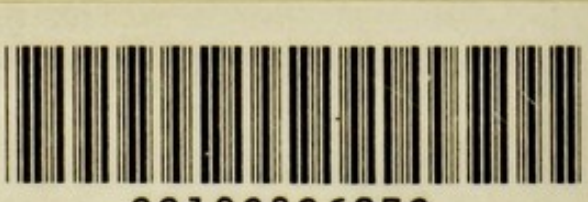
Chemistry of the proteids / by Gustav Mann ; based on Professor Otto Cohnheim's 'Chemie der eiweisskörper.'.

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CHEMISTRY OF THE PROTEIDS

CHEMISTRY

OF

THE PROTEIDS

BY

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BASED ON PROFESSOR OTTO COHNHEIM'S

'CHEMIE DER EIWEISSKÖRPER'

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PREFACE

It was in the first instance my intention to present to English readers a translation of Dr. Otto Cohnheim's *Chemie der Eiweisskörper*, the first book of its kind dealing with this abstruse subject. In the performance of my self-imposed task I found, however, that, apart from bringing the subject-matter up to date, there was room for extension, and on certain questions it seemed to me necessary to express opinions at variance with those held by the distinguished author. Under these circumstances it soon became apparent that the work, as presented now to the reader, was no longer a transcript of the German text-book, so that, whilst readily admitting that the present volume is largely based on Dr. Cohnheim's second edition, I thought it better to incur the entire responsibility for all matter therein contained, whilst publicly admitting my indebtedness to Professor Otto Cohnheim of Heidelberg. Proof-sheets of this book were submitted to Professor Cohnheim, and my action in regard to this matter has met with his entire and cordial approval.

The points on which special stress has been laid are as follows :—

1. Cellular metabolism is a cyclic event and not a question of anabolism and katabolism. That, throughout, the biological aspect of chemistry has been specially kept in mind will be seen by referring to the Index under the heading of 'Physiological considerations.'
2. Every care has been taken to give each investigator his due, for I hold Science to be above any consideration of nationality. The reactions in daily use have all been traced back to their originators, and in the chapter dealing with the synthesis of albuminous compounds the respective merits of the different workers have been duly set forth.

3. The arrangement of the primary dissociation-products is based on the conception that in the amino-acids we have a recapitulation of the same set of changes by which a paraffin becomes converted into an alcohol, then into an aldehyde, and finally into an acid. After this change has occurred at one end of the open-chain-amino-acids, it reoccurs at the other end, and so we pass from mono-carboxylic acids to di-carboxylic acids, and finally, by the central carbon-atoms also becoming 'alcohol-carbons,' we arrive at the very important poly-hydroxy-compounds, which seem to play so great a part in the metabolism of carbohydrate-radicals. After having dealt with the mono-amino-acids the di-amino-acids are discussed on the same principle. Lastly, the union of amino-acids with pyrrol- and benzene-compounds and with sulphur is entered into.
4. The nitrogen-radicals of the albumin molecule have been fully discussed.
5. The secondary dissociation-compounds have, however, not been classified, although the action of alkalies, steam, oxidising media, and sulphur, as well as the changes produced by metabolism, are fully described.
6. The synthesis of albumins, as far as known up to September 1905, is given in full.
7. The action of ferments has, on the whole, been dealt with very shortly, so as not to increase the size of the book. The view has been advanced that the chief function of both pepsin and trypsin is to facilitate the disintegrating action of the H- and OