact investigation it would be necessary to take into account also the elimination of the sulphur. But even here we cannot be entirely satisfied. Only by combining the examination of the urine and fæces with that of the remaining elimination products, especially the gaseous ones, shall we obtain an exact insight into the influence upon the total metabolism. In many cases even with such experiments the results are not entirely satisfactory. We should know oftentimes more accurately to what extent kinetic energy has been changed into potential energy, in order to judge correctly the physiological nutritional value of the individual foodstuff.

Before considering the details of an experiment in metabolism, we must bring forward the fact that an exact insight into the questions concerning metabolism can only be expected when influences which have no bearing upon the problem at hand are excluded as completely as possible. Comparative experiments in which the basal conditions are as nearly alike as possible, should be carried out as a rule upon the same animal. Individual peculiarities which sharply influence metabolism should never be disregarded. One of the most important requirements to be satisfied in a metabolism experiment is that the test should be carried out for a considerable length of time. It is not possible to draw any exact conclusions from observations made only during a period of twenty-

four hours or less. The metabolism of the food eaten the previous day will affect the results; and, moreover, the metabolism of the food taken during the day of the experiment will not be complete during such a short time. Atwater [1] showed how much more valuable the results were when the experiment was continued for some considerable time. A great many contradictions and differences in the researches concerning metabolism, which are to be found in the literature, may be traced to the fact that this requirement has not been satisfied.[2]

As a foundation for a *metabolic balance*, an accurate knowledge of the composition of the *income* and of the *outgo* is essential. As regards the *income*, the foods are to be considered in two directions. We can, on the one hand, evaluate them according to their chemical composition, and, on the other, according to the energy which they contain. We obtain the former values by means of chemical analysis. We have the organic and inorganic constituents to estimate. In the former the carbon, hydrogen, and nitrogen are determined. By multiplying the nitrogen found by 6.25, the amount of albumin is obtained. Of course this method of estimating the amount of albumin is not an exact one. It is perfectly possible that the food may contain nitrogen in some other form than as albumin. In general, however, the amount of such nitrogenous matter is inconsiderable. The fat content is obtained by extracting the food with ether, in which connection it is to be remembered that a part of the fat will go into solution only after the product examined has been "opened up" [3] by digestion with pepsin-hydrochloric acid, or with two per cent hydrochloric acid. Then, when the ash is known, the amount of carbohydrate may be determined by difference. By drying an aliquot part of the weighed mixture, and weighing the dry residue, the amount of water in the food is determined. Oxygen is naturally also to be considered among the substances taken into the system. Unfortunately, up to the present it has not been found possible to measure accurately the amount of this gas that is utilized. This thwarts the exact answering of many questions in the field of metabolism. The potential energy of the food may be ascertained by the heat of combustion. In exact experiments we must naturally also take into consideration the temperature of the food and drink as it is taken into the system.

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[1] Ergeb. Physiol. (Asher and Spiro) Jg. **3**, 497 (1904).

[2] Studies of metabolism under pathological conditions are important. Clinical investigation and experimental pathology are closely connected with the progress of the knowledge of metabolism under normal conditions. By such means many new questions have arisen, and certain disturbances have given us insight into this or that process. It would be beyond the scope of these lectures to attempt to mention the numerous discoveries which have been made in this way. We can give only the outline here and the more important results. The physiology of metabolism has become such an important branch of science that it can be studied only upon a broad basis.

[3] Cf. Lecture XIV, p. 325.

The income is to be contrasted with the *outgo*. There are three principal ways in which the products of metabolism leave the organism, — through the kidneys, the intestine, and the lungs. The fæces contain not only products of metabolism, but also the unabsorbed food. The determination of the nature of the fæces is highly important. It gives us an idea how completely the nourishment has been utilized. In the urine, besides the inorganic salts, we have to consider the nitrogen, sulphur, phosphorus, carbon and hydrogen content. The first three elements give us information concerning the decomposition of the protein. Usually the nitrogen is alone determined and the amount of consumed protein is obtained by multiplying the nitrogen value by 6.25. In many cases the decomposition of the protein is also traced qualitatively, and it is determined how much nitrogen is present as urea, and how much in the form of other compounds. In order to get an idea of the energy economy, the heat of combustion of the entire excreta is determined, further the heat given off by the body, and the amount of heat equivalent to the muscular work performed.

The gas metabolism is studied with the help of apparatus of special construction,[1] by means of which the amounts of carbon dioxide and water vapor eliminated are determined. From the amount of carbon dioxide the equivalent weight of carbon may be computed. This, together with the amount of carbon contained in all the remaining excreta, gives us, first of all, an idea as to the utilization of the carbon in the food by the organism. The question then arises how can we tell from the total amount of carbon the amount that has resulted from the separate foodstuffs, albumin, carbohydrate, and fat. To estimate this we start with the total amount of nitrogen eliminated, and from that compute the amount of albumin decomposed. Since the average carbon content of protein is known, it is easy to compute how much of the carbon came from proteins. This is naturally on the assumption that the combustion of the remaining protein molecule takes place simultaneously with the elimination of the nitrogen. As a rule, the relation of nitrogen (16 per cent) to carbon (53 per cent) in protein is as 1 : 3.3. If, then, we multiply the nitrogen value by 3.3 we obtain the amount of carbon which was obtained from protein; and by deducting the product from the total amount of carbon in the egesta, the amount obtained from nitrogen-free food is given. If there is no remainder, then only protein was consumed. By comparing the total amount of carbon and the remainder after deducting the carbon from

[1] Descriptions of such apparatus may be found as follows: Regnault and Reiset: Annal. **73,** 92, 129, 257 (1850), and Ann. chim. et phys. (3) **26** (1849). Hoppe-Seyler: Z. physiol. Chem. **19,** 574 (1894). Pettenkofer: Annal. II, Suppl.-Band, p. 1 (1862). Voit: Z. Biol. **11,** 541 (1875). Sondén and Tigerstedt: Skand. Arch. Physiol. **6** (1895); and Atwater: Ergeb. Physiol. (Asher and Spiro) Jg. **3,** 498 (1904).

protein in the egesta with that in the ingesta, we can tell whether all of the carbon has been eliminated or whether more or less. In the two latter cases we can tell whether the organism has consumed its own protein or fat, or whether it has added to its supply of albumin and of nitrogen-free substances.

We have disregarded the losses which the body sustains by its secretions, and the losses of epidermis from the skin, intestinal canal, and other mucous membranes. These losses are very hard to estimate. To some extent they come into consideration with the eliminations from the alimentary canal in the analysis of the fæces. They may be disregarded, or not be estimated by themselves, because they are so small in amount that the error introduced does not assert itself in the nutrition balance. Particular attention should be paid to the nitrogen content of the fæces, which arises largely from intestinal depositions. The fæces of man contain 0.5 to 1.4 grams of nitrogen, even when the food contains but little nitrogen or none. Among the nitrogen-free products, the fæces contain principally fat. In starvation man eliminates 0.6 to 1.4 grams of fat per day. With nourishment free from fat, 3 to 7 grams are eliminated daily.

For the determination of the heat given off by the organism, a special apparatus is also necessary. A calorimeter which permits the simultaneous determination of the respiratory exchange and the amount of heat liberated is Atwater's *respiration calorimeter*.[1] This apparatus, in which the person, or animal, experimented upon may remain for a week, consists of a chamber which is large enough to be comfortable. This space is supplied with a ventilating arrangement, so that the volume of the air can be accurately measured. It is so regulated that the air enters and leaves at exactly the same temperature. Now and then samples of air are taken as it enters and as it leaves the chamber, whereby the amount of carbon dioxide and water given off by the lungs and by the skin is determined. Suitable arrangements are also provided for the introduction of food and drink, and for the removal of the solid and liquid excretory products. The amount of heat given up by the organism, together with the heat equivalent of the external muscular work, is measured at the same time. The heat given off is carried away by a stream of cold water, which flows in tubes around the chamber. By carefully regulating the temperature of the water and the velocity at which it flows through the tubes, it is possible to carry away the heat as fast as it is produced, thereby keeping the temperature of the chamber constant. By determining the amount of water that has flowed through the tube, and the change in temperature, it is possible to estimate the amount of heat given off by the body.

By determining the amount of water vapor in the air as it enters and as it leaves the chamber, it is possible to find out how much water is given off by

[1] *Loc. cit.* p. 499.

the body. The amount of heat energy required for its evaporation must be added to the amount of heat energy carried away by the water current. Atwater measured the heat equivalent of the external work performed in a very interesting manner. He provided a bicycle which was connected with a dynamo. The electric current produced was led through an incandescent lamp, and there transformed into heat energy, which can be directly measured as such. From the duration of the experiment, and the amount of electric current produced, the amount of work performed can be estimated.

According to this method of investigation, the kinetic energy of the body is computed from three factors. First, there is the total amount of heat carried away as heat, including that carried away by the air current; second, the latent heat of the water vapor which is carried away from the body; and third, there is the heat equivalent of the external work. The first amount of heat has several sources. It comprises the heat given off by radiation and conduction from the skin, that obtained from the excreta on their being cooled to the room temperature, and further there is the cooling of the expired air (carbon dioxide and water vapor) to the room temperature.

By means of such an apparatus not only the influence of different kinds of nourishment upon metabolism can be determined exactly, but also their relations to the external work. As we have already seen,[1] it is by the help of such experiments that we have been able to prove that the fats, in order to be utilized for muscular work, need not necessarily be transformed into carbohydrate, but that their calorific value may be applied directly.

			Transformed Energy.			
Name, Nature of the Experiment.	Laboratory Number.	Duration in Days.	Sum	Excess Energy in Period of Work Over Period of Rest.	Heat Equivalent of the External Work.	Utilization in Per cent.
E. O.						
Rest experiment . . .	13	42	2279	...	...	...
Work experiment . . .	3	12	3892	1613	214	13.3
J. F. S.						
Rest experiment . . .	4	12	2119	...	...	...
Work experiment . . .	6	18	3559	1440	233	16.2
J. C. W.						
Rest experiment . . .	1	4	2357	...	...	...
Work experiment						
Minimum work . . .	4	16	5056	2699	529	19.6
Maximum work . . .	2	8	5332	2975	601	20.2
Average	14	46	5143	2786	546	19.6

[1] See Lecture XV, p. 338 *et seq.*

Atwater succeeded in solving a number of important problems concerning metabolism with the help of his apparatus. If we consider metabolism simply from the standpoint of energetics, i.e., consider the organism simply as a machine, then one of the first questions to interest us is as regards the utilization of the food for the performance of external work. We must remember that an ordinary steam engine on an average converts but 15 per cent of the energy contained in the fuel into work. The rest is set free as heat.

The table on page 625 shows the relation of the external muscular work to the total amount of transformed energy, from which the efficiency of the human body as a machine may be computed.[1]

The work performed in these experiments was measured by the bicycle arrangement described above. In the table its heat equivalent is taken into consideration. The objection might be raised that it is hardly possible to distinguish sharply between a period of rest and one of work, for in the latter case there is merely additional work over that required by the organism for the exercise of the remaining physiological functions. It is difficult, and in fact entirely impossible, to make sure that the mental and nervous expenditure of energy will be exactly the same in two experimental periods. *A priori* it is conceivable that there is a considerable transformation of energy in these two kinds of work. To meet this objection Atwater[2] compared the results obtained where the person experimented upon was resting mentally and physically as completely as possible, with results obtained in a period where the person was engaged in severe mental effort, and found that there was no appreciable increase in the transformations of matter or of energy in the latter case. This does not necessarily mean that mental activity does not correspond to a considerable consumption of energy. It is entirely impossible to stop completely at will all mental effort. The mental work continues, whether the person experimented upon, as in Atwater's experiments was the case, is busied with the results of experiments, a German treatise, or the study of physics; there is no apparent difference from the results obtained in metabolism when the brain is as much at rest as is possible to make it voluntarily. As far as the above experiments are concerned, therefore, it makes no difference to what extent brain and nervous activity affect the total consumption of energy; for, as Atwater has shown, the consumption of energy from these causes remains the same, whether mental work is performed intentionally or unintentionally. A glance at the above table shows that all human organisms do not work with the same degree of efficiency. That of E. O. was less efficient than that of J. S. F. and J. C. W. At all events, however, as a machine the human organism is more efficient

[1] Atwater: *loc. cit.* p. 608.

[2] Atwater: U. S. Dept. Agr. Office of Exper. Stations. Bull. **44.**

than an ordinary steam engine. We must admit, on the other hand, that the way this was proved by Atwater's experiments is not altogether beyond reproach. It is not an exact determination. We do not know just how much effect the increased muscular work had upon the functions of the other organs. At the same time it is probable that the values obtained are not far from the truth.

While the advance in technique and in methods results in more precise investigations, still, on the other hand, we are often compelled to resort to indirect methods with all their sources of error which we have so often mentioned. This is true in many cases when we attempt to determine the participation of nitrogen-free foodstuffs in metabolism. We can, it is true, get some idea of this by comparing the volumes of inspired and expired air. In normal breathing the volume of the expired air is always greater than that of inspired air. This is due to the fact that the outer air is warmed to the body temperature after it is inspired, and at the same time it is almost completely saturated with water vapor. In order to obtain actually comparable figures it is necessary to measure the two volumes of air under precisely the same conditions. Both must be brought to the same temperature and pressure; and, furthermore, they must be dried. When this is done, it will be found that the volume of the expired air is almost invariably smaller than that inspired. This is due to the fact that in the combustion of the foodstuffs, the carbohydrates alone yield a volume of carbon dioxide equal to that of the oxygen consumed, while in the combustion of protein and fat this is not the case. In the case of the latter, a part of the inspired oxygen is utilized for the formation of water, sulphuric acid, and other substances. The amount of oxygen consumed in this way does not appear in the volume of the expired air when measured as above. In the combustion of carbon, one volume of carbon dioxide gas is formed from one volume of oxygen. In this case the ratio $\frac{CO_2}{O_2} = 1$. This relation of expired carbon dioxide to inspired oxygen is called the **respiratory quotient.** In the combustion of carbohydrates this is 1. A diet in which protein predominates causes the quotient to fall to about 0.80, while if fat is chiefly concerned in the metabolism, the value of the quotient falls to 0.70. According to the value of this respiratory quotient, therefore, we can draw some conclusions regarding the nature of the food upon which the subject experimented upon is working. If, for example, a dog which has a good supply of glycogen stored up is made to fast, then the high respiratory quotient, which is approximately 1, shows that at the given moment the dog is maintaining its economy chiefly by drawing upon its carbohydrate stores. When the value of the quotient begins to fall, it shows that fat is being consumed, and finally it will be compelled to utilize its own protein. Naturally, it is not, in general,

advisable to depend upon this respiratory quotient alone, but we should also take into consideration the other eliminations, especially that of nitrogen.

Now that we have roughly sketched the outlines of the methods employed for studying metabolism, we will briefly discuss, before taking up the important facts that have been ascertained concerning metabolism under definite conditions, the influence of the conditions created by the subject experimented upon itself. First of all, there is the question of size. It is perfectly clear that the total metabolism will be more extensive in proportion to the size of the organ in function. Eventually the consumption of material is to be traced to the work of the individual cells, and the more cells there are the greater will be their total requirement. Thus a small animal will require absolutely less nourishment than a larger one. Of course individual peculiarities play a part which must not be left out of consideration. If, on the other hand, instead of paying attention to the absolute amount of material consumed, we consider the energy transformed per kilogram of body-weight, provided we are working under otherwise parallel conditions, we shall find that the metabolism of the smaller animal is greater than that of the larger one. In order to obtain values which shall be actually comparable, the separate experiments upon metabolism must be carried out with the animal at rest, and also fasting. In this way the so-called **fasting value** is obtained.[1] The reason that a small animal decomposes more substance in proportion to its own weight lies in the following: — The smaller an animal is, the larger the surface of its body in comparison to the volume and weight of the body. It may be assumed that about four-fifths of the total heat given off by the body is through the skin. The amount of heat lost by the skin is very nearly proportional to the amount of surface covered by it, so that the smaller animal with its relatively larger surface loses more heat than the larger animal. Consequently the smaller animal requires a greater supply of heat energy than the larger one, as otherwise its body temperature, which is regulated by two factors, the amount of heat generated and that given off, will not be maintained at the proper height.

The tables on the following page show how the amount of oxygen consumed depends upon the size of the body,[2] and give also a comparison of the metabolism of energy in animals of various sizes with their relative surface development.[3]

The influence of the greater surface becomes apparent when we compare the metabolism of younger and older individuals of the same species.

[1] Cf. Max Rubner: Z. Biol. **19**, 535 (1883). Slowtzoff: Pflüger's Arch. **95**, 158 (1903). Karl Oppenheimer: Z. Biol. **42**, (1901).

[2] Max Rubner: Z. Biol. **19**, 536 (1883).

[3] Max Rubner: *ibid.* p. 549.

This, however, of itself does not by any means explain the considerably more active metabolism on the part of the younger individual. This is evident when we compare not the metabolism of the whole external surface, but rather that per unit of surface. On comparing, for example, the metabolism per square meter in fully developed dogs of different sizes, we will find that the value is the same for all dogs within certain narrow limits. This is, however, not the case if we compare the metabolism of young dogs with that of older ones. In the case of the former the metabolism is greater per unit of surface than it is in the older dogs.

I.

CONSUMPTION OF OXYGEN, ARRANGED IN THE ORDER OF THE ANIMAL'S WEIGHT.

Species.	Weight in Kilograms.	Grams Oxygen Absorbed per Kilogram in 1 Hour.	Species.	Weight in Kilograms.	Grams Oxygen Absorbed per Kilogram in 1 Hour.
Male calf . .	115	0.481	Rabbit . . .	3.43	0.735
Male calf . .	115	0.428	Mountain rat	1.55	1.198
Sheep	70	0.464	Hen.	1.51	0.846
Sheep	66	0.490	Drake. . . .	1.22	1.382
Sheep	65	0.400	Cross-bill . .	0.028	10.974
Hen-turkey .	6.2	0.702	Green-finch .	0.025	13.000
Dog	5.59	0.902	Green-finch .	0.025	9.742
Goose	4.60	0.677	Sparrow. . .	0.022	9.595
Rabbit . . .	3.58	0.763			

II.

COMPARISON OF THE EXCHANGE OF ENERGY IN ANIMALS (DOGS) OF DIFFERENT SIZES WITH THEIR RELATIVE SURFACE DEVELOPMENT.

Number.	Weight in Kg.	Surface in Cm^2.	Surface in Cm^2. per 1 Kg. Weight.	Calories per 1 Kg. in 24 Hours at 15° C.	Calories per 1 Cm^2. Surface.
I.	31.20	10.750	344	35.68	1036
II.	24.00	8.805	366	40.91	1112
III.	19.80	7.500	379	45.87	1207
IV.	18.20	7.622	421	46.20	1097
V.	9.61	5.286	550	65.16	1183
VI.	6.50	3.724	573	66.07	1153
VII.	3.19	2.423	726	88.07	1212

In the case of sucklings the metabolism per kilogram of body-weight is likewise much greater than it is with fully developed dogs; but, on the

other hand, that per square meter of external surface is smaller than in the case of the older dogs. This is not remarkable. The suckling performs as a rule but little external work. It sleeps the greater part of the time, so that the consumption of material is not so extensive as during latter periods.

In old age, the metabolism is greatly diminished, even when we compare the amount per square meter of external surface with the values obtained in the same way at middle age. In human beings between the ages of 22 and 56 years the amount of carbon dioxide eliminated in an hour per square meter of body surface is about 11.2 grams, while in old age (70 to 77 years) the value is only 9.2 grams in the case of males; with females the elimination between the ages of 17 and 40 years is about 11.75 grams, while at 71 to 86 years it is only 9.79 grams.[1]

The fact that the extent of metabolism in different periods of life is of different intensity need not surprise us. In considering metabolism as a whole, we must not forget that it is the sum of the metabolism of innumerable units, the cells. In the developing tissue of youth the transformations are unquestionably much more extensive than in the adult organism. We know from a great many observations that the organism soon accustoms itself to certain functions, and usually expends more energy the first time that a demand is made, whereas, later on, it accomplishes the same result much more economically and with the expenditure of far less effort. It is indeed conceivable that the cells of the adult organism learn to work more and more economically. At present we are not in a position to study the metabolism of the individual cells, i.e., that of the protoplasm. We obtain the impression, instinctively rather than as a result of scientific investigation, that the cells of the individual are not all equally efficient. This hypothesis gains form when we recall the numerous cases of pathology whose etiology is paraphrased with the conception of *disposition*. There is no doubt that the various diseases of metabolism, such as diabetes, gout, rachitis, etc., are eventually to be traced to a disturbance in the metabolism of individual cells. This may affect a larger or smaller cell-complex, and the whole cell-work of the individual may be affected apparently without our being able to explain precisely the way in which the metabolism of such a *weakened* or *weak* individual is changed. As long as the cell itself belongs in the domain of the unknown, we cannot expect to be able to gain precise information concerning the physiology and pathology of cells. While we must emphasize the fact that at present the designation of a pathological derangement of a cell-function, or of the metabolism as a whole of individual cells, only represents a conception in accordance with our present knowl-

[1] Magnus-Levy and E. Falk: Arch. Anat. Physiol. 1899. Suppl. 314.

edge, still, on the other hand, the importance of cell-life, of the metabolism of the cells and their functions, must not be overlooked in considering the metabolism of the body as a whole.

Interesting glimpses into the course of general metabolism have been obtained by studying the results under definite conditions. The metabolism taking place after the complete withdrawal of the food has been studied especially. Under this condition the animal lives at first upon its own stored-up material, and finally attacks its own tissues. The duration and the whole course of the metabolism during starvation depends largely upon the condition of the body at the beginning of the experiment. As soon as a certain definite fraction of the body-substance has been used up, death takes place. Naturally the activity of the metabolism also affects the duration of starvation period. According to the principles enunciated above, therefore, we should expect, *a priori*, that young individuals would suffer from the withdrawal of nourishment much more quickly than adults. Similarly in the case of animals which in general have a lower metabolism, as with cold-blooded animals, they will survive starvation longer than will the warm-blooded animals. Dogs can live without food for six weeks. Birds live on an average from 5 to 20 days, while fish and reptiles may survive for from 6 months to a year. Even in the case of human beings, long periods of fasting have been observed.[1]

The first marked change that results from the withdrawal of all nourishment is the loss in weight of the body, which within a relatively short time is followed by a loss in muscular power. The subject sleeps much, and towards the end of the period of starvation is in a somnolent condition. The whole metabolism of the animal diminishes simultaneously with the loss in weight. If, however, the amount of material transformed is compared to a kilogram of body-weight, it will be found that the metabolism is only slightly changed from that of a well-nourished animal. In a short time the fasting animal adjusts itself to a minimum metabolism, which remains constant for quite a while. First of all the animal makes use of its stores of carbohydrates and fat. The former are soon exhausted. From the beginning of the starvation period, albumin is continuously being decomposed. The amount of albumin which the fasting organism must decompose in order to accomplish the necessary metabolism, depends chiefly upon the amount of nitrogen-free substances which are present. If the animal is able to consume a considerable amount of the latter, then

[1] L. Luciani: Das Hungern, Hamburg-Leipzig, 1890. J. E. Johannson, E. Lundgren, Klas Sondén, and Robert Tigerstedt: Skand. Arch. Physiol. **7**, 29 (1896). C. Lehman, F. Müller, I. Munk, H. Senator, and N. Zuntz: Virchow's Arch. **131**, Suppl. 1 (1893). R. Tigerstedt: Nordisk, Medic. Arch. No. 37 (1897). C. Voit: Z. Biol. **41**, 113 (1901). Siegfried Weber: Ergeb. Physiol. (Asher and Spiro) Jg. **1**, Abt. 1, p. 702 (1902).

the albumin in the organism is protected to a certain extent from consumption. On the very first day of fasting, there is in the majority of cases a noticeably high elimination of nitrogen, and especially when the food has been rich in proteins. The rise in the amount of nitrogen eliminated, which in individual cases, as with rabbits, for example, reaches a maximum on the third to the fifth day from the beginning of the fasting, is taken as a guide for determining the time when the carbohydrate stores have been exhausted; i.e., when the albumin-sparing factor has been eliminated.[1] Moreover, this increase in the decomposition of albumin is not a constant phenomenon, and evidently depends upon the species. In the case of rabbits, especially, it is hard to determine the exact day when the starvation period begins. These animals have an extremely voluminous intestine, and particularly in the cæcum there is always a mass of only partly utilized material upon which the animal may subsist for some time. In the case of dogs, in general there is a uniform, slowly diminishing elimination of nitrogen.[2] Voit has shown the influence of a preliminary diet, rich in protein, upon the elimination of nitrogen during the first day of fasting, by the following three experiments.[3] He determined the amount of nitrogen eliminated daily. Before beginning the experiment the first dog was fed daily with 2500 grams of meat, the second dog received 1500 grams of meat, while the third dog was fed with a mixed diet, poor in proteins.

	Nitrogen Eliminated in Grams per 24 Hours.		
	Experiment I.	Experiment II.	Experiment III.
First day of fasting	60.1	26.5	13.8
Second day of fasting	24.9	18.6	11.5
Third day of fasting	19.1	15.7	10.2
Fourth day of fasting	17.3	14.9	12.2
Fifth day of fasting	12.2	14.8	12.1
Sixth day of fasting	13.3	12.8	12.6
Seventh day of fasting	12.5	12.9	11.3
Eighth day of fasting	10.1	12.1	10.7

A summary of the nitrogen elimination in a five-day fasting experiment with men, is given by the following values published by Tigerstedt:[4]

[1] R. May: Z. Biol. **30,** 1 (1894).

[2] M. Kumagawa and R. Miura: Arch. Anat. Physiol. **1898,** 431.

[3] Voit: Hermann's Handbuch, **6,** 1, p. 89 (1881).

[4] Robert Tigerstedt: Lehrbuch der Physiologie des Menschen. 3 Aufl. Bd. **1, p. 111** (Leipsic, 1905).

	Body Weight in Kilograms	Nitrogen.	Decomposed in Grams.			Total Transformation in Calories.	Total Transformation per Kilogram of Body Weight in Calories.
			Fat.	Carbohydrate.	Alcohol.		
Last day of eating	67.8	23.41	87	267	28	2705	39.9
First day of fasting	67.0	12.17	206	...	...	2220	33.2
Second day of fasting	65.7	12.85	192	...	...	2102	32.0
Third day of fasting	64.9	13.61	181	...	...	2024	31.2
Fourth day of fasting	64.0	13.69	178	...	...	1992	31.1
Fifth day of fasting	63.1	11.47	181	...	...	1970	31.2
First day of eating	64.0	25.44	64	250	22	2437	38.1
Second day of eating	65.6	18.07	72	248	37	2410	36.8

From these values it is apparent that the starving man quickly adjusts himself to a definite minimum consumption.

In the further duration of the fasting period the organism lives exclusively at the cost of its protein and its stores of fat. The carbohydrates are quickly consumed, and in the later periods come scarcely into consideration at all. The starving organism is very economical with its protein. Of the total calories 84 to 90 per cent come from the fat, and only from 10 to 16 per cent from protein. This holds naturally only for fat animals. After a time the stores of fat are exhausted, and the organism is then compelled to obtain the requisite amount of energy (calories) at the expense of its own protein. At this time there is a rapid increase in the elimination of nitrogen through the urine. This was observed by Voit,[1] and has subsequently been much discussed. As a matter of fact, the animals experimented upon were found to be not entirely free from fat at the time when, just before death, there was an increased elimination of nitrogen in the urine. Often quite a considerable amount of fat has been found to be present at such a time. On account of this fact the conclusion has been drawn that the increased elimination of nitrogen is not altogether due to the fact that the store of fat has been entirely consumed, but that there must be other causes. F. N. Schulz[2] assumes that the increased decomposition of protein just before death by starvation is to be attributed to the sudden destruction of numerous cells in the body. It is indeed conceivable that the cells, whose ability is taxed to the utmost during starvation, in order to provide the necessary material for the general metabolism, at last disintegrate. They are constantly giving

[1] E. Voit: Z. Biol. **41**, 113, 502, 550 (1901).

[2] F. N. Schulz: *ibid.* **41**, 368 (1901), and Pflüger's Arch. **76**, 379 (1899).

up material but receive nothing from without. The increase in the elimination of nitrogen shortly before death may be prevented by feeding cane-sugar. Thus Kaufmann [1] fed starving rabbits with 25 to 30 grams of sugar daily, and when they died, at the end of 18 or 19 days, it was without the appearance of the increased nitrogen elimination. This experiment does not permit us to decide satisfactorily the question as to the cause of the increased decomposition of albumin which has been so often observed just before the end of the fasting period. It is possible that, when the cane-sugar is fed to the starved animals, the destruction of the cells is prevented. On the other hand naturally the carbohydrate acts as a protein-sparer. Perhaps the increased elimination of nitrogen is prevented for this reason. Furthermore, it must not be left out of consideration that, as we have already frequently mentioned,[2] there is no question but that fat acts as a solvent for many substances, and plays an important part in this direction, besides its function of acting as reserve material. Especially in the extensive transportation of material from one cell to another, it is possible that the fat content of the tissues may play an important part. If the amount of fat present has been reduced to a minimum, the exchange of material will necessarily suffer, and thereby the entire metabolism. We must also not forget that evidently the fat, as it lies deposited in the fat-cells, cannot be consumed as such by the body. It must first be removed from the cell. It is very probable that a cleavage into fatty acid and glycerol takes place. This eventually affects cell activity, for only the cell can yield the required ferment. It is perfectly clear that when the cells are working with a limited supply of material, the formation of the ferment will finally be influenced. It might be thought, *a priori*, that the oxidation power of the starved organism would likewise become considerably lessened. This is, however, not the case, or at least not of the oxidation processes as a whole. M. Nencki and N. Sieber [3] injected subcutaneously one gram of benzene into a rabbit weighing 2.517 kilograms. They subsequently found 0.307 gram of phenol in the urine. The animal was then starved for three days, at the end of which time it weighed 2.425 kilograms. Once more a gram of benzene was injected, and this caused the elimination of 0.334 gram of phenol. Analogous results were obtained in experiments with dogs. On the other hand, the observation that in starvation the ratio of the neutral sulphur to the oxidized sulphur increases in value indicates a lessened oxidation power of the starving organism. We should not yet attempt to draw definite conclusions from the experiments at hand. It is perfectly possible that the power of oxidation is

[1] M. Kaufmann: Z. Biol. **41**, 75 (1901).

[2] Cf. Lecture VI, p. 112.

[3] Pflüger's Arch. **31**, 319 (1883).

lessened only in certain directions. On the other hand, in discussing animal oxidations we called attention to the importance of the preliminary cleavage for a complete combustion.[1]

If food is no longer withheld from the animal, it recovers rapidly. It replaces first of all what it has lost of its own body-substance, and seeks to regain its former condition.

Now it is very interesting to find that during starvation the animal organism attacks the different parts of its own body-material to quite different extents. It might have been expected, *a priori*, that those organs would suffer most upon which the greatest demands are placed. On the contrary, there is a constant transference of material to those organs which are most useful and are consequently most indispensable to life. This was shown very clearly in considering the life and development of the salmon,[2] and led to the question whether we must not assume that the starving organism is obliged to effect extensive syntheses from the building-stones of the less important cells. We can easily believe that the heart, whose function is so essential for the maintenance of life, retains its material unchanged and carries out its work at the expense of other tissue. On the other hand, it is also possible that the muscle-cells of the heart are constantly being broken down and rebuilt. At present we have no means of estimating the life of a cell, and cannot decide how long it can retain its corporate existence, or whether it is constantly assimilating and giving up material. If the latter is the case, then the tissues of the starving organism must be the scene of remarkable transformations.

Voit[3] gives the following values for the loss of body-weight during starvation in the case of doves and cats.

	Per cent of Original Weight.			Per cent of Original Weight.	
	Doves.	Cats.		Doves.	Cats.
Fat	93	97	Testes	...	40
Spleen	71	67	Skin	33	21
Pancreas	64	17	Kidneys	32	26
Liver	52	54	Lungs	22	18
Heart	45	3	Bones	17	14
Intestine	42	18	Nervous system	2	3
Muscles	42	31			

[1] Cf. Lecture XIX, p. 451 *et seq.*

[2] Cf. Lecture XVI, p. 351.

[3] Handbuch der Physiologie des Gesamtstoffwechsels und der Fortpflanzung. Part I. Physiologie des allgemeinen Stoffwechsels und der Ernährung by C. von Voit, Vol. 6, p. 96, 97 Leipsic (1881).

It is evident from this summary that the organs which are in constant activity, as the heart, lungs, kidneys, and nervous system, suffer much less loss of weight than the other organs. Voit also tested the influence of the activity of an organ upon keeping its composition constant by feeding doves with food which was deficient in lime, but contained a sufficient amount of other materials. A post-mortem examination showed that those bones which were in constant use had suffered less from the lack of lime than relatively inactive bones, such as the breast-bone and the bill. The last two bones had become perfectly porous. Evidently the composition of the bones which were used most was maintained at the expense of the others. In this direction the observation of E. Pflüger on dogs is worth mentioning. He found, as has already been mentioned,[1] that when the pancreas was extirpated the liver of these animals tended to gain rather than lose in weight. Evidently the liver is an important place for transformations, such as fat into sugar, etc.[2]

The loss in weight affects all the different substances contained in the organs. The starving animal constantly loses water even when it is given none to drink. Water is formed by the combustion of fat and albumin. Mineral substances are likewise being eliminated constantly. The organs maintain their functions as long as possible even in those cases where, as far as we know, the organ is dispensable. Thus while the secretion of bile diminishes, to be sure, during starvation, still, on the other hand, it continues to form for quite a length of time. Likewise the secretion of milk continues for a time. On the other hand, the gastric secretion soon ceases, as was shown by the examination of the contents of the stomach of the fasting professional, Succi.

A very important result of these starvation experiments is that the animal organism constantly eliminates nitrogen under all conditions. To be sure, the amount of decomposed albumin may become quite small, but it never ceases altogether. Albumin assumes, as we have already shown in discussing the Law of Isodynamics,[3] a peculiar position among our organic foodstuffs. It cannot be entirely replaced by any other material. It is possible to nourish a dog upon albumin alone, and even to fatten it somewhat. This is never the case, however, when the total calorific

[1] Cf. Lecture V, p. 85.

[2] The observations concerning the relative loss of weight for the different parts of the body are as a rule very inadequate and unsatisfactory. We have stated above the generally accepted view concerning these relations, but we must say that the field has not yet been well covered. There is no question but that a careful examination of the losses of the different organs in their various constituents, naturally referred to average values, will give us a new point of view concerning intermediate metabolism and of the relations existing between the individual organs. The well-known experiment of Miescher is the first step in this direction.

[3] Cf. Lecture XV, p. 336.

requirement is met with nitrogen-free food. It is then impossible to prevent the animal from losing weight even when far more fat and carbohydrate are fed to it than corresponds to the calories required when the dog was in metabolic equilibrium with albumin present in its food. As soon as the albumin is wanting in the food supply, starvation metabolism begins, i.e., body albumin begins to be decomposed. It is true that the animal lives a few days longer than if it were absolutely starving on account of lack of all food, but it will gradually die as a result of albumin starvation. The decomposition of albumin shows a peculiar behavior when varying amounts of albumin are present in the food. The more albumin the animal eats, the more there is decomposed. To be sure, by greatly increasing the amount of albumin it is possible for the organism to store away material, but usually not in the form of albumin itself. The cells of the body evidently strive to keep the albumin content of the organism at a constant level. It is possible to bring an animal into so-called *nitrogen equilibrium* with different amounts of albumin. Equilibrium is reached when the organism experimented upon eliminates the same amount of nitrogen that it receives. This relation is most apparent if instead of estimating the amount of nitrogen eliminated during a single day, a period of several days is studied.

The fact that an increase in the albumin income also causes an increase in the total metabolism apparently helps to enable us to decide whether the cell-material, or protoplasm, itself takes part directly in the decompositions and combustions, or whether we have to distinguish sharply between the cell-nutriment and the cell-building-stones. Here we meet with the most important problem of metabolism. It is quite generally assumed that in animal combustions it is chiefly the albumin in the nutriment, also designated as circulating albumin, which is consumed, while the *living* protoplasm is only drawn upon for the outgo when there is a deficiency in the supply of albumin. There are a number of observations which support this assumption. Above all, it is remarkable how quickly the albumin is oxidized after its introduction into the organism. Within a few hours the total amount of nitrogen reappears in the urine. It is hardly absorbed before its elimination begins. Although the presence of fat and carbohydrate in the food somewhat diminishes the decomposition of albumin, still on the whole the decomposition of the albumin is about the same as with a purely meat diet. The fact that the extent of albumin decomposition is, within wide limits, independent of the albumin content of the body itself, has also been cited as supporting the above conception. The assumption that the tissue-cells of the fully developed animal organism are in a relatively stable condition, and work essentially by means of the energy obtained from the food, is an attractive one. According to this, the cell takes up from the blood, or the plasma, the substances which it

requires for the exercise of its functions. It retains its own cell-material. The fact that now and then a few cells are broken down and renewed, hardly affects the metabolism as a whole.

This very simple representation of metabolism is, however, as we shall find on closer examination, not entirely in accordance with certain facts. As we have already repeatedly stated, it has become recognized during the last few years that the digestion of the food is not for the sole purpose of making it capable of absorption. Unquestionably, one of the principal objects is to make the material which is obtained from different sources conform to the material out of which the body is made up. The substances contained in the food are, by means of the various ferments, not only made capable of absorption, but of assimilation as well. Starch, which is the glycogen of plants, is broken down into molecules of *d*-glucose, only to be changed later into animal glycogen. We do not yet know how much of the absorbed glucose is changed into the latter compound, nor whether the glycogen throughout the entire animal kingdom is all of the same nature, or whether perhaps there are not different kinds of glycogen corresponding to the different species of animals. Here we cannot decide definitely whether the preliminary preparation is all-important as regards assimilation, or whether it merely serves the purpose of making the material capable of absorption. The question is similar in the case of the fats. In this case one might even get the impression that the nutriment is deposited in an unchanged condition. The fat is split by the digestive ferments into its constituents, fatty acids and glycerol, which, however, unite again in the intestinal wall. It has been found possible to cause foreign fat to be deposited in the body. We have already shown that the fat stores occupy a peculiar position in animal economy. It is a question whether the animal organism can also utilize substances foreign to it for physiological functions in cell-metabolism. At the same time we may quite safely assume that in the case of the fats, digestion only serves to prepare it for absorption. To be sure, we must confess that the fat stores of different animals under normal conditions are not homogeneous as regards their composition. How far these differences in chemical composition depend upon differences in the nature of the food, remains an open question. In general we may indeed assume that the fat contained in the stores has a specific composition for every animal, in case the animal is not deprived of the power to construct its own fat by decomposition and selection, on account of being limited to fat of a definite kind.

At all events, it is very remarkable how quickly and easily the animal organism decomposes, by means of the ferments, the fats and complicated carbohydrates into their simple components, only to rapidly reconstruct them again, and eventually, at the time they are consumed, carry away the resulting products equally rapidly. These very complicated processes

become still more remarkable when we follow, as we have done, the behavior of albumin in the animal organism. We find that in the alimentary canal it undergoes a far-reaching decomposition. Now the different albumins are very similarly constituted in respect to the amino acids which they contain. With few exceptions they all contain the same building-stones. Their chief difference lies in the relative amounts which they contain of these acids. In spite of these differences the serum albumins are, as far as our knowledge goes, of the same composition, no matter whether the albumins contained in the food are closely related to the serum bodies in their composition or not.[1] Although the researches in this direction have only just begun, still a number of observations indicate that the albumin in the food is so transformed before it reaches the circulation and the tissues that it loses its original character and becomes of a nature corresponding to the body albumins, first of all to the serum albumins. It is, in fact, not the albumins of the food that circulate in the blood and tissues, but rather those of the body itself.[2] Possibly, if we were able to trace more closely the transformations of the albumins on their path of absorption and assimilation, and had a better understanding of the albumin molecule, the decompositions would then appear to be very simple ones. At present it appears as if we could not neglect the above-described relations in the conception of albumin metabolism. According to them, the decomposition which albumin undergoes does not appear to be a relatively simple process. It is certain that the albumin before it is consumed must be decomposed again in the tissues into simpler components. The great question is only with regard to the reason for the changes in the albumin in the food, so that it becomes like the albumin of the body, when it is to be consumed so quickly.

It seems to us as if the answer to this question, as we have previously stated,[3] will give the solution to the enigma of the large albumin requirement. To be sure, the animal organism effects all these changes chiefly in order that nourishment may be offered to the cells in a form such that they are able to utilize it. We must always remember that the cells eventually accomplish their work by means of ferments, and that these are regulated very sensitively so that they will react only with certain definitely constituted compounds. Undoubtedly the tissue-cells cannot break down and utilize starch, or the fat and albumin of the food. The intestine works over these materials and gives to the cells nutriment of quite specific composition. The cells are capable of utilizing these definite products and only these. Their whole construction corresponds to these compounds.

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[1] Emil Abderhalden and Franz Samuely: Z. physiol. Chem. **46,** 193 (1905).

[2] In Lecture XXIX we shall discuss a biological method for determining whether the albumin nutriment passes directly into the blood and lymph circulation.

[3] Cf. Lecture XI, p. 223 *et seq.*

This is a satisfactory explanation as regards the carbohydrates and fats, which, at least with fully developed organisms, can, under certain conditions, be regarded solely as combustible materials. The amount of these substances consumed is determined by the amount of energy which is transformed in the body. If the supply of carbohydrate or fat is greater than the organism requires at a given time, some of it is laid aside. It is quite different with albumin. The amount at hand regulates the entire metabolism. The more there is present, the greater the metabolism. Why is so much albumin required? The distinction between *circulating* albumin, i.e. albumin which is to be used as fuel, and *organized* albumin, or that which is used for the construction of cell-material, does not help us here.[1] As a matter of fact, there is no proof that there is any justification for a sharp distinction between these two kinds of albumin. As we have previously stated, we prefer to consider the total albumin metabolism from a single standpoint and to trace the high requirement of albumin on the part of the animal organism to the fact that in the adaptation of the albumin in the food to the form required by the body there are quite a number of the simpler constituents which are not suitable for synthesizing the new albumin, and these are eliminated. The intestine contains a mixture of different amino acids from which it selects those which suit it and in quite definite porportions. Here again the Law of the Minimum holds. The amount of amino acid which is utilized in the synthesis of new albumin is governed by the amount of that amino acid which is present to the slightest extent relatively. This conception holds only while we have no positive proof that the animal cell is capable of forming one amino acid from another to any considerable extent. For the present our knowledge of the relations concerning the decompositions in the tissues makes any such assumption appear improbable. Now the most widely different cell complexes likewise possess their own characteristic albumin. We refer, for example, to the histones, which differ again from the albumin of their nutriment, in this case the serum albumin. Here again the nutriment is broken down, and again the cell chooses those building-stones which it can utilize, and rejects the others. Thus we can imagine that in this reconstruction of the cells, which, although not very extensive of itself, is nevertheless constantly taking place, a considerable supply of albumin is required. When the albumin supply is large, the cell is assured of a sufficient supply of all the most varied building-stones. The unutilized amino acids are at once robbed of their amino group, and perhaps the nitrogen-free chains are further utilized. Even in starvation metabolism the consumption of albumin must be remarkably high, for, in this case also, the albumin trans-

[1] Cf. Emil Abderhalden: Zentr. gesamte Physiol. u. Pathol. des Stoffwechsels, N. F. **1** (1906).

ported from organs of minor importance cannot be utilized directly. Here again there is a preliminary decomposition and selection of material.

Now the only other striking phenomenon is that an increased supply of albumin increases the extent of the entire metabolism; i.e., not that of albumin alone. Perhaps this discovery may be accounted for by the fact that the animal organism evidently has no depôt for storing up the excess of albumin. This is evident because it is so difficult under normal conditions of nutrition to cause a deposition of albumin in the fully developed organism. Under these circumstances it is perfectly conceivable that when there is an increased supply of albumin there is a greater amount of cellular transformations so that the other materials are also required.

In this explanation we wish to call particular attention to the importance that is attached to the maintenance of the perfectly specific cell construction in the case of each species of animals and perhaps of every single individual. Here unquestionably the proteins play the most important part by virtue of the fact that they offer such a variety of forms. The abundant supply of albumin guarantees to the animal organism its own individuality and that of its cells as well as its own metabolism.

We must at this place once more mention the fact that albumin metabolism has been studied almost entirely from a single point of view. The rapidity of the albumin decomposition has been identified with the rapidity of the nitrogen elimination. We have, however, no precise reason for assuming that the splitting-off of the nitrogen is, as a matter of fact, the signal for the disruption of the entire molecule. After the formation of urea, carbon chains remain which may be utilized in a number of different ways. It is possible that the cells prefer to have so much albumin because it provides them with all the different materials which they require. It is perfectly thinkable that these carbon chains can be used to form sugars, or they might equally well be utilized for the production of fat.

It is, as the above discussion shows, perfectly impossible to give an explanation of albumin metabolism which shall be based upon exact experimentation. We can indeed formulate hypotheses, but for the present there is no preference to be given to any particular one. None of the present hypotheses satisfactorily unites all the known facts, in such a way that in every respect all the results of experimentation are clearly accounted for. We must leave these questions entirely open, and suggest that new theories and new experiments can alone cause progress, and consequently a discussion of the various attempts at explanation would be scarcely worth our while. Again and again in the questions arising from all sorts of different kinds of metabolism we run against the metabolism of the cell, and cell activity. The conception of intermediary metabolism is a very accessible one. We are constantly coming in contact with it. It is here the place to state that contrary to what one might expect by reading

the literature, as far as we are concerned it is an unknown quantity, and at present we have practically no clear insight into cell-metabolism. For the time being we recognize only the general stages of metabolism as a whole. There is here a vast field for experimentation which has scarcely been touched upon. It is only by a clear recognition of this fact that it will be possible for us to penetrate fully unprejudiced into this obscurity, and with new methods and new resources succeed in gradually developing more and more facts which will replace the hypotheses. We have gone into albumin metabolism upon a somewhat broad basis, because eventually all questions concerning metabolism, no matter what their nature may be, directly or indirectly penetrate into the problem of albumin metabolism in the animal organism. The uncertainty which at present envelops the latter to some extent affects all other investigations in this field, and explains, at least to some extent, the different answers which have been given the apparently similar questions concerning metabolism.

In this connection we must once more remember that it is exceedingly difficult to cause a deposition of albumin in the animal organism. Muscular work has a remarkably favorable action in this direction. The significance of work for accomplishing an albumin "fattening" has been recognized by the physician. A retention of nitrogen has alone been satisfactorily established in this connection. Less nitrogen appears in the urine than the organism receives. We do not know what becomes of this nitrogen that remains in the body. From the experiments of Schreuer[1] we know that the "albumin" retained in the body is not equivalent to the remaining albumin in the body. The deposited albumin is readily lost again, for when the ordinary diet is resorted to, the organism soon returns to its usual albumin condition. This is true especially of the nitrogen retention which is brought about by a large supply of albumin. If, at the same time, demands are placed upon a function of the body, e.g., that of the muscles, we can easily imagine that the individual cells will attempt to utilize this for increasing their cell material.

In the fully developed organism there is also some occasion for albumin being retained. It requires constructive material. Direct experiments teach that the developed organism in fact constantly acquires nitrogen. The adult organism during pregnancy finds itself placed under quite similar conditions as during youth. Here again there is a constant formation of new tissue to an unusual extent. Corresponding to this, P. Bar and R. Daunay[2] showed that in the case of a gravid bitch, nitrogen was constantly held back from the nourishment. This nitrogen retention was also noticeable when the food was the same as that with which the animal was in

[1] Pflüger's Arch. **110**, 227 (1905). Cf. Karl Bornstein: *ibid.* **106**, 66 (1904).

[2] J. physiol. et pathol. général, **1**, 832 (1905).

nitrogen equilibrium before the period of gestation. From this fact it follows that the developing fetus does not live at the expense of the organism of the mother, but to a certain extent is included in the general nourishment. The organism of the pregnant mother utilizes to better advantage than usual the substances contained in the food and especially the protein.

In discussing the replacement of one foodstuff by another and the position of each in animal economy, we have considered the suitability of each kind of food for definite functions. We have seen that the carbohydrate decomposition is proportional to the amount of muscular work, and that fats and also albumins can replace carbohydrates as sources of energy.[1]

It remains for us to mention briefly the influence of certain external conditions upon metabolism. Above all, the influence of the surroundings is of interest. Up to the present time the effect of temperature has alone been studied to any extent. In this respect the poikilothermous animals are very different from the homothermous ones. In the former, metabolism runs parallel to the variations in temperature, i.e., it decreases with a fall in the external temperature and increases with a rise in temperature. This can be demonstrated very clearly by studying the respiratory exchange. The warm-blooded animals, on the other hand, behave quite differently. With them metabolism increases with falling temperature and decreases with rising temperature. This is due to the fact that the warm-blooded animals seek to keep their body-temperature the same, irrespective of the external conditions. The loss of heat caused by a fall in the external temperature is compensated by an increased metabolism. The muscles are the principal seat of this change in the extent of the metabolism. If the muscular activity which may be expressed by movements, by shivering, or also by muscular tensions, is prevented by curare poisoning or by severing the spinal cord high up, the heat regulation ceases. The principle that the metabolism of homothermous animals increases with falling temperature and conversely diminishes with rising temperature holds only in part. It has been shown that a considerable rise of temperature also has a similar effect upon the warm-blooded animals as upon the cold-blooded ones; the metabolism increases so that there is an increased production of heat against which the organism seeks to protect itself by giving off more heat. The chief difference, then, between the homothermous animals and the poikilothermous ones is their opposite behavior with regard to a fall in temperature.

[1] There are a great many experiments concerning metabolism under varying conditions. We cannot consider them here, because in most cases it is difficult to establish the exact effect of the different factors. We would refer to the influence of high altitude upon metabolism, and of a sea-shore climate. Cf. N. Zuntz, A. Loewy, F. Müller, and W. Caspari: Höhenklima und Bergwanderungen in ihrer Wirkung auf den Menschen. Bong & Cie, 1906. A. Jaquet and R. Stähelin: Arch. exper. Path. Pharm. **46,** 274 (1901). Loewy and Müller: Pflüger's Arch. **103,** 1 (1904).

LECTURE XXVIII.

GENERAL METABOLISM.

II.

The human and animal organism requires for the maintenance of its bodily condition and for the exercise of its functions a perfectly definite amount of nutriment. The nutritional requirement depends, naturally, upon various external conditions. Unquestionably individual peculiarities also come into play here, at least to some extent. One very important factor is the amount of work to be performed. It is also self-evident that the large amount of new tissue which is being formed in the growing organism also influences the amount of food required.

There are a number of different ways for getting an idea of the amount of food required under definite conditions. For one thing we can ascertain the diet chosen by different people of various callings, and estimate from its composition the calorific value, and use this as a basis. If this is done with a number of different individuals for each class, then we shall obtain very useful average values. A single observation does not give a reliable indication of the food requirement. Certain circumstances, such as the nature of the food chosen, its utilization, especially in individual cases, lessen the value of the calculated amount of calories from a single observation. The greater the amount of material worked over, and the more uniform the external conditions are, the less influence will be exerted by individual peculiarities. It is perfectly clear that all such estimations will involve more or less error, and at best we can only arrive at approximations.

It is usually not possible to determine in such investigations the extent to which the food material is utilized. Such computations must be based upon exact experiments on metabolism. Nevertheless, such observations have great value from a hygienic-sociological standpoint. The physiology of metabolism has become epoch-making in this direction. By means of it, attention has been called to the altogether insufficient nourishment of certain classes. It is perfectly clear that a permanent underfeeding and too low standard of life must eventually tend to weaken the individual. The resistance towards injurious external influences, towards infectious diseases, etc., becomes lessened, morbidity and mortality increase, the growth of children becomes retarded, the number of able-bodied men for

the army becomes smaller, and before long the inadequate nourishment of a class of people casts its shadow in many directions.[1]

Occupation.	Protein.	Fat.	Carbohydrate.	Calories.[2]
Laborer at moderate work	118	56	500	3091
Laborer at hard work	137	173	352	3678
Well-paid craftsman	151	54	479	3148
Cabinet-maker (40 years old)	131	68	494	3242
Street porter (36 years old)	133	95	422	3214
Young physician, Munich	127	89	362	2890
Lawyer, Munich	80	125	222	2437
Carpenters, coopers, and locksmiths in Bavaria	122	34	570	3206
University professor, Munich	100	100	240	2373
Bavarian woodsmen	135	208	876	6091
Brewery man at hard work	190	73	599	3993
Peasant man	143	108	788	4848
German laborer (mean from 11 families)	72	49	451	2608
Miners at hard work	133	113	634	4240
Weavers' families in Saxony	65	49	485	2710
Two laborers' families in Frankfurt a. M	68	49	419	2424
Laborer in Berlin	98	69	490	3075
Italian brickmaker	167	117	675	4605
French laborer	138	80	502	3419
English laborer	140	34	435	2733
Scandinavian laborer	198	109	710	4811
Well-nourished tailor, England	131	39	525	3096
Hard-working weaver	151	43	622	3618
Hard-working blacksmith	176	71	667	4179
Seamstresses, London	54	29	292	1699
Students, Japan	83	14	622	3019
Salesman	55	6	394	1898
Swedish laborer at moderate work	134	79	485	3322
Swedish laborer at hard work	189	101	673	4545
Transylvanian farm-hand	182	93	968	5217
Factory people in Central Russia:				
Men and women	132	80	584	3708
Boys	98	51	487	2896
Country folk near Moscow:				
Men	129	33	589	3236
Boys	102	28	471	2637
Fishermen on the Wolga:				
Men	319	57	486	4369
Women	219	43	563	2909

[1] Cf. Paul Mombert: Das Nahrungswesen. Gustav Fischer. Jena, 1904. Here unfortunately we can but touch upon these problems which are so very important as regards the common people. It is highly important that these relations should be studied closely.

[2] These values should be at least 8 per cent lower to correspond to the calories actually utilized by the organism. A part of the food is not absorbed. This amount varies with different food, as we have seen. The results given are comparable without making this deduction. For accurate data it would be necessary to know the amount utilized from case to case. The above table is from a summary in J. König's "Die menschliche Nahrungs- und Genussmittel, ihrer Herstellung, Zusammensetzung und Beschaffenheit," Julius Springer, Berlin, 4th edition, Vol. 1, p. 388 (1904).

In the table on page 645 are given the results of a few observations with regard to the amount of nourishment taken by different classes of people.

Atwater has published the following values for the different classes in the United States.[1]

Class and Occupation.	Food Purchased.			Food Eaten.			Digestible Nutrients in Food Eaten.			Fuel Value in Calories.		
	Protein in Grams.	Fat in Grams.	Carbohydrates in Grams.	Protein in Grams.	Fat in Grams.	Carbohydrates in Grams.	Protein in Grams.	Fat in Grams.	Carbohydrates in Grams.	Purchased.	Eaten.	Utilized.
Farmers' Families (9)[2] . .	101	128	476	97	121	465	88	117	453	3560	3435	3305
Mechanics' Families (9) .	113	153	420	106	142	406	97	137	395	3605	3420	3295
Professional Men's Families (9)	110	136	442	107	129	437	99	124	426	3530	3430	3305
College Students' Clubs (5)	127	181	402	106	146	363	98	141	354	3880	3305	3170
Laborer's Family	109	102	434	108	100	432	99	96	422	3175	3145	3030
Mason . . } at hardest	180	365	1150	. . .	. . .	. . .	. . .	. . .	. . .	8850	. . .	. . .
Blacksmith } work	200	304	365	. . .	. . .	. . .	. . .	. . .	. . .	6905	. . .	. . .
Man in Adirondacks in midwinter	. . .	. . .	. . .	200	216	367	190	209	358	. . .	4335	4190
Football Player	181	292	557	. . .	. . .	. . .	. . .	. . .	. . .	5740	. . .	. . .
Sandow, "The Strong Man"	244	151	502	. . .	. . .	. . .	. . .	. . .	. . .	4462	. . .	. . .
Teachers' Families:												
Illinois	124	158	487	101	113	441	. . .	. . .	. . .	3975	3275	. . .
Indiana	111	110	349	106	102	340	. . .	. . .	. . .	2910	2780	. . .
Officials' Families with little work:												
Connecticut	110	136	442	107	129	437	. . .	. . .	. . .	3530	3430	. . .
Pennsylvania	98	155	396	91	145	380	. . .	. . .	. . .	3465	3280	. . .
Mechanics' Families with little work (5)	114	170	436	105	154	407	. . .	. . .	. . .	3826	3524	. . .
Students' Clubs:												
Young Men (16)	105	147	465	. . .	. . .	. . .	. . .	. . .	. . .	3705	. . .	. . .
Young Ladies (4) . . .	101	139	414	. . .	. . .	. . .	. . .	. . .	. . .	3405	. . .	. . .

C. Voit and Max Rubner[3] compute the total food consumption per day for adults to be per head:

[1] W. O. Atwater: Storr's Agricul. Experim. Station, Storr's Conn. Ann. Report, **9**, 152 (1896) (1891–1896).

[2] The numbers in parenthesis represent the number of dietary studies upon which these average values are based. In several cases experiments were made with a family in the fall and spring of the year, thus making two dietary studies with one family. Thus in the first case there were five farmers' families which were under observation. (Translator.)

[3] Cf. M. Rubner in "Handbuch der Ernährungstherapie und Diätetic," by E. von Leyden, Part 1, 154 (1857), and König: *loc cit.*

Place.	Protein.	Fat.	Carbohydrates.	Calories.
	Grams.	Grams.	Grams.	
Königsberg	84	31	414	2350
Munich	96	65	492	3036
Paris	98	64	465	2929
London	98	60	416	2696

From the values published by Atwater it is apparent that the diet chosen by people of various callings is very different both as regards the composition and calorific value. In order to be able to estimate the extent to which the food chosen suffices to meet the requirements which are placed upon the organism, it is necessary to make use of the average values obtained by direct experiments upon metabolism. In order to establish the minimum requirement of the human organism, the metabolism is studied under conditions at which the muscles are as much at rest as possible. In twenty-four hours the requirement per kilogram of body-weight has been found to amount to from 30 to 36 calories. The average man may be assumed to weigh about 70 kilograms, and on this basis the minimum requirement per twenty-four hours would be 2100 to 2520 calories. This value does not hold for absolute rest, when the requirement is considerably less. The resting organism is satisfied with considerably less food, as has been shown by metabolism experiments with a woman in hysterical coma.[1] The minimum requirement was then only 1680 calories. It is impossible for the muscles to be in such a state of rest when the person is awake. Now from the computed values for a person resting as much as possible, we may pass judgment upon the values given in the above tables. Unquestionably some of the above individuals were underfed. According to the wide experience of Voit, the daily diet of an adult engaged during nine or ten hours in ordinary work should be about 2749 calories.[2] This amount may be distributed among the different nutrients as follows: Protein 118 grams, fat 56.0 grams, carbohydrates 500 grams. The more work there is to be done, the more calories are needed. Thus Voit estimates that soldiers engaged in maneuvers require 135 grams albumin, 80 grams fat, and 500 grams carbohydrates, amounting to 3018 calories. In time of war, however, the requirement he states to be 145 grams protein, 100 grams fat, and 500 grams carbohydrates, which is equivalent to 3218 calories. Working women, on the other hand, require but 2200 calories, which should be composed of 94 grams protein, 45 grams fat, and 490 grams carbohydrates.

[1] Sondén and Tigerstedt: Skand. Arch. Physiol. **6**, 1 (1895). J. E. Johansson, E. Landgren, K. Sondén and R. Tigerstedt: *ibid.* **7**, 29 (1896).

[2] After subtracting the calories lost by insufficient utilization. This deduction is not made in the case of the values given for the separate nutrients.

In order to get a better idea of what these values mean, we will state that [1]

100 grams of protein are contained in

5000 grams potatoes,	650 grams fat pork,
4200 grams human milk,	620 grams yolk of hens' eggs,
3000 grams cabbage,	600 grams fat beef,
3000 grams cow's milk,	500 grams lean beef,
1250 grams rice,	430 grams peas.
800 grams wheat,	

For each 100 grams of protein there is present

	Carbohydrates.	Fat.
Cow's milk	140	107
Peas	230	21
Human milk	270	170
Wheat	580	14
Rice	990	11
Potatoes	1000	0

There has been much discussion as to whether the amounts of abumin given above as representing the average albumin requirement are too high or not. It has been found possible under certain conditions to get along with less protein. It is a question, however, whether these results obtained in single experiments, which, moreover, cover as a rule but a brief period of time, should be used for determining the ordinary requirement. Aside from individual peculiarities which unquestionably come into play here, there are certain external conditions which are to be considered as exerting an influence in a way that it is difficult to define accurately. The food requirement is different with different nations according to the climate in which they live. It is certain that habits also play a part. The human and animal organism must also be independent, within certain narrow limits, of the amount of protein in the food. The amount of protein which the organism receives from day to day changes considerably when the diet is a varied one. The amount of non-nitrogenous material which the organism receives, and which is almost invariably sufficient in quantity, enables the organism to be satisfied with as little as 100 or even 90 grams of protein per day. It would of course be wrong to base a definite standard of the food requirement upon the investigations which have been made up to the present time. It would be possible to formulate this requirement more sharply if we knew more precisely the conditions under which the values were obtained in different cases. Even the body-weight as such is not an exact factor. A very muscular individual will require

[1] These values are taken from König's "Die menschliche Nahrung- und Genussmittel, etc." They refer to the food in its natural condition.

more protein than a bony person with but slight muscular development. On the other hand, we are at present unable to measure the actual amount of work done by people of different callings, which would give us exact data for the calorific requirements. At all events, the protein content of the food is practically of chief importance, as regards a definite food requirement. From a practical standpoint, furthermore, it is hardly desirable to have a definite minimum established as regards the amount of protein required by the organism. If it should be attempted to make the food contain such an amount, it would be very easy to fall below it from time to time, and this would lead to albumin losses from the body. There is absolutely no reason, aside from the expense, of being afraid that too much albumin will be eaten. The body easily assumes a state of equilibrium with a larger supply of protein. We have seen that it is very difficult indeed to cause an accumulation of albumin in the body.

The question of expense is the most important factor as regards the best way to meet the calorific requirements with non-nitrogenous food after the organism has received sufficient albumin. Carbohydrates are cheapest. The amount of these in the above-mentioned diets is also considerable. They can be easily taken care of by the intestine. Fat, unfortunately, is very expensive, although, to be sure, an equal weight of it has more than twice as much calorific value as the carbohydrates.

In this connection we must call attention to a peculiarity. When persons are obliged to resort to a definite diet which is about the same from day to day, they often lose in weight, and show even in their outward appearance that they are not well-nourished, even although there may have been enough calories in the food. We can to some extent understand that a diet which is absolutely non-irritating, and remains exactly the same from day to day, will not be as nutritious as one of different composition which may not have any greater calorific value. We have seen that the smell, taste, and other sensations play an important part in the preparation of the digestive fluids. Now when the sensation is exactly the same from day to day, and the food is free from irritating substances, it soon fails to cause any stimulation of these sensations. The way in which the food is prepared also exerts an important effect. This governs largely the extent to which the smell and taste nerves are stimulated, and moreover provides for a greater utilization of the food material.

One question which has been considerably discussed is whether the human organism should obtain its food preferably from the vegetable kingdom exclusively, or whether a mixed diet is preferable.[1] Certain

[1] Cf. G. von Bunge: Der Vegetarianismus, 2d edition. A. Hirschwald Berlin, 1901. Ferdinand Hueppe: Der moderne Vegetarianismus. A. Hirschwald, Berlin, 1900. W. Caspari: Physiologische Studien über Vegetarianismus. Martin Hager, Bonn, 1905.

anatomical observations apparently indicate that man is adjusted to take care of a diet containing both meat and vegetables. The intestine is neither as short as in the case of the pure carnivora, nor as long as that of the herbivora. It is interesting to find that nations, such as the Chinese and Japanese for example, which are accustomed to a diet in which vegetables predominate, have a longer intestine than individuals of a nation which is accustomed to a meat diet. As regards the shape of the teeth, it is not possible to draw any definite conclusions; and it is also true as regards the historical development of the human race, that there is no proof that man was originally accustomed to a vegetable diet. Our knowledge of cookery has to a certain extent made us independent of the nature of the raw material. This is particularly so with regard to the products of the vegetable kingdom. Cooking enables us to get at the contents of the plant cells better, which were originally enveloped by cellulose; and important foods, such as the potato, are made more accessible to the action of diastase. If we really wish to make a definite decision with regard to the relative values of vegetables and meats, we must, in the first place, compare the extents to which each is utilized. This may be ascertained by the analysis of the fæces, determining the amount of unabsorbed material. Certain factors come into play here which make it hard for us to decide. A great deal depends upon the nature of the food. A diet rich in starch may have an unfavorable action upon the absorption of the other nutriment, on account of the fermentation processes resulting and the formation of acid (butyric acid), which accelerates the peristalsis of the intestines, thereby causing a prompt evacuation. Food rich in cellulose will have the same effect. Individual peculiarities also undoubtedly come into play here. At the same time it is perfectly possible for us to obtain approximate values for the extent to which the various foodstuffs are utilized in the human organism. Such values are given in the table on the following page.

The more incomplete utilization of the protein in vegetables as compared to that of flesh foods is also shown by the results of experiments by Atwater and Langworth[1] with vegetable, meat, and mixed diets. This is shown in the following summary.

Food.	Experiment No.	Nitrogen in Grams per Day.			
		In Food.	In Urine.	In Fæces.	Nitrogen unutilized.
Purely vegetable	55	13.8	13.9	3.9	28.3%
Mixed diet { Average amount of meat.	74	19.4	15.6	2.4	12.6%
Mixed diet { Large amount of meat .	56	33.1	24.5	2.9	8.9%

[1] A Digest of Metabolism Experiments, Washington, 1897.

Food.[1]	Food Absorbed in Per cents of that Eaten.				
	Dry Substance.	Nitrogenous Substance.	Fat.	Carbohydrate.	Mineral Matter.
1. ANIMAL FOODS.					
Milk —					
Children	96.0	95.5	97.0	99.0	60.0
Adults	94.5	93.5	95.0	99.0	50.0
Cheese	92.0	95.0	90.0	98.0	60.0
Eggs	95.0	97.0	95.0	...	80.0
Flesh —					
From slaughtered animals	95.5	97.5	94.0	...	82.0
From fish	95.0	97.0	91.0	...	77.5
Slaughter-house scraps	90.0	89.0	92.0	...	70.0
Fat —					
Butter	...	...	97.0	...	...
Oleomargarine	...	...	96.5	...	...
Lard	...	...	96.0	...	...
2. VEGETABLES.					
Wheat flour or wheat bread —					
Fine	95.0	81.0	75.0	98.5	60.0
Medium	93.5	75.0	60.0	97.5	70.0
Coarse	90.0	72.0	55.0	92.5	55.0
Rye meal or rye flour —					
Fine	93.0	73.0	...	95.8	50.0
Medium	88.5	68.0	...	93.3	57.4
Coarse	84.0	60.0	...	90.0	38.0
Rice	96.0	80.0	93.0	99.0	85.0
Indian meal	93.5	83.0	70.0	96.5	70.0
Whole as meal —					
Legumes	81.5	70.0	30.0	84.5	70.0
Peas, beans	90.5	84.5	40.0	95.0	63.0
Potatoes	93.0	78.0	97.5	95.8	85.0
Green vegetables	82.0	72.0	93.0	83.5	73.5
Mushrooms	80.0	70.0	...	...	...
Cocoa	...	41.5	94.5	98.0	...
3. MIXED DIET.					
Largely animal	95.0	91.0	95.0	97.0	...
Largely vegetable	90.0	78.0	86.0	93.0	...
Average diet	94.0	85.0	92.0	95.0	...
The same with white bread	95.0	88.0	92.0	96.0	...
The same with rye bread	91.0	82.0	92.0	93.0	...

Metabolism experiments with purely vegetable diets show that it is perfectly possible to nourish a youthful, vigorous organism with vegetables

[1] König: *loc. cit.*

alone and to maintain it at the highest stage of bodily and mental vigor.[1] A diet consisting entirely of vegetables is inadvisable for the following reasons: In the first place, vegetables are not utilized very advantageously, as the above tables show; this is particularly true of the protein which they contain. It must be stated, however, in this connection, that the values in the tables were determined solely by the amount of nitrogen in the food. This is not quite right, for meat contains nitrogenous extractive substances which are not of an albuminous nature. For this reason the values given for the protein in the meat were a little too high. This, however, does not materially influence the comparison. A vegetable diet has the further disadvantage that it lacks savor. To be sure, this may be remedied by artificial additions, and by exercising especial care in the preparation of the food. A vegetable diet is especially objectionable on account of the greater volume of the food.

All our present knowledge, both from the standpoint of experiments on metabolism and practical experience, justify us in assuming that a mixed diet is to be preferred as food for a people. There is no reason why we should attempt to eliminate animal food from our rations.

It has never been positively proved that a flesh diet, even when it preponderates, is harmful. All statements with regard to the injurious effects of a meat diet are based upon indirect conclusions, which are capable of two interpretations. We must admit that the human organism is capable of deriving sufficient nourishment from a vegetable diet, if it is provided in sufficient quantity. It does not seem true, from the above experiments, that the organism accustoms itself to vegetable food in the sense that the vegetable material is consumed to better advantage after a time. It would not be at all advisable, on the other hand, to restrict the diet for any length of time to meat, and chiefly because of the fact that there is then a lack of material which tends to promote the peristalsis of the intestines. In the case of the carnivora, the same effect as that produced by cellulose is obtained from the fragments of bone and other difficultly-digestible material which the animal swallows with its food.

The whole question concerning the relative advantages of vegetable, meat, or mixed diets rests largely upon one important point, namely, which kinds of material are utilized in the body to the best advantage. We have again and again stated that the food does not, under normal conditions, become part of our bodies in the form that it is eaten, but it is the constituents which result from a complete disintegration of the food that are suitable for the body. All nourishment is eventually assimilated in our tissues. If we hold to the standpoint that the tissue cells — of course in a restricted sense — are quite independent of the nature of the food which

[1] Cf. Caspari: *loc. cit.* p. 122.

is eaten, and are affected solely by the nutriment which they receive from the circulation, and which has already been assimilated, then we can formulate the whole question regarding the value of animal or vegetable food in such a way that we shall have to know merely which of the two contains the building-stones of protein in the proportions corresponding more closely to the albumins of the body. As regards the assimilation of the albuminous substances in the animal organism, the first thing to consider is whether the protein introduced can be decomposed into its constituents by means of the ferments contained in the intestine, and then whether these building-stones are present in the right proportions. If we examine the proteins contained in vegetable and animal food from this point of view, we shall find that the latter correspond more closely to the composition of the protein contained in our own tissues. This is shown particularly plainly in the case of glutamic acid, as is shown by the values given in the following table: [1]

One hundred grams of the protein contain of glutamic acid in grams:

Gliadin from wheat	37.17	Conglutin from Lupinus luteus	20.96
Gliadin from rye	33.81	Casein	10.7
Hordein from barley	36.35	Egg-albumin	8.0
Zein from indian meal	16.87	Albumin from fish-muscle	8.9
Glutein	23.42	Albumin from ox-muscle	11.9
Legumin	16.6	Serum-albumin	7.7
Vignin from peas	16.89	Serum-globulin	8.5

Unquestionably our present knowledge indicates that the albumin from vegetable foods gives rise to more waste products in the intestine than that from flesh foods. On the other hand, it is perfectly conceivable that a mixture of animal and vegetable proteins would enable the animal organism to utilize certain constituents of the latter in common with the former which might otherwise be worthless perhaps on account of a deficiency in glutamic acid. This is, at present, merely a suggestion. It is well, however, to consider such questions from all possible points of view.

The fact that vegetarians are often under-nourished is worthy of mention. They are then obliged to draw upon their own albumin, and live, so to speak, upon animal protein. They are then false to their own doctrine!

In order to determine the nutritive value of a foodstuff, we must in the first place know its composition. In the following table the composition of one of the most important foods is given. In infancy milk is the chief, if not the only, form of nourishment. We have already discussed the composition of its ash, and have found that it is characteristic of the milk of every

[1] Cf. Lecture IX, p. 172 *et seq.*, and T. B. Osborne and R. D. Gilbert: Am. J. Physiol. **15**, 303 (1906).

species of animals. This is also true of the organic constituents, especially protein, fat and carbohydrate, as the following table shows:

One hundred parts by weight of milk contain:[1]

Species.	Casein.	Albumin.	Total Protein.	Fat.	Sugar.
Dog I	4.80	2.64	7.44	11.62	3.24
Dog II	4.84	2.43	7.27	12.19	3.23
Pig I	3.76	1.45	5.21	9.54	3.30
Pig II	3.26	1.55	4.81	7.09	3.44
Pig III	3.71	1.65	5.36	6.32	3.19
Sheep	4.08	0.80	4.88	9.29	5.04
Goat	2.91	0.76	3.67	4.33	3.61
Guinea pig I	4.60	0.49	5.09	7.31	2.31
Guinea pig II	4.79	0.61	5.40	6.96	2.02
Rabbit	8.17	2.21	10.38	16.71	1.98
Cat I	3.79	3.30	7.09	4.49	4.79
Cat II	3.79	3.11	6.90	4.80	4.80
Cat III	3.69	3.29	6.98	4.98	4.71
Cat IV	3.59	3.49	7.08	4.76	4.82
Cow	2.90	0.50	3.40	3.70	4.95
Buffalo[2]	4.26	0.46	4.72	7.51	4.77
Zebra[2]	...	...	3.03	4.80	5.34
Camel[2]	3.49	0.38	3.87	2.87	5.39
Lama[2]	3.00	0.90	3.90	3.15	5.60
Reindeer[2]	8.38	1.51	9.89	17.09	2.82
Horse[2]	1.30	0.75	2.05	1.14	5.87
Ass[2]	0.79	1.06	1.85	1.37	6.19
Mule[2]	...	...	2.63	1.92	5.69
Elephant[2]	...	...	3.45	20.58	7.18
Caaing-whale[2]	...	...	...	43.76	...
Hippopotamus[2]	...	...	...	4.51	...

Human milk differs from all of the above varieties except ass's milk, in containing more albumin than casein. One hundred parts by weight of human milk contain:[3]

Casein.	Albumin.	Total Protein.	Fat.	Milk-sugar.
0.80	1.21	2.01	3.74	6.37

Milk, besides containing casein and albumin, also contains a small amount of globulin.[4] The composition of the milk with a uniform diet varies but slightly during the nursing period, with the exception of that

[1] Cf. Emil Abderhalden: Z. physiol. Chem. **26,** 487 (1899); **27,** 408 (1899).

[2] Computed from several analyses. Cf. König: *loc. cit.* pp. 661, 663, and 664.

[3] Average values from König: *loc. cit.* p. 598.

[4] Wroblewski [Z. physiol. Chem. **26,** 308 (1898–99)] also believes that a different protein which he calls *opalisin* is present, but its isolation and description are not very convincing.

which flows shortly after birth. This contains far more protein, and is called the colostrum. Human colostrum contains in 100 parts by weight in grams:

Nitrogenous Substances.	Fat.	Milk-sugar.
3.07	3.34	0.40

The colostrum is found in all species of mammals. With the cow the relations are as follows: 100 parts by weight of milk contain 2.90 grams casein, 0.50 gram albumin, 3.70 grams fat, and 4.95 grams milk-sugar. One hundred parts by weight of colostrum contain 4.19 grams casein, 12.99 grams albumin and globulin, 3.97 grams fat, and 2.28 grams milk-sugar. The exact significance of the colostrum is not known. We can indeed imagine that the tissues and cells of the new-born, which now exercise certain functions for the first time, require a considerable supply of protein.

The composition of the milk is dependent upon a number of external conditions.[1] Cow's milk, especially, has been much studied as regards the influence of various factors upon the composition and amount produced per day. One of the chief factors is the breed, and another the nature of the nourishment the animal receives. Moving about and work have an effect.

The variations in the composition of the milk are not great under normal conditions. This is very important. The fact that the milk of different species of animals varies greatly is of much significance. The composition of the milk evidently has an effect upon the rate of development of the suckling.[2] It is natural to expect that the richer the milk is in its organic and inorganic constituents, the more rapidly the suckling is able to build up its tissues. If the milk of different species of animals all had the same composition, then the desired effect could be produced only by means of a much greater production of milk, and similarly a correspondingly greater quantity would have to be taken into the system of the suckling. The question arises whether the milk of one species of animals can be substituted for that of another. Our experience concerning metabolism does not show us *a priori* any reason why this could not be done, provided of course that the suckling should receive the same amounts of nutriment, both qualitatively and quantitatively.[3] It is, to be sure, con-

[1] Cf. König: *loc. cit.* p. 601.

[2] Cf. Lecture XVII, p. 404.

[3] Cf. Max Rubner and Otto Heubner: Z. exper. Path. Therap. **1** (1905). Franz Tangl: Pflüger's Arch. **104**, 453 (1905). Camerer: Z. Biol. **16**, 24 (1880); **20**, 566 (1884).

ceivable that the albumin of one kind of milk may be differently constituted from that of another in a quite specific way. In the case of sucklings the most important function of the food is to build up the cells. Within a short time the animal doubles its original weight. We can imagine that some kinds of protein are not suitable for being introduced into the cell. Such ideas were very well justified at the time when it was assumed that the protein was decomposed in the intestine only to the peptone stage and that these products were absorbed, and when there was no evidence at hand concerning the composition of the different proteins of the body. After it was ascertained, however, that the suckling was able from the protein in its food to construct all the different proteins contained in the various fluids of the body and in the tissues, the composition of which is quite different from that of casein,[1] it hardly seemed right for us to lay too much stress upon the quantitative composition of the protein in the food. Even casein is decomposed while it is in the alimentary canal. Outside the intestine the various cleavage-products unite in various ways to form new proteins. Even the albumin contained in milk is made capable of absorption by means of changes which take place while it is in the intestine. This does not imply by any means that the chemical composition of the various proteins is entirely a matter of indifference. There are no grounds for any such assumption. It is also conceivable that the caseins from different varieties of milk contain certain specific groups. At present, to be sure, we do not know of any such. All that we do know is that up to the present time the different kinds of casein which have been studied all contain the same building-stones and apparently in about the same quantitative relations.[2] We would, however, far exceed the present state of our knowledge if we were to conclude definitely that all the different varieties are identical because they are composed of the same constituents. It is perfectly clear that the same amino acids may be combined in a number of different ways in the complex molecule. The number of possible isomers is very large. We have already seen in studying fermentations that very slight differences in the construction of the molecule are of much biological significance.

The casein of human milk differs from that of cow's milk in that rennin throws it down in the form of a much finer flock. It is also easier to precipitate casein from cow's milk by slightly acidifying it with acetic acid. From human milk it is very difficult to precipitate the casein by means of acetic acid. At ordinary temperatures the precipitation is at best very incomplete, and in most cases no precipitate at all is formed. In order to throw down completely the casein from human milk, it is necessary to carefully acidify it slightly, dilute it with water, and keep it at 37° C. for

[1] Cf. Lecture X, p. 211.

[2] Emil Abderhalden and Alfred Schittenhelm: Z. physiol. Chem. **47** (1906).

some time. In spite of this different behavior of the casein from the two kinds of milk, which may be due to several causes, we are not justified in assuming that there is any great difference in the nature of the casein.

On the other hand, just as we cannot safely assume from our present chemical knowledge that the composition and nature of the protein from different kinds of milk are dissimilar, so we are not justified in assuming that the milk of the different species of animals is quantitatively but not qualitatively different. The present state of our knowledge concerning the composition and nature of the different constituents of milk does not tell us how completely the milk of one species may be replaced by another. This does not by any means imply that it is impossible to effect a satisfactory replacement. We only wish to emphasize at this place how far our present knowledge is from the desired goal, and how far the present demands and concessions have stretched beyond the boundaries of our actual knowledge. At present we are obliged to depend almost entirely upon practical experience which receives but slight support in the analytical values obtained from the investigation of milk. We must emphasize the fact that our present methods of examining milk, particularly the analysis of the ash, show us merely what elements are present and in what proportions. The presence of sulphuric acid in the ash may be accounted for in several ways. It may occur in the milk as such, or the sulphur may be present in some state of combination other than that of sulphate. On the other hand, the old idea that the intestine is only able to bring about certain slight changes in the food, which is then absorbed after having been reconstructed as little as possible, is more and more to be discarded. As a matter of fact, the changes which take place while the food is in the intestine are quite considerable. The assimilation begins in the intestinal canal. The synthetic capabilities of the animal organism are much greater than was formerly assumed. It is far less dependent upon the nature of the food which it receives than was once believed to be the case.

Practical experience has shown that it is not possible to replace entirely satisfactorily the mother's milk with that of some other species, or by means of a milk substitute. The mortality of infants nourished at the breast is much less than that of infants brought up in some other way. It is an open question, however, whether this increased mortality is wholly due to insufficient nourishment. In many cases it is perfectly true that the children of women who are not able to nurse their children, or at least only for a short time, are in many cases not as strong as the children of normal women. Statistics in this direction, therefore, to be useful must take into account not merely whether the child was brought up on mother's milk, or upon a milk substitute, but it should also be stated why the mother's milk was abandoned. It is perfectly clear that if a sickly child is placed

upon artificial feeding, it will be hard for it to utilize the nutriment to best advantage. The child has at the start a defective circulation. It is hard for the weakened cells to carry out a thorough assimilation and transformation of the food materials. They tend to become weaker and weaker, and lose more and more the ability of reconstructing the material. Numerous complications lessen the value of the artificial nourishment which the child receives. If milk from an animal is used, large amounts of microorganisms are invariably present which cause unfavorable effects in the bowels of the child. These organisms may be killed by sterilization, and then their harmful effects will not be felt, but on the other hand it has been found that in sterilization changes are produced in the milk itself which make it more difficultly digestible. Perhaps it serves to "denaturize" the proteins in the milk, so that it is harder for the ferments to act upon them. It has been found possible to carry out the sterilization process in such a way that this injury to the milk itself is reduced to a minimum. One great danger to be feared in the artificial feeding of infants is the overloading of the alimentary canal. Under normal conditions the infant has to work pretty hard to get its food. In sucking out the milk the child becomes tired, so that after a time it stops feeding.

Social relations undoubtedly exert a great influence upon the prevailing conditions. Many people resort to artificial feeding because they believe the conditions are unfavorable, and even when the mother's milk is given, it is so seldom in accordance with natural conditions, that even these infants do not develop normally. It should be our task to educate people to believe that mother's milk is the proper food for the child, and that it alone affords a positive guarantee for the normal development of the infant. At the same time it should also be our aim to carry out researches in the hope of discovering more satisfactory substitutes for the mother's milk when it is not available. As we have said before, the nourishment of the child should in no case be regulated solely with regard to the fuel value of the food. The most important thing is to make sure that it will serve for the construction of tissue. A milk substitute may be absolutely worthless in spite of the fact that it has a high calorific value. Especially at this time of rapid development the Law of the Minimum holds in its entirety. By no means should the nature of the organic constituents alone come into consideration. It is equally important that the inorganic requirements should be satisfied. Furthermore, it is not even sufficient to know the total amount of the inorganic material.

In the case of mammals the milk nourishment continues only during the lactation period, and is then abandoned entirely within a relatively short time. We have already seen in studying the iron content[1] that

[1] Cf. Lecture XVII, p. 386.

it would be dangerous to continue milk as the sole food for too long a time. In the case of the human race, milk plays a more or less important function as food during the whole period of growth and even for adults. The utilization of the material in milk is somewhat greater on the part of the infant than is the case with the adult, but even then it remains very satisfactory. Cow's milk is utilized to good advantage by the human offspring.

One very important food for the growing organism, as well as for the adult, is the egg. In the raw state, as well as when hard boiled, the egg is equally well utilized. As far as we are concerned, the eggs of birds alone come into consideration, and especially those of hens.

In 100 grams of fresh eggs there are present : 73.7 grams water, 12.6 grams nitrogenous matter, 12.0 grams fat, 0.7 gram nitrogen-free extractive substances, and 1.07 grams of ash. The percentage composition of the last-mentioned is as follows: [1]

	Per cent Ash of the Dry Material.	K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	SO_3	SiO_2	Cl
Total contents of hen's eggs	3.48	17.37	22.87	10.91	1.14	0.39	37.62	0.32	0.31	8.98
White of the eggs . . .	4.61	31.41	31.57	2.78	2.79	0.57	4.41	2.12	1.06	23.32
Yolk of the eggs . . .	2.91	9.29	5.87	13.04	2.13	1.65	65.46	...	0.86	1.95

The greater part of the phosphorus contained in eggs (about 80 per cent) is present in lecithin, nuclein, and other organic compounds. Only a small part is found in the form of inorganic salts.

Flesh foods form one of the most important articles of diet for adults. The amount eaten varies with different nations and with different classes of people. According to Ostertag [2] the consumption per head in different localities is as follows:

	Australia.	U.S.A.	Great Britain.	France.	Belgium and Holland.	Austria-Hungary.	Russia.	Spain.	Italy.
Per year in kgms.	111.6	64.4	47.6	33.6	31.3	29.0	21.8	22.2	10.4
Per day in gms.	306	149	130	92	86	79	59	61	29

In China even less meat is eaten than in Italy. Similarly certain negro races live almost entirely upon vegetables. The composition of meat as

[1] König: *loc. cit.* p. 576.

[2] R. Ostertag: Handbuch der Fleischbeschau, p. 4. Stuttgart, 1899.

it comes upon the table varies greatly. The species of animal from which it is obtained, the amount of waste (tendons, bones, etc.), and the state of nourishment of the animal, all constitute important factors. Meat contains, besides albumin, other nitrogenous substances, such as creatin, creatinin, sarkosin, xanthin, and carnin. It is not right, therefore, to estimate the amount of protein present by the nitrogen content alone. The composition of the flesh of fish is quite similar to that of mammals. Fish is, as a rule, utilized by the human organism as well as other flesh foods.

The values given in the following table illustrate the composition of different kinds of food:

	Water.	Nitrogenous Material.	Fat.	Ash.
Meat of mammals	76.0	21.5	1.5	1.0
Salmon	64.0	21.1	13.5	1.2
Pike	79.6	18.4	0.5	1.0
Shellfish	81.5	16.9	0.3	1.3
Sole	82.7	14.6	0.5	1.4
Oysters	80.5	9.0	2.0	2.0
Lobsters	81.8	14.5	1.8	1.7
Fresh-river crabs	81.2	16.0	0.5	1.3
Edible snails	80.5	16.3	1.4	1.3

Meat as well as milk is used in the manufacture of certain prepared foods. The latter is used to make butter and cheese; the former in the manufacture of sausages and other cured products. It may be said that the value of flesh food from an economic standpoint is largely dependent upon the amount of the product which may be utilized. With fish, for example, there is a relatively large amount of waste.

Among the vegetable foods the different kinds of grain are very important; these are used in the form of meal and flour in a number of different ways, especially in bread-making. They also serve, as well as potatoes, for the manufacture of starch. Then again there are the large number of green vegetables and fruits. As regards their nutritive value and utilization in the human organism, we have found that on the whole they are not utilized as completely as the flesh foods. A vegetable diet gives rise to a large amount of excreta. In the case of the different grains it makes considerable difference whether the whole grain is used in the flour, or only that which has been freed from the hulls. The more of the hull there is present, the less the percentage utilization. We cannot include within the scope of these lectures all the different data which have been acquired concerning these relations, and which are so important in considering the food-supply of a people. We shall have to refer to the special works on the subject. Here we only desire to point out the great importance of the

study of such problems for the complete understanding of the principles of practical nutrition.

It is very significant that even the adult organism requires nutriment not only as fuel, but also for the constant construction and renewal of the cells. It must never be forgotten that the organism is adjusted for a mixed diet, and that certain kinds of stimulation are necessary for the production of the digestive juices. It is not at all practical to consider replacing our food with chemical products. Our knowledge regarding the necessary nourishment for the organism is far too limited for us to attack such problems. The attempt has been made quite recently to provide albumin in as pure a form as possible for the use of the sick. Our understanding of metabolism is not such that we can approve of such experiments unreservedly. In paying a good deal of attention to a particular nutriment we are pretty sure to neglect something else. We have seen from the Law of Isodynamics that the carbohydrates and fats replace one another in accordance with their calorific values, and that protein may be replaced by these two foodstuffs to a certain extent. There is always danger, in making use of chemically-pure foods, that too little attention will be paid to the amount of inorganic salts which are required. The knowledge of the calorific requirements for the performance of a definite amount of work is very important for the establishment of a ration. The calorific requirement forms a foundation. It must not, however, be regarded as the sole requirement. The composition of the ration is by no means a matter of absolute indifference.

In the following table the calorific values of a few foodstuffs are given:[1] one gram of substance yields the following number of heat units, expressed in small calories:

(A) PROTEINS.

	Stohmann.	Berthelot.		Stohmann.	Berthelot.
Plant-fibrin	5941.6	5832.3	Flesh fibers (with fat removed)	5720.5	5728.4
Serum-albumin	5917.8	...	Flesh (with fat rem'd)	5662.6	...
Hemoglobin	5885.1	5910.0	Blood-fibrin	5637.1	5529.1
Milk casein	5867.0	5626.4	Peptone from blood-fibrin	5298.8	...
Yolk of eggs	5840.9	...	Chondrin	5130.6	5342.4
Legumins	5793.1	...			
Vitellin	5745.1	5780.6			
Egg-albumin	5735.2	5687.4			

(B) FATS.

Tissue fat	9484.5	Linseed oil	9623.0
Butter	9231.3	Olive oil	9328.0

[1] Cf. König: *loc. cit.* p. 283 *et seq.*

(C) CARBOHYDRATES.

Grape-sugar	3742.6	Maltose	3949.3
Fruit-sugar	3755.0	Starch	4182.8
Galactose	3721.5	Dextrin	4112.5
Cane-sugar	3955.2	Cellulose	4185.4
Milk-sugar	3951.5		

(D) ORGANIC ACIDS.

Oxalic acid	571	Citric acid	2397
Tartaric acid	1745	Benzoic acid	6281

This is all that we care to mention with regard to metabolism as a whole. We realize that we have merely touched upon most of the questions without attempting to consider them from all the different points of view. Metabolism physiology has during recent years developed a field of its own. In pathology it finds much that is kindred in nature. Both fields are intimately connected with one another, and this is largely the result of recent efforts. Without going into clinical experience in detail, it would be hardly possible to give a complete picture of general metabolism from all directions. We shall, therefore, be obliged to refer the reader to the special works on the pathology and physiology of metabolism. Our discussion has been only to bring out the more important principles, and will, it is to be hoped, prove an incentive to further studies.

LECTURE XXIX.

OUTLOOK.

I.

We have not even approximately exhausted the large domain of physiological-chemical investigation in the discussion of our knowledge concerning the chemical processes which take place in plant and animal organisms. We have merely been able to touch upon the fundamental principles upon which the science rests. To be sure, our knowledge is still incomplete, and the explanation of many phenomena has resulted solely from the play of the imagination. On the other hand, the progress of the exact sciences is constantly bringing new methods to the aid of physiological chemistry, and in this way we are being led to more definite problems, so that we are hopefully looking forward to the further development of the field. Little by little the unknown becomes the known. Direct proofs gradually replace the indirect conclusions. The physiological chemist is gradually breaking away from the observation of a single individual. It is becoming very evident that satisfactory results can be obtained only when the investigation is carried out with as many different organisms as possible. The broader the foundations, the greater the scope of observation, and the more varied the conditions are under which certain physiological processes are studied, the less danger there is in arriving at biased conclusions. Just as morphology developed into an independent science only by extensive comparative investigations and by the careful consideration of the anthropogeny of each individual species, so we shall expect to obtain from comparative physiological-chemical investigation the answer to many problems and to receive new impulses for further inquiry. Just as an organ which is functionally unimportant — e.g., an apparently superfluous bone — may be in the eyes of a zoölogist an eloquent proof of a common origin with a certain class of animals, and just as the botanist is able to conclude from the similarity of the flora of our highest Alpine peaks and that of the Far North that there is an intimate relation between these two regions, so we may certainly hope to meet here and there with chemical processes which will lead us from the present into the far-distant past. What an infinite perspective is opened to us by a glimpse at the wonderful flower tapestry of the Alpine heights, this so foreign and so characteristic

witness of long-forgotten ages! We also find many insects which are sharply confined to the Alps and to the Far North. Many a paleontologic discovery serves to form a bridge between two apparently foreign fields, and at one stroke changes assumptions to indisputable proofs. The bottoms of our Alpine lakes are covered with forms of life which we encounter again only in the arctic regions.[1] How interesting it would be to turn our physiological chemistry into similar channels! We have hardly begun to advance in this direction, for our present methods are still unable to follow the flight of thought, and our understanding of the chemical processes taking place in the different organisms is still too limited for us to make comparative studies. Nevertheless this goal should be regarded as most worthy of attaining. To be sure, there are a great many isolated facts and a multitude of observations concerning the organisms of different kinds of plants and animals, but they are far from being of equal value, and it remains to unite our knowledge of certain processes into a continuous chain. We can, however, call attention to certain facts which indicate that not only every species of animal but even each individual is to be regarded as one which is characteristic and limited in its general metabolism.[2]

Let us consider for the moment the great multiplicity of forms in the animal kingdom. In what a contrast the tissues of these morphologically so different beings must stand! Consider the vertebrates. Everywhere we find the same physiological function, the same tissue, the same organ. Not only is this true of the external appearance, but even the finer structure shows a great similarity. In spite of this fact, the same organs of different species of animals are very different in their metabolism, and again the chemical composition of these organs and tissues must be characteristic for each species of animals and perhaps for every individual. Herein lies the reason for the differences in their metabolism. Let us see what right we have to compare the purely morphological differentiation of the various kinds of life into classes, families, and species with physiological-chemical limitations, especially as regards the species. If we consider physiological-chemical investigation as a whole, we shall find that it extends in two directions. On the one hand many apparently dissimilar elements are united by a common band to form a large whole, and on the other hand many functions which were apparently identical have, after careful study of the individual processes, been found to be different in nature, and thus limitations have been found to exist where none were suspected. Thus it was formerly believed that there was a

[1] Cf. F. Zschokke: Die Tierwelt der Schweiz in ihren Beziehung zur Eiszeit, Basel, 1901.

[2] Cf. Huppert: Ueber die Erhaltung der Arteigenschaften, Prag, 1896. Franz Hamburger: Arteigenheit und Assimilation, Leipsic and Vienna, 1903. Emil Abderhalden: Naturwissenschaftliche Rundschau, **19**, No. 44 (1904).

great gulf between the animal and vegetable worlds. There was not supposed to be anything in common between them as regards their chemical processes and their metabolism. The plant cells were alone assumed to build up organic substances, or, in other words, effect syntheses, whereas the animal cells were assumed to break down only. Wöhler's discovery that benzoic acid is changed to hippuric acid in the animal organism made the first breach in the wall separating the two kingdoms. Then in rapid succession bridge after bridge has been built between these apparently so distinct fields, so that to-day a common band unites the animal and vegetable worlds. With this knowledge as a basis, it was then desirable to study more closely the differences between these two kingdoms, and also to unite them more closely by numerous intermediate stages.

A few examples may illustrate the significance which, from a physiological-chemical standpoint, governs the conception of the species.

The characteristic mark of distinction of mammals, the mammary glands, deliver a secretion, the milk, which is quite uniform in nature. The milk from an animal almost always has a very similar qualitative composition, although it varies quantitatively somewhat. Each species, however, has its own characteristic milk, and this is true not only of the mineral constituents, but of the organic matter contained in it as well.[1] In fact, we have reason to believe that even qualitatively the different kinds of milk differ from one another. Although our present knowledge does not suffice to characterize these differences more precisely, — and indeed the different kinds of casein appear to us as identical, — we must not forget that we are never justified in deciding from the qualitative and quantitative composition of the cleavage-products whether the original proteins under investigation are identical. In the arrangement of these constituents in the original molecule, to say nothing of the other kinds of isomerism, there are countless possibilities.

Furthermore, let us consider the blood of different animals. In every case it has the same function, the same physiological significance, and morphologically the most far-reaching similarity. We always find blood-corpuscles and plasma. What a remarkable similarity there is between human blood and sheep's blood, and yet the sad experiences which have resulted from the attempts at substituting the latter for the former have proved that deep-seated differences must exist. The blood-corpuscles of mammals all contain hemoglobin as a characteristic constituent. Its function is invariably the same, and yet the hemoglobin is specific for each different species, as is apparent from the external relations alone, such as the crystalline form and solubility. Thus the hemoglobin of the

[1] Emil Abderhalden: Z. physiol. Chem. **26**, 487 (1899); **27**, 408, 594 (1899). Cf. Lecture XVII, p. 404.

squirrel crystallizes in the hexagonal system, and that of the mouse in the orthorhombic. From a mixture of these two kinds of blood, each crystallizes in its own specific form, and to the same extent as corresponds to the original mixture.

Comparative quantitative analyses of different kinds of blood [1] indicate that, within fairly narrow limits, there is a definite composition of the blood for every species. In closely related animals the relative amounts of the individual constituents are similar, while in unrelated ones the differences may be very marked. It is noteworthy that the serum appears to be of very similar composition in the case of all mammals. The product in this case is apparently identical when prepared from various classes of different animals. We must not forget, however, that the examination of the ash can give us at best only a rough idea of the composition of the serum. It only serves to tell us what elements are present, and nothing at all concerning the manner in which they are contained in the circulating blood. But even if it were possible to prove that the inorganic and simple organic constituents were qualitatively and quantitatively the same in the sera of widely different species, there still remains the far more difficult problem of determining the identity of the proteins. It is perfectly possible that the different proteins in the blood contain groupings which are characteristic of each species of animals.

Let us return to the oft-discussed observations concerning digestion. We have seen that the nature of the proteins contained in serum [2] is evidently independent of the kind of food that is eaten. This is probably true for all the other substances, or at least for the more complicated organic ones. The cells of the body never know what the nature is of the food eaten. They always receive a modified nutriment. The ferments of the intestine and the accessory glands have the function of resolving the complicated organic constituents of the food into cleavage-products and, on the other hand, the cells of the intestine have the property of effecting a chemical reorganization of these building-stones into new products which are suitable for the cells of the body. The intestine thus regulates to a certain extent the general metabolism and guarantees the maintenance of a constant composition of our tissues. It is, therefore, perfectly clear that the entire course of the metabolism in the cells of our organs is dependent upon their composition. The composition of the protein molecule, or perhaps better the protein molecules, is particularly influential in imparting to each individual cell its characteristics. The cells of the body produce the ferments, and these are probably transformation products of the proteins. We can easily understand that their finer construction is

[1] Emil Abderhalden: Z. physiol. Chem. **23**, 521 (1897); **25**, 65 (1898).

[2] Emil Abderhalden and Franz Samuely: Z. physiol. Chem. **46**, 193 (1905). Cf. Lecture X, p. 212.

dependent upon that of the cell proteins. Thus their activity is regulated very delicately, and the foundation is laid for a specific cell-metabolism. The original structure of the cell determines its characterization for the whole of its existence. Although we do not doubt that the various tissues possess, corresponding to their functions, variously constituted cells, the differences of which are apparent not only in the metabolic end-products, but especially in the nature of the secreted substances, we can, on the other hand, imagine that all the cells of one and the same nature have common outlines along certain lines, so that a common character marks the whole cell structure of an individual.

Now an individual results from the union of two cells, the egg and sperm-cells of the same nature. Each must contain within itself the common form of cell composition, and thereby possess the kind of cell-metabolism which is characteristic of this particular species. All the cells which result in rapid succession from the fertilized egg-cell can only assume these characteristic tendencies, and thus the chemical unity of the original cell guarantees the maintenance of the species. This is subsequently maintained by the activity of the intestine, which only permits such material to reach the tissues as has been previously prepared in a definite manner for the entrance into cell-metabolism and into the cells themselves. We do not mean to assert that the body cells have lost the ability themselves to transform and adjust to their composition and to their metabolism any foreign nutriment, or especially any foreign protein. The unicellular organisms must be able to cause all these processes to take place side by side. With the higher animals, the function of transforming the foodstuffs is relegated almost exclusively to the intestine. There is here a division of labor.[1] We can easily imagine that by reason of an imperfect function of the intestine, an insufficient amount of prepared material may be carried to the tissues, and that under some circumstances the entire chemism of the cells, their structure and at the same time their metabolism, may become altered so that finally degenerations result which are apparently inexplicable.

In mammals the individuality of the cells of the species is in a very great measure guaranteed by the longer or shorter period during which the new being remains associated with the organism of the mother. The fœtus obtains its nourishment from the blood of the maternal organism; the suckling from her milk.

We have thus arrived at a purely chemical explanation for the conception of species and its maintenance. We admit that we are reasoning from a limited number of observations, and are not yet in a position to demonstrate experimentally the truth of our conception. We realize fully

[1] Cf. Ulrich Friedemann and S. Isaac: Z. exper. Path. u. Therapie, **1**, 513 (1904).

that we are now only constructing as it were a scaffolding which will perhaps serve to lead future investigation into definite channels.

We are not alone in this idea. Franz Hamburger,[1] starting from entirely different experimental results, has likewise traced in a most interesting manner the individuality of a species and its maintenance to a definite composition of the cells and body fluids. This idea is closely related to the so-called **biological reaction,** which we shall discuss briefly. Its discovery is associated with the names of Bordet, Tchistowitsch, and Nolf.[2] It represents merely a generalization of the Law of Immunity, and depends upon the formation of very specific substances after the introduction of products *foreign to the species.* The knowledge of this principle is of great significance for the further development of physiological chemistry. It forms a bridge to the domain of pathology; and we become more and more convinced that pathological processes are not sharply distinct from physiological ones, but are common manifestations of body cells under definite conditions. The limitations of purely physiological-chemical investigation are thus being more and more eliminated. By the improvement of methods, it continually enters new fields, and on the other hand other fields constantly attach themselves to it, and await new impulses for further fruitful work. Here we must introduce the name of an investigator to whom, more than any one else, our thanks are due for the expression of this unity between physiological and pathological processes, namely, Paul Ehrlich. We shall return to his theory, which has served as a foundation for important investigations in this domain. In this connection also we must call attention to the great importance of Pawlow's work.[3] He likewise clearly recognized the numerous transition stages between physiological and pathological processes, from his observations on the functions of the alimentary tract under varying conditions.

It is quite out of the question for us to give here even a brief summary of all the investigations which are based upon the conception of the "biological reaction." In a very short time it has developed into an important, independent branch of biological science. We shall here very briefly call attention to a few fundamental experiments. If we inject, for example, horse-blood into a rabbit, the serum of this animal soon shows characteristic new properties towards the injected blood. It dissolves the blood-corpuscles and forms a precipitate with the new serum, called the *precipitin-formation.* This reaction is a specific one. The serum of the rabbit which has been

[1] *Loc. cit.*

[2] Jules Bordet: Annales de l'Institute Pasteur, **1899,** 240. Tchistowitsch: *Ibid.* **1899,** 413. Nolf: *Ibid.* **1900,** 299. Cf. Rostoski: Zur Kenntnis der Präzipitine. Würzburg, 1902.

[3] Pawlow-Walther: Das Experiment als zeitgemässe und einheitliche Methode medizinischer Forschung. Bergmann, Wiesbaden, 1900.

treated previously with horse's blood will not react, for example, upon the blood of the ox, sheep, or goat. We may add that the formation of such specific products is not peculiar to the blood and its serum. The property is common to all the cells, body-fluids, and secretions. If we inject the spermatozoa of the sheep into a rabbit, the blood-serum of this animal when added to the living, motile spermatozoa of the sheep will restrict their activity. And this serum also has a solvent action upon the blood-corpuscles of the sheep's blood; i.e., the effect of the spermatozoa is the same as if sheep-blood itself had been injected. Hamburger assumes that every cell, and all the other substances which circulate in the body-fluids, possess definite atomic groupings, which we must regard as imparting the specific nature to these products. Consider the ferments! These are substances of which we know merely the effect. Emil Fischer has, as we have often mentioned, called our attention to their very specific action, and indicated clearly their dependence upon the configuration of the different compounds with which they react. The ferment molecule must possess certain definite groups by reason of which it can react with certain other molecules, and only these. Fischer has aptly compared the relation between the ferment and the compound with which it reacts as that of a key, and the lock which it fits. Just as a given key fits only a certain kind of lock, and conversely the lock can only be opened by just such a key, so the specific atomic grouping of the ferment molecule probably harmonizes exactly with the compound to be acted upon. We can easily imagine that slight changes in the arrangement of the atoms in the ferment molecule will be sufficient to modify the efficiency of the ferment. On the other hand, we can also believe that when this characteristic group is in combination with some other substance, the ferment will be prevented from exercising its function. It is possible that the zymogen stage of the ferment is brought about by some such combination or different arrangement of the atoms in the ferment molecule. These views are advanced merely to indicate that just as the individual cells can produce ferments, they themselves may be endowed with specific atomic groups, which, as Ehrlich has suggested, may perhaps stand in certain relation to the assimilation of the food. In this case there would be a certain analogy between fermentation and cell-activity. We know of the formation of anti-ferments when the ferments are introduced into the circulation. These anti-ferments are also specific in their action. We may look upon the formation of the precipitins, and related bodies, as taking place in a perfectly analogous manner. We introduce into the blood and cells of a foreign species of animals a certain atomic grouping which is peculiar to a different species, and which is perfectly foreign to the first animal. The animal organism reacts towards this exactly in the same way as towards the toxines which result from micro-organisms. Substances are evidently

produced which are so constituted that they exactly correspond to the structure of the foreign product. In this way the active groups which would be injurious to the body-cells are rendered harmless. The "biological reaction" of the animal organism is, from this point of view, to be regarded merely as a means of protection.

Let us see what subsequent investigation has done for us in this direction towards establishing our conception of species. In the first place it could be shown that the formation of precipitins was not confined to one species, but that the specific result of the reaction was limited to related animals within such narrow limitations that the relationship between animals which from morphological and other similarities are usually grouped together could be confirmed by means of the "biological reaction." Nuttal [1] found, for example, that the serum of a rabbit into which the serum from dog's blood had been injected would give a precipitate with the blood of eight different kinds of *Canidæ*, but not with the blood of any other species. Friedenthal [2] showed, furthermore, that only the anthropoid apes showed a marked blood-relation to man, whereas the lower apes showed but slight indication of a common origin. We may add that the different kinds of birds have also been compared in this way, and that recently Uhlenhut [3] has succeeded in so perfecting the method of carrying out the biological reaction that it has become possible to differentiate and distinguish between closely related kinds of blood. The reaction has become of considerable importance in forensic blood determinations.

It would be, of course, desirable to ascertain what compounds the cells and body-fluids make use of for carrying out this specific reaction. We would naturally think of the proteins in this connection, for, on account of their extremely complicated composition, they are most suited to serve as carriers of specific groups of atoms. As a matter of fact, it has been found possible to prove that after the injection of protein, e.g., serum-albumin, perfectly specific precipitins are formed; [4] and, indeed, it was even found possible to distinguish by this means between the casein of different kinds of milk. We do not yet know whether in such cases the individual protein substances come into consideration, or whether the effect is produced by impurities which adhere to them. At all events, it is of great interest to

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[1] Cf. Nuttal: Proc. Roy. Soc. **69,** 150 (1901). Blood Immunity and Blood Relationship. Clay & Sons, London, 1901.

[2] Arch. Anat. Physiol. **1900,** 494. Sitzsber. Berliner Akad. **1902.** Verhandl. Berliner physiol. Gesell. **1904.** Uhlenhut: Arch. Rassen- und Gesellschaftsbiologie, **1,** 682 (1904).

[3] Uhlenhut: Deut. med. Wochschr. 1905, 42.

[4] Cf. among others, L. Michaelis: Deut. med. Wochschr. 1902, 41. L. Michaelis and Carl Oppenheimer: Arch. Anat. Physiol. Sup. **1902,** 336. F. Obermayer and E. P. Pick: Wien. klin. Wochschr. **1904,** 10. Andrew Hunter: J. Physiol. **32,** 327 (1905).

find that if the proteins enter metabolism in any other way than through the intestines, i.e., in such a way that the substances have not lost their identity by the activity of the intestine and changed so that they are suited to the body-albumin, then the only effect to be observed is the formation of these specific products. This result supports our views regarding the great importance of the digestion processes and assimilation in the intestine for the maintenance of the species.

With the assumption of atomic groups of specific nature in the individual cells, and thus in the egg and sperm, new aspects are given to the problem of heredity. Although it has not yet been found possible to cause morphological changes that have been artificially produced to be inherited, still there remains the possibility of effecting hereditary variations by influencing the chemical composition. We may mention the interesting experiments of Engelmann and Gaidukow [1] who succeeded for the first time in proving satisfactorily that a property which had been acquired could be inherited. If cultures of *Oscillaria sancta*, a kind of alga, are kept for months at a time in light of a definite color, then the single threads of the alga gradually assume a complementary color, i.e., a shade which is favorable to the assimilation in such light. The change of color takes place only with the living organism. Aqueous solutions of the dye do not show any such change in shade under the same conditions. We have, therefore, a case of a vital, physiological adjustment. Engelmann designates it as *chromatic adaptation*. Now, strange to state, this acquired change of color is retained when the *Oscillaria* are placed in ordinary light. In the case of rapid propagation, the new color prevails, so that the assumption may be made safely that there is a new formation of chromophyll in the younger generations of cells. In reality we have here a case of the inheritance of a change in chemical composition, and in fact in the formation of a pigment, the synthesis of which remains the same as formerly in the new environment.

It might have been thought that by feeding compounds of quite definite composition it would be possible to effect a change in the chemical composition, and thereby in the cell-metabolism. Such experiments must be without much prospect of success, because of the fact that, as we have seen, the intestinal wall frustrates the entrance of such foreign substances. The unicellular beings are likewise unsuitable for deciding such questions, because they are also provided with the necessary means for maintaining their constancy of chemical composition. At best, the only way we can conceive of any such experiment being successful would be to continue for a long time the introduction of such substances to the body-cells in some other way than through the alimentary canal, for it would be expected that

[1] T. W. Engelmann: Arch. Anat. Physiol. **1902**, Suppl. 333. Sitzsber. Berliner Akad. Wissensch. **1902**. Arch. Anat. Physiol. **1903**, 214.

in the course of time the cells would lose to some extent the ability of transforming rapidly the substances which are brought to them. At all events, this is the only way in which the metabolism of more highly organized beings could be affected.

It is almost generally assumed that in the inheritance of certain properties the nucleus of the cell plays a particularly important part, and in fact it is usually assumed that it alone is able to transmit the characteristics of the parents. This is certainly not justifiable, for although the protoplasm represents an apparently homogeneous mass, which is but slightly differentiated and excites but little the interest of the histologist, it is not at all apparent why, with its infinitely complicated composition, it should not be capable of at least taking part in the processes named. In this direction the following experiment of Godlewski[1] is interesting. He fertilized nucleus-free fragments of *Echinus* eggs with spermatozoa of *Antedon rosacea*. It was found possible in some cases to effect a development. Even these nucleus-free pieces did not develop to be of the *Antedon* type. In order to understand this experiment better, we should recall the investigations of Loeb,[2] which we have previously mentioned, who succeeded in a great number of cases in bringing eggs to spontaneous segmentation by the action of certain salts in definite concentrations. Of great interest is his discovery that under certain definite conditions the egg of a definite species, e.g., that of a star-fish, could be fertilized by the spermatozoa of an entirely different nature. We shall not go into the significance of these discoveries any more deeply, but will briefly consider the outlook which Loeb himself obtained from his experiments. He believes it is not at all improbable that the greater part of the many different forms of life, particularly those of deep sea, may have resulted from conditions which are not at all unlike those of his experiments. It is indeed conceivable that in the course of time the composition of the sea-water in certain localities may change so that the conditions for the fertilization of one species are replaced by those corresponding to a different one. We mention these experiments merely to illustrate in what widely different ways biology seeks to penetrate the mystery of life. To be sure, we are not justified in considering the interesting results of Loeb's experiments as by any means solving the problem of egg development, or even in hoping that by such a way we shall obtain an insight into the laws of heredity. Loeb's experiments merely show that it is possible, by changing the concentration of a salt solution in which the unfertilized egg is placed, to bring about cell-division. They do not tell us anything at all about the causes of cell-multiplication, and the reason

[1] Anzeigen der Akad. Wissensch. Krakau, **1905**, 501.

[2] University of California Publications, **1**, No. 1, p. 1 (1903); **1**, 39 (1903); **1**, 83 (1904); **2**, 5 (1904). Pflüger's Arch. **99**, 323 (1903); *ibid.* **104**, 325 (1904). Cf. Emil Abderhalden: Arch. Rassen- und Gesellschaftsbiologie, **1**, 656 (1904).

for the regular arrangement and the gradual differentiation of their functions.

Similarly, Godlewski's investigation fails to shed light upon the problem of heredity itself. It shows us, on the other hand, quite clearly and definitely that it is not right to regard the process of cell-division as a function of the nucleus alone, and consider that the protoplasm plays a passive part. The cell of the egg, in its entirety and according to its chemical composition, must have, more than any other cells of the body, the ability to subdivide and increase. The entire nature of cell propagation, in all its particulars, has been found to be bound up with it.

Loeb's experiments have shown that the egg-cells of various species of animals may be easily induced to take part in such a process of cell-division. The cells of one kind develop up to a certain stage apparently of their own accord, others require a slight stimulation, while others need quite a considerable impulse. The entire development of the individual is bound up with countless problems. When we remember that we have before us a mode of development which has been inherited for ages and has been taking place over and over again in paths which have been well defined, the wonderful thing is that there is apparently no limitation to it. We can understand perhaps how, in the case of the mature organism, lost tissues may be regenerated; and, again, it does not appear so remarkable to us that from the cells of a certain tissue others may be produced with the same function and the same chemical composition, so that the whole corresponds to a morphological and functional unit. On the basis of our present knowledge, however, it is a mystery how all the different tissues can develop from a single cell, and how in each kind of tissue chemical processes will take place which are peculiar to that particular tissue, as is evident from a study of the secretions and of the end-products of their metabolism. The complicated picture of the morphological development of the individual seems to us far less remarkable than the much more intricate and more involved differentiation of the chemical construction and the chemical processes which take place in the individual cells.

Now we know that the individual, in the case of the more highly organized animals especially, shows not only a development of species, but in its beginnings there are stages of development to be detected which we can understand only from the history of the ancestry of the animal organism. We need recall merely the formation of the gill-clefts or gill-slits, a stage of development which even the human fœtus passes through. Is it not probable that there are differences in the chemical composition of the tissues during the separate stages of its being, which are likewise suited for tracing the entire group of vertebrates back to a common ground plan? We must thank G. von Bunge for an answer to this question.[1]

[1] Z. physiol. Chem. **28**, 452 (1899).

He pointed out that the land vertebrates are richer in sodium chloride in proportion as the stage of development is young. This fact was illustrated particularly clearly by comparing the sodium chloride content of the cartilage of embryo and that of later stages of development of the same species. The older the animal, the lower sinks the content of sodium and chlorine. There must be some reason why the cartilage of the embryo is so rich in common salt. This fact is all the more striking because the land is poor in this salt, potassium salts preponderating. Typical inhabitants of the land, such as insects, have in their bodies the elements sodium and potassium in about the same proportion as that in their food. The remarkably high content of sodium chloride in the cartilage of the early stages of development of vertebrates which inhabit the land may be regarded, like the appearance of the gill-slits and other similar phenomena, as an ancestral reminiscence. It is not at all strange that even the chemical composition should indicate long-forgotten conditions.

Not alone the development of the individual leads to such various problems, but in later life also we meet with processes the nature of which we can understand only very imperfectly. We usually assume that the development of the entire organism can be traced back eventually to three germ-layers. We can indeed distinguish these as regards their chemical function and their construction even although one and the same layer, as the ectoderm, may be of very heterogeneous construction. We can imagine that the cells of each of these three germ-layers among themselves have certain common characters so that eventually each individual cell is in a position of replacing other cells which have resulted from the same germ-layer and even to form these anew. It is not sufficient, however, to conceive that these three germ-layers correspond to three great classes of different cells. They must, as we have already suggested, all show common characteristics. To what extent the body-cells of even adult organisms have the ability of changing their composition, and thereby their function, is shown by the following experiment.[1] If the crystalline lens is entirely extirpated from a water salamander, after a short time there will be found in its place a newly formed transparent structure which perfectly corresponds to the original lens. It is very interesting to find that the new formation of the lens starts from the epithelial cells of the iris. Now the cells of the latter are normally as opaque as possible. Nevertheless these cells multiply after the removal of the lens, and the pigment, which causes the opacity, disappears. In this process comprehensive chemical processes must take place. The cells

[1] Cf. Vincenzo L. Colucci: Memor. della R. accad. delle scienze dell' inst. di Bologna, Serie 5, T. 1, p. 593 (1890). Also G. Wolff: Arch. Entwicklungsmechanik der Organismen, **1**, 380 (1896).

of the crystalline lens and of the iris have unquestionably quite different construction corresponding to their different functions. Certainly their metabolism is different.

Thus when we find that from one tissue another of entirely different characteristics may be formed, with a quite different function, we cannot help asking whether pathological new formations do not arise from similar processes. Certainly the cells of the newly-formed crystalline lens of the Triton renew themselves and form cells of the same kind, and show hardly any tendency to form the pigment which is required by the cells in the iris. Similarly it is possible that a body-cell may retain its individual nature only with difficulty if for any reason its chemical nature and function become seriously altered, and the progeny of such a cell will possess the characteristics of the mother-cell so that gradually a whole cell-complex will develop which is of a nature foreign to the entire organism and to its metabolism, and in fact the metabolic end-products of this new cell-complex may exert a disturbing influence upon the metabolism of the remaining cells of the body.

Apparently there is a connection between these cells and the mother soil — we refer especially to sarcoma and carcinoma — for it has been frequently doubted whether such cells can be successfully transmitted to organisms of a different species. We are, in making these suggestions, very far from explaining the formation of these peculiar, atypical tissues. We only wish to bring forth the fact that with the further development of our physiological-chemical knowledge new tasks will be set, and that even problems of purely morphological investigations will, in the course of time, become closely allied to those of physiological chemistry. If it is once found possible to compare the metabolism of the cells of a cancer, or other malignant growth, with normal cells, we may certainly expect to obtain a more accurate insight into the nature of such mysterious processes.

Taking into consideration all that we know concerning metabolism, and what we have deduced indirectly, it does not appear to us impossible that, among the more complicated processes, here and there a link in the chain of separate processes may be missing, or react in a faulty manner, thus giving rise to degenerations which eventually may be inherited, or at least there may be indications of the transmission to several members of a family. We would recall certain anomalies,[1] such as cystinuria, alcaptonuria, and albinoism, and finally familiar types of degenerations in the nervous system. Gout and diabetes may also be caused by disturbances in some phase of the functions of cell-metabolism which are partly inherited and partly acquired. Although we may imagine that, for example, an anomaly in the composition of the body-albumin is inherited

[1] Cf. A. E. Garrod: Pflüger's Arch. **97**, 410 (1903).

in such a way that the egg-cell, as a part of the mother's organism, is affected by the disturbance, still this will not hold for the transmission of many other properties. In this direction we have the peculiarity in the inheritance of hemophilia, as we have already indicated.

If we consider the cells in their entire chemical construction, and constantly bear in mind that herein lies their entire function, we can indeed imagine that a faulty organization of the cell will have an influence upon the general metabolism. The conception of the *disposition* which plays such an important part in pathology is certainly well founded, and the cause of it is to be sought in the state of the function of certain groups of cells, or the whole cell state of the body may be affected in such a way that the individual appears to be functionally deficient. Naturally such speculations, which have no experimental justification, do not penetrate at all into the nature of a *disposition;* but, nevertheless, it seems fitting to suggest that an alteration in the chemical processes of the cells themselves may come into consideration here.

Let us now turn back to the limitations of the concept of species on a basis of physiological-chemical investigation. We have mentioned only a few of the species which have been most thoroughly studied. We might multiply these examples, but a few suggestions should prove sufficient. We know that the general metabolism of different animals is unlike. This is partly due to the different kinds of nourishment which they receive. Thus we can readily understand that the urine of herbivora will be of quite different composition from that of pure carnivora, and that of the latter will likewise be different from that of omnivora. We even find differences in the same animal species. We recall in particular the amount of kynurenic acid present in the urine of dogs. We encounter other characteristics which indicate that each species of animal has a peculiar cell-metabolism. We do not doubt at all that there are also individual differences. The fact that there is a perfectly definite endogenic uric acid value for each individual points in this direction. Also the peculiar characteristic color of the skin, hair, eyes, etc., gives one the impression that it is the expression of a specialized individual metabolism of certain definite cell-complexes. Each individual possesses its own characteristic smell. This is usually not observed by human beings, but that there are such distinctions is evident from the ability of certain animals, such as the dog, to trace a person by the scent.

The differentiation of the species increases infinitely in the case of lower animals, especially the anthropods. What a richness of coloring, and what splendor in the groups of butterflies and beetles! Each separate color is an expression of the chemism of the cells producing it. We meet with the same phenomena in the plant world, where the beautifully colored flowers indicate an unsuspected variety of peculiarly modified chemical processes.

To be sure there are certain relations between the pigments that the vegetable kingdom produces and those of the living animal organism, which find expression particularly when it is a case of assuming a color for purposes of protection. It would be interesting to compare the pigment used in mimicry with that of the plants upon which the animals feed. We would also refer to the large number of poisonous substances which animals produce partly as weapons of attack and partly for other purposes.

The specific character of cell-metabolism does not by any means stop at causing differentiated individuals. It also concerns the individual cell. We shall refer in this connection merely to the specific metabolic substances, especially the toxines, which the cells produce to combat every kind of bacteria.

The intricate, specific organization of the cells of certain kinds of animals is also indicated indirectly, as, for example, by their behavior towards certain poisons. Thus we know that a man who is not accustomed to morphia will usually sleep after taking two centigrams, whereas with dogs, ten times this amount does not have the slightest effect toward producing sleep. A goat will stand twenty grams of morphine hydrochloride without showing any desire to sleep, although other indications of poisoning appear. Whereas atropine is a violent poison for man, rabbits are perfectly immune toward it. They can feed unharmed upon leaves of belladonna. It is also known that all kinds of animals do not furnish equally good fostering soil for pathogenic bacteria, and that different organisms react differently toward certain infections.

If we summarize all these details, we shall recognize how manifold the processes are which stamp their outlines upon every kind of species and every individual. Here lies before us an immeasurable, almost uncultivated field of investigation. New problems and new methods become more and more intricate, and the demarcations of the conception of species become more and more exact. The purely morphological boundaries of species, families, and classes will pass away. Comparative physiological-chemical investigation will, in the future, take the lead and control the results of purely morphological investigation.

We must consider one other field which is gradually coming into more intimate contact with physiological chemistry. We refer to pharmacology. We are no longer satisfied with determining the effect of individual drugs. We desire to determine, by comparative experiments, whether it is this or that group which represents the active principle, and whether the compound exerts its effect as such, or whether it first undergoes a transformation.[1] Finally, we are interested to know how the

[1] Cf. S. Fraenkel: Die Arzneimittelsynthese auf Grundlage der Beziehungen zwischen chemischen Aufbau und Wirkung, Berlin, J. Springer, 1906. H. Bechold and P. Ehrlich: Z. physiol. Chem. **47**, 173 (1906).

substance introduced is broken down, and in what form it leaves the body. Conversely our interest is fastened upon the way in which the body reacts toward the influence of certain compounds. Here also innumerable specific cell reactions come into play, and we constantly meet with indications of the functional differences of the cell-complexes of different organs. Investigation in this field is only just beginning. We lack methods for tracing the course of each individual substance in the body from cell to cell. We should like to know whether the different body-cells have a different affinity for certain products which are introduced, and whether perhaps the specific reaction of the organism is not the expression of the ability for selection on the part of certain particular tissue-cells. On the other hand, we are interested, in every case, to know how the animal organism wards off the action of all the different substances which are introduced into the body and are foreign to it. In considering the functions of the cells we constantly met with such problems and have seen in how many different ways the organism protects its cells from the action of such substances. Sometimes the substance which is introduced is oxidized, sometimes it is reduced, and at other times it is conjugated either directly, or after certain preparatory attacks, with different substances which are produced in the intermediate metabolism. Thus we have seen how the animal organism behaves toward glycocoll, sulphuric acid, urea, and glucuronic acid.[1] We know that the proteins themselves play an important part in these processes. They combine with many of these harmful substances which are introduced into the body and form insoluble compounds. Often this combination involves the breaking down of the cell. The cell-albumin becomes incapable of exerting its function. The effect of a great many poisons is due to their affinity to the proteins of the cells and tissues. Here again we meet with certain specific differences according to the nature of the cell and the proteins which have taken part in their construction. In this connection we would recall the different coloring of the cells, the cause of which is likewise attributed to a different chemical construction of the cells. It is this which lies at the root of the various functions of the different kinds of cells, and the separate organs, and upon it depend also all of the various reactions which have been mentioned, whether the cell plays an active part, or whether a mere passive one.

[1] Cf. E. Fromm: Die chemischen Schutzmittel des Tierkörpers bei Vergiftungen. K. J. Trübner, Strassburg, 1903.

LECTURE XXX.

OUTLOOK.

II.

We have seen in discussing the boundaries of physiological-chemical investigation, that such exist only artificially, and that the domain is immensurable. The tendency is becoming more and more marked to refer sciences which were formerly sharply defined to a common basis. This is particularly true in the domain of pathology, which is becoming more and more closely related to physiology. In fact, pathological processes in a certain sense are nothing else than physiological processes of our body-cells under specific conditions. This applies particularly to a group of processes, interesting alike to pathologists and physiologists, namely, the formation of certain substances in response to the action of other products on the body-cells. We have already encountered these processes at various times. We have seen that the animal organism responds to the introduction of ferments by the formation of anti-ferments; that is, the cells produce substances which prevent the activity of the ferments. We again met this problem in considering the so-called "biological reaction." Here also, it was a case of the formation of distinct metabolic products. The most significant thing about these processes is the fact that the products formed act in a very specific manner. We thus involuntarily return to an analogous process which we have already mentioned. It is known that the human and animal organism is capable, not only of withstanding the infection of specific pathogenic bacteria, but also, that it possesses peculiar characteristics, which prevent a second attack of the same bacteria for a long time after their first repulse. This, of course, only applies to certain infectious diseases. Other diseases do not leave such a condition of affairs behind them. This is evident from the fact that one may be afflicted several times with the same disease, — e.g., pneumonia, — whereas other sicknesses, such as typhoid or scarlet fever, usually occur but once.

The far-reaching specificity is also noticeable in these cases. This is best demonstrated by the following example. We can determine, by experiment, the quantity of cholera bacteria necessary to cause the death of a guinea pig of a given age and weight. If we take a smaller quantity, the animal will only become sick, and will gradually recover. This guinea

pig has now obtained an entirely new property, which sharply differentiates it from the other animals of the same class, which have not been treated in the same manner. We usually speak of this as an *acquired immunity*. This is evident from the following. The immunized animal can then be infected with an amount of cholera bacteria which would formerly have produced death, and it now does not even cause illness. If we inject these bacteria into the abdominal cavity, and after a time withdraw some of the fluid, we shall observe a very characteristic picture under the microscope. The cholera bacteria have lost their activity, and form small balls, which are sometimes collected into "clumps." A control animal, inoculated for the first time with these cholera bacteria, will show an entirely different picture. The organisms in this case are endowed with great activity. We find it necessary to assume that the immunized animal possesses substances in its organism which injure the cholera bacteria, and restrict their activity. This belief is strengthened by the investigations of R. Pfeiffer.[1] If, together with the cholera bacteria, serum from an immunized animal is injected into the abdominal cavity of another animal which has not been previously treated, we shall observe the same phenomena which were characteristic of an animal which had already been infected with cholera bacteria. The latter become non-motile, and form balls or clumps. M. Gruber[2] has shown that this phenomenon may be verified in a test-tube, by bringing cholera bacteria into contact with serum from an animal infected with cholera. We immediately observe under the microscope, that the cholera bacteria lose their mobility, and unite in clumps. This is spoken of as an "agglutination." Its appearance is indicated by the turbid liquid becoming clear, and a precipitate forming on the bottom of the vessel. It is interesting to find that a specific reaction is taking place here, for it is not possible to detect an influence upon the cholera bacteria by the serum of an animal which has withstood some other infection. Thus an animal which has become immune against typhoid bacteria does not possess a serum which acts upon cholera bacteria, and conversely the serum of an animal which has been immunized against cholera does not have the slightest effect upon typhoid bacteria.

The animal organism not only produces a specific protecting material against bacteria, but also against their poisons. Thus, the medium on which diphtheria bacteria have been cultivated shows toxic properties after the bacteria have been filtered off. Diphtheria toxine acts even in

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[1] R. Pfeiffer: Z. Hygiene, **15**, 268 (1894); **20**, 217 (1895); Deut. med. Wochsch. Nos. 7 and 8, pp. 97, 119 (1896). R. Pfeiffer and Kolle: Z. Hygiene, **21**, 203 (1896). R. Pfeiffer and Wassermann: *Ibid.* **14**, 46 (1893). R. Pfeiffer and Marx: Deut. med. Wochschr. **1898**, 47, 489; Z. Hygiene, **27**, 272 (1898).

[2] M. Gruber: Münchener med. Wochschr. **1896**, 206. M. Gruber and H. E. Durham: *Ibid.* **1896**, 285. M. Gruber: *Ibid.* **1899**, 1329. Cf. R. Kraus: Wiener klin. Wochsch. **1897**, 32.

small doses. By gradually increasing the dose of this poison to an animal under experiment, we can immunize it, so that it is able to stand relatively large amounts of it.[1] We cannot here go into all the developments arising from these observations, which are so interesting from a biological standpoint. We can state merely that bacteria yield to the body substances which we call **toxines.** They have a harmful effect upon the normal cell-metabolism. They are, in part, constantly given up by the bacteria, while to some extent the toxines are retained by the bacteria in their cell structure. In the latter case these poisons only become active on the death of the micro-organism. It is questionable whether we are justified in looking upon these two groups of poisons as being unlike. It is possible that the eliminated toxines are the end-products of metabolism. It is just as plausible to believe that we have here a phenomenon which more closely resembles a secretion process. From this point of view it is easier to understand why the micro-organisms eliminate such highly complicated substances; while, on the other hand, the conception that the toxines are end-products of metabolism seems doubtful, because we usually find that such substances require but little expenditure of energy for their formation; i.e., they are lower decomposition products. The strictly specific nature of the toxines also harmonizes more readily with the idea of a typical secretion process. In such a case we have to deal with products which are analogous to the ferments. The toxines given off would then correspond to the "unorganized" ferments; those remaining in the cells, to the "organized" ferments.

Just as this classification of the ferments is a purely superficial one, and has nothing to do with their nature and their manner of action, so it is perfectly possible that the toxines which are given up freely by the cells and those which are firmly attached to the cells are essentially identical. Again, the comparison of the toxines and ferments is also superficial, and should not prejudice us with regard to the toxines. We do not know anything definite about their nature. They are classed with the proteins, and justly so, for only to this class of chemical compounds can we imagine that such complicated bodies belong. For the same reason we have concluded that the ferments belong to the protein group, being probably transformation products of the cell-proteins. Just as the ferments exert a specific action, so the bacterial poisons have well-defined characteristics. We know of poisonous substances which are produced by highly organized plants and by animals the action of which is quite similar to that of the toxines. Thus we have *ricin* from the castor-oil plant (*Ricinus communis*), and *abrin* from the seeds of Jequirity (*Abrus precatorius*). Both are extremely poisonous, and an immunity may be

[1] E. Behring: Deut. med. Wochsch. 1890. E. Behring and Kitasato: *Ibid.* 1890.

established to offset their action.[1] Snake venom also belong to this class. The skin and blood of toads likewise contain such poisons, and they are also found in the garden-spider. The blood of the eel contains a toxine belonging to this group. Indeed the number of plant and animal poisons which have been studied is far greater and the poisons are of a more varied nature than we can attempt to describe. We are not yet ready to discuss their constitution, and in fact none of these poisons has been obtained in a perfectly pure state. It is perfectly clear that this fact affects investigation in the whole field of toxines and antitoxines. To-day we cannot depict, as sharply as we should like, the effect of the toxines and the cause of the formation of antitoxines. At present we must resort to hypotheses, and a state of certainty will prevail only when it is found possible to establish the constitution of at least one of the toxines. From our study of the ferments, we can readily believe that the antitoxines are similarly constituted to the toxines. This seems probable from the specific relations between these two products. It is important as regards the development of the modern conception of toxines and antitoxines that we should recognize clearly where the facts end and speculation begins, for nowhere has this been forgotten more than in this particular field. Perfect clearness in this respect is essential for the sound development of this branch of knowledge, because one of the most fruitful and most beneficial hypotheses of the age governs our conception of the nature of toxine action and the formation of antitoxines. We have in mind the theory to which Paul Ehrlich refers all the investigations in this field, and which with unexpected rapidity has been confirmed by observation after observation, and discovery after discovery.

This hypothesis is quite generally known under the name of Ehrlich's **side-chain theory.**[2] Ehrlich attempted with his theory, which is closely related to chemical representations, to bridge over the gaps in our inadequate knowledge concerning the chemical structure of the toxines. He makes certain assumptions concerning the nature of the active groups. In place of the chemical composition, he uses certain names which may be replaced by definite chemical radicals with the advance of our knowledge. Paul Ehrlich has not only succeeded in correlating by means of his ingenious theory many processes in this large field which apparently took place side by side quite independently of one another, but his theory has, moreover,

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[1] Paul Ehrlich: Deut. med. Wochschr. **1891,** 976, 1218. Fortschritte d. Medizin, **1897,** 41.

[2] Cf. Rostoski: Zur Kenntnis der Präzipitine. A. Stuber, Würzburg, 1902. Carl Oppenheimer: Toxine und Antitoxine. G. Fischer, Jena, 1904. P. T. Müller: Vorlesungen über Infektion und Immunität. G. Fischer, Jena, 1897. Ludwig Aschoff: Ehrlich's Seitenkettentheorie und ihre Anwendung auf die künstlichen Immunisierungsprozesse. G. Fischer, Jena, 1902. Paul Römer: Die Ehrlichsche Seitenkettentheorie und ihre Bedeutung für die medizinischen Wissenschaften. A. Hölder, Wien, 1904.

the great advantage that upon it as a foundation link after link of a tangled chain of processes has been disentangled, so that as a result we have before our eyes a continuous picture of separate processes. Problem after problem has accumulated, and gradually a new structure has been built which serves to bring under one roof all the various processes which stand in any relation to the formation of the anti-bodies. The investigations of Ehrlich appear especially important to us, because they are the first to bridge over the chasm which has previously been assumed to exist between physiological and pathological processes in the animal kingdom, so that to-day a sharp line can no longer be drawn between these two fields. Ehrlich has pointed out that the formation of the anti-bodies stands in direct relation to the cell-metabolism. In order to make this relation clear, we shall explain briefly how Ehrlich represents the assimilation of the nutriment by the cell as taking place. The individual cells are only capable of taking up and uniting with their structure those substances which correspond to their entire composition. The substances taken up must fit into the cells. The protoplasm possesses groups which are chemically active, and these have a maximum affinity to a certain arrangement of the atoms in the nutriment, which it unites to the cell-body. Paul Ehrlich calls these groups *side-chains*, or receptors. On the basis of this theory we can easily picture to ourselves why certain cells reject this and that substance, and on the other hand assimilate other products. One is tempted to deduce a purely chemical theory for the process of assimilation, though by doing so we may be making a grave error. We can easily imagine that a chemical compound, for instance the benzene ring, may carry side-chains, and that these may enter into reaction with other complexes. A new compound would result, but such a reaction is, as a rule, complete when this has been accomplished. The cell, however, behaves in an entirely different manner. It constantly utilizes material, and must be continually forming new side-chains, for it is always confronted with the necessity of taking up nutrient substances; that is, the new groups must always be present to combine with the nutriment. It follows from this assumption, that the "side-chains," as conceived by Ehrlich, do not correspond to our present idea of a purely chemical phenomenon. These side-chains are only hypothetical as yet, and have nothing definite to substantiate their existence. If we assume that the various cells have differently constituted side-chains, we will then have reached the idea of the specific nature of the cells. This also permits us to venture the assumption that the different nutrients are completely disintegrated during digestion, and are transformed into homogeneous products in the intestines. Ehrlich's theory is only completely comprehensible from this point of view. The specific groups of the cells must exactly correspond to those of the nutrient materials, the latter being established only at the time of assimilation.

With the above in mind, let us apply the side-chain theory to the formation of antitoxines. We have already stated that the bacterial poisons are probably very closely related to the proteins. We can easily imagine that they may have atomic groupings very analogous to those of the nutrients, and that, for this reason, they are attached to distinct cells. The cell immediately loses its ability to assimilate any nutrient material at those points where any toxines have attached themselves. If the cell has not been permanently injured by the poison, it will try to repair the damage by a fresh supply of side-chains. Under these conditions there may be an over-production of side-chains, to such an extent that they will not all have room to attach themselves to the protoplasm; they will consequently be pushed off, and circulate in the blood. We must not forget that these new side-chains must correspond exactly in their composition to those to which the toxines have attached themselves. This "first" side-chain must certainly have had a definite affinity for the toxine before it combined with it, in its transport in the organism.

Those analogously constituted side-chains which circulate in the blood must also have the ability of uniting with toxines, thus making them harmless, before they reach the cells. According to this view, the formation of antitoxines is not a new process — the free side-chains are nothing more than antitoxines — but merely a repetition of a normal function of the cell. It corresponds to the secretion of the individual cells to which we have repeatedly called attention. It is impossible for us to go into the details of the facts which go to substantiate Ehrlich's assumption. We only wish to add that it has been shown that definite bacterial poisons, for instance tetanus poison, enters into combination with tissue-cells, and that, on the other hand, we are acquainted with poisons which can be recognized as having very distinct affinity for specific tissues. Thus, it is known that abrin possesses very close relationship to the components of the tissues of the conjunctiva. Ehrlich designates the group of the toxine molecule, which unites with the side-chains of the cells, or the free side-chains, as the *haptophor group*. It is clear, if this conception of the combination of toxines and antitoxines is correct, that only a definite amount of the latter can combine with a given quantity of the former. The whole process must evidently correspond to a neutralization.

The toxine also contains a *toxophor* as well as a haptophor group. This is the carrier of the specific poisonous effect of the toxine. That this assumption of different groups in the toxine molecule is well founded follows from the fact that antitoxines may be produced even after the toxophor group itself has been destroyed. It has, itself, nothing to do with the immunity reaction of the organism. In the latter case the haptophor group only must be taken into consideration. If this be removed, for instance by antitoxine, then the toxine will be rendered valueless for immunization.

According to this conception, every body-cell must be able to form antitoxines, although such an assumption is not absolutely necessary. We can also imagine that individual cell-complexes possess the ability of producing toxines; in fact, there seems to be evidence of a selective action within certain limits. The whole subject of the formation of antitoxines is analogous to that of the general process of metabolism. We can easily imagine that the cell-metabolism is so altered by the introduction of toxines that an over-production of side-chains results. This assumption only serves as an assistant hypothesis, which is to act as a prop to the main idea which is likewise hypothetical in its nature. It is entirely possible that the idea of such an enlarged production of atomic groups, and their expulsion beyond the influence of the cell, is not at all necessary. We must call attention to a process which we have already discussed in detail. We have repeatedly remarked how from the carbon dioxide of the air products of entirely different properties are formed as soon as it comes in contact with the plant cells containing chlorophyll. They are in the first place optically active, and contain in their composition hydrogen as well as carbon and oxygen. We are accustomed to assume that the first product formed is a carbohydrate, although this belief has no substantial foundation. Other compounds, as well as carbohydrates, might be formed just as easily. Why is an optically active, very specifically constituted substance, formed from carbon dioxide and water? We are unable to answer this question. We must assume that this phenomenon is mainly dependent on the composition of the protoplasm of the chromophyll-containing cells. This is, itself, asymmetrically constituted, and can consequently only produce asymmetric compounds. When we consider the question why the cells of the stomach only deliver pepsin or hydrochloric acid, and those of the pancreas likewise give a very specific secretion, we must answer that in this case also the constitution of the cells is the fundamental cause of the individual functions. Every body-cell evidently endeavors to maintain its composition, for its permanency guarantees that its function remains the same and that there is a normal progress of its metabolism. The whole organization of the animal body is so adjusted that the cells shall maintain their specific composition. This is already evident from our consideration of the subject of digestion. We do not in the least doubt that every individual body-cell continually forms a definite secretion, thus participating in the general metabolism. But the same food is being normally presented to the cells by the blood. If a foreign substance passes beyond the intestine, manifold assistance is offered as quickly as possible all over the organism to forestall any damage. The liver, especially, guarantees the constant composition of the blood. It captures material, unites it with other products, etc. If its functions are not sufficient, other organs come to assist, while the different glands

finally begin to participate in removing the foreign substance by an increased elimination of secretions. The animal organism under normal circumstances constitutes a well-protected entity. Nothing foreign can penetrate into the cell-metabolism, consequently the general metabolism proceeds along its usual course. It becomes an entirely different matter when material is presented to the cells which can turn the whole organization toward an entirely different direction. There are constantly cells in our body which are engaged in process of destruction, and others, which here and there renew an important foundation stone, or even entirely reconstruct it. The body-cells have become adapted to a definite nutrition through many generations, and confine themselves to material which is useful to the whole organism. During infection, the blood will transport substances which are evidently closely related to normal nutrient materials.

This assumption seems all the more probable when we suggest that we can easily imagine how in the preparation of the building-stones of a cell, not only the cell in question is active, together with its neighboring cells, but there must be an intimate exchange of the products of metabolism on the part of the separate cells. Now the toxines are merely products resulting from the metabolism of cells. If these products become a part of a body-cell, then immediately the entire function of such a cell is changed. It will, as before, receive and give up substances to the fluids of the body, but the substances now given up will be of an entirely different character, for the function of a cell is a result of its own composition. We can easily understand how, if a single constituent of a cell is altered, all the chemical processes may take place in a different direction. If the cell is not badly injured it will continue to function. Naturally the subsequent absorption of material will take place in accordance with the altered conditions, and thus the peculiar nature of such cells will gradually make itself felt. Among the thousands of body-cells, it is not necessary that many of them should be attacked, perhaps only those which were in the process of undergoing a transformation. We know that in chemical processes the slightest deviation in the conditions may cause the reaction to take place differently. How much more must a continuous change in the nature of the metabolic products affect the normal course of processes which take part in the cell construction! We wish to affiliate this conception of Ehrlich concerning the formation of antitoxines more closely with processes of metabolism, and especially in order to avoid leaving the impression that the formation of these side-chains is an abnormal function of the cells. The different toxines are naturally differently constituted, and it does not seem at all strange that the cells of the various tissues should take up these toxines in different degree, and that, for instance, the cells of the nerve tissues should be peculiarly adapted to unite with tetanus poison. This toxine

shows very clearly that Ehrlich is right in his conception of the production of toxines by the cells. Wassermann and Takaki[1] carried out the following experiment. They triturated the spinal cords and brains of normal guinea pigs with a physiological salt solution. Into this emulsion they introduced a single, double, treble, and ten times deadly dose of tetanus poison and injected these mixtures subcutaneously into mice. The animals did not die. It is noteworthy that this antitoxic action of tetanus poison is confined solely to the nerve tissues. It was also shown that the brain and spinal cord emulsions likewise act antitoxically if they are injected subcutaneously into mice and then the various lethal doses afterward administered. Similarly the poisonous effect is much lessened if the emulsions in question are introduced after the toxine into the body. It is possible that we shall be able to isolate the poisonous group in tetanus poison and find out its composition. Centrifugalized emulsions of nerve tissue are perfectly inactive, which proves that we are not concerned with the substances in the surrounding fluids but with the cells themselves. The discovery that the antitoxine from the brain is destroyed by boiling, and that the protective effect of the emulsion obtained from the spinal medulla or brain is lost in the same way, is of great significance. Blumenthal[2] has at last succeeded in proving that the tetanus poison combines with the brain substance, by adding the tetanus poison, which itself can readily pass through a filter, to brain substance and then filtering. The filtrate contains no toxine. It might be thought that perhaps the solids of the nervous tissue had held it back merely mechanically. That this is not the case is shown by the fact that the mixture of tetanotoxine and nerve substance no longer has a toxic effect. Finally Blumenthal succeeded in proving that the protective action of the brain and cord grew less in proportion to the amount of toxine which had been administered to the animal in life. It is easy to explain why this is true. The brain substance cannot, of course, combine with an infinite amount of toxine. The amount taken up naturally depends upon the number of the reacting groups that are present which have an affinity to tetanotoxine. If some of these groups have already been satisfied, then naturally this tissue will be capable of removing only a fraction of the amount which it is otherwise capable of uniting with. In fact, we can determine quantitatively how much antitoxine is present in a certain amount of nerve substance by estimating the point at which it ceases to combine with more toxine.

We cannot here go into further details concerning the toxines and antitoxines, nor discuss the development of Ehrlich's side-chain theory in this direction any further. It is sufficient for us to have sketched the

[1] Wassermann and T. Takaki: Berliner klin. Wochenschrift, **1**, 5, 1898.

[2] F. Blumenthal: Deut. med. Wochschr. No. 12, p. 185 (1898).

extent of the investigation in this field and to have shown how closely related it is to physiological conceptions. We shall now turn our attention to the results of Ehrlich's hypothesis in the study of hemolysis, which is more closely related to our subject.

It is a well-known fact that the sera of many varieties of blood are able to dissolve the red corpuscles of other species of animals. This fact is the cause of the bad results which have resulted from the attempts at the transfusion of blood into human beings. For a long time little was known concerning the nature of hemolysis. Recently Belfanti and Carbone[1] have established the fact that the serum of a horse into which the red corpuscles from rabbits have been injected, has a much more poisonous effect upon rabbits than does normal horse serum. Bordet,[2] whom we have to thank for developing our conception of hemolysis, showed that the serum of guinea pigs into which there had been repeated intraperitoneal injections of from three to five cubic centimeters of defibrinated rabbit's blood, would, when placed in a test-tube, rapidly dissolve the red corpuscles of the rabbit, whereas the normal serum of the guinea pig either did not have this property at all, or showed but slight evidence of it. Here again we are dealing with quite specific effects, and there is really a formation of anti-bodies here. This discovery is of especial interest because Bordet has shown that even cells to which we are not accustomed to ascribe toxic effects have a quite similar effect to that of the bacteria. The phenomenon is not remarkable. It merely shows us that every species of animal has its own peculiarly constituted cells and thereby its own specific metabolism.

We must, first of all, consider the explanation of how the hemoglobin is removed from the red corpuscles under the action of the serum which is employed. It cannot be a question of variations in the osmotic pressure, for even a fraction of a milligram of the serum exerts this effect, and again the specific action also contradicts any such assumption. All of our knowledge points to a poisonous effect upon the red corpuscles themselves. We shall now try to apply Ehrlich's side-chain theory, as far as possible, to the phenomenon of hemolysis. Bordet succeeded in proving that a hemolytic serum loses its effect if it is heated to 55° C. The serum is then designated as inactive serum. In order to avoid any misconceptions, we had best take up a specific example. Let us assume that we have a guinea pig which has previously been treated with rabbit's blood. If the serum of this animal is allowed to fall drop by drop upon an opaque solution of red corpuscles from a rabbit, contained in isotonic

[1] S. Belfanti and P. Carbone: Giorn. della R. Accad. di med. di Torino, 1898.

[2] J. Bordet: Ann. inst. Pasteur, **12**, 688 (1898); **13**, 273 (1899); **14**, 257 (1900); **15**, 303 (1901). Cf. Von Dungern: Münchener med. Wochschr. Nos. 13 and 14, pp. 405, 449 (1899). K. Landsteiner: Zentr. Bacteriol. **25** (1899). P. Ehrlich and J. Morgenroth: Berliner klin. Wochschr. **1899, 1900, 1901.** P. Ehrlich and H. Sachs: **1902.**

salt solution, we shall find that within a short time a solution of the red blood-corpuscles takes place. The solution becomes transparent and a clear red. If exactly the same experiment is carried out with inactive serum, the red corpuscles will remain unaffected. Now if in a third test-tube the serum of a normal guinea pig is added to the suspension of blood-corpuscles, there is again no hemolysis. On the other hand, the solvent effect is obtained if the normal serum from normal guinea pigs is added to the inactivated serum. This proves that at least two substances are necessary for bringing about hemolysis. One of these is found already formed in the immune serum and also in normal serum, and the other is only yielded by immunized serum, i.e., in this case in the blood of guinea pigs which have been previously treated with the blood of rabbits. The two substances are different as regards their behavior toward heat. That which is present in normal serum is stable towards heat, while that present in the other is unstable. Quite a number of different names have been given to these substances. We shall choose for the thermo-stable substance the designation *amboceptor*, and for the thermo-unstable one the name *complement*.

Ehrlich's theory fits in at this point. He was especially interested in explaining why the different sera should act so specifically, and what the relations of amboceptor and complement are to the red blood-corpuscles and to themselves. For simplicity's sake we will designate the two components, amboceptor and complement, which produce hemolysis, by the name *hemolysin*. This must have, judging from its analogy to the toxines, a very distinct affinity to some constituent of the red corpuscles. Ehrlich and Morgenroth's experiments have proved this. They injected sheep's blood into a goat. The serum from this animal completely dissolved the sheep-blood corpuscles, although it lost this property on heating to 55 degrees. The complements were destroyed. The amboceptors must have remained unchanged, as they are thermo-stable at this temperature. The above investigators then added sheep-blood corpuscles, and centrifugalized the mixture after standing half an hour. To the resulting serum they added more sheep-blood corpuscles, and also some fresh normal serum. No hemolysis resulted. When the previous addition of sheep-blood corpuscles was omitted, and normal serum added to the inactivated serum, the solution of the red corpuscles immediately set in. It follows from these experiments that the sheep-blood corpuscles had removed one of the components of the hemolysin which was, in fact, the amboceptor. That this assumption is correct, is evident from the fact that the blood-corpuscles, centrifugalized as above, immediately went into solution on the addition of normal serum in an 0.85 per cent salt solution. We must also mention that normal goat's-blood serum does not attack the normal sheep-blood corpuscles.

One phase of hemolysis was, therefore, explained. The amboceptors present in immune serum unite with the red blood-corpuscles of that species of animals to which they are suited. No other kind of blood is able to combine with the amboceptors which react so readily with sheep's blood. For this reason alone we cannot assume that it is a case of mere absorption of the blood amboceptors by the red blood-corpuscles. The combined amboceptors cannot be removed by washing, and in fact the affinity of these for the red corpuscles may be determined. It has been found that different red corpuscles are capable of combining with very different amounts of amboceptors.

We must now attempt to explain how the complement stands in relation to the process of hemolysis. In the first place, Ehrlich and Morgenroth have proved that normal red corpuscles do not unite with the complements. The simplest explanation is that the amboceptors possess at least two differently constituted groups. One unites with the blood-cell, and the other with the complement. This effects the solution of the blood-corpuscles. The complement alone cannot act upon them. The groups which are adapted to act upon the red corpuscles are wanting. Only by the aid of the amboceptor is the complement able to react with the erythrocytes. Just what this influence is we cannot tell, but it is possible that a fermentation takes place. We may state that hemolysin has been considered to be analogous to the toxines. It is in a sense a compound toxine. The haptophor group of the toxine corresponds to the amboceptor, and the toxophor group to the complement. The comparison seems even more justifiable when we add that it has been found possible to form anti-bodies to the hemolysins. Certain poisons of the animal and vegetable kingdom are analogous to the hemolysins.[1] We may mention snake venom, garden-spider poison (*Arachnolysin*), and toad poison (*Phrynolysin*). It is also possible to obtain poisons with a hemolytic action from bacterial cultures. We may refer to *stapholysin* which is obtained from staphylococcus cultures, and *tetanolysin* from tetanus bacteria.

Here at this point we may also refer to an observation which we have already discussed in detail.[2] One of the many poisonous effects of snake venom is its hemolytic action. If, for example, we add the poison of the cobra to blood, hemolysis soon sets in. If the blood-corpuscles, however, are well washed, i.e. freed as completely as possible from every trace of serum, and then placed in 0.85 per cent sodium chloride solution, no

[1] Cf. Flexner and Noguchi: J. exper. Med. **6**, No. 3 (1902). H. Sachs: Hofmeister's Beitr. **2**, 125 (1902). F. Pröscher: *Ibid.* **1**, 575 (1901). R. Kraus and P. Clairmont: Wiener klin. Wochschr. **1900**, No. 3, and **1901**, 1016. M. Neisser and F. Wechsburg: Münchener med. Wochschr. **48**, No. 18, p. 697 (1901) and Z. Hygiene, **36**, 299 (1901).

[2] Cf. Lecture VI, p. 115, *et seq.*

hemolysis takes place on the addition of cobra poison. The addition of a little serum, or of lecithin, now suffices to cause hemolysis. It is natural to designate lecithin as the amboceptor which enables the poison of the cobra to attack the erythrocytes. It is, furthermore, interesting to find that cholesterol can prevent this action of the lecithin. We mention these interesting discoveries here because they perhaps explain why the two compounds, cholesterol and lecithin, are found in every cell.

There is no doubt that red blood-corpuscles are constantly being destroyed in our organisms. Hemolysis undoubtedly takes place. It may be brought about by changes in the osmotic pressure, but it is also possible that the normal destruction of the red corpuscles results from a process similar to that just described.

We may add that the formation of precipitins may also be explained on the basis of Ehrlich's side-chain theory, and many other discoveries find their proper place in complicated processes by means of the same theory.

We have now reached the end. We are aware that we have just placed considerable stress upon a mere hypothesis, and realize the danger that may result from such a generalization. On the other hand, we are able, in each particular case, to decide whether we are justified in comparing a given process with those which have been just described. It is clear that the final goal of our investigation in the field of immunization is never to be attained by the mere advancement of any special theory. We find ourselves temporarily in a region which is not accessible to chemical or physical investigation, for both require as a starting-point the employment of chemically pure substances. As long as we are unable to prepare any one of these complicated products in a pure state, and establish its chemical constitution, we must not expect to obtain an exact insight into all the complicated processes upon which the actions of the toxines and related substances are based.

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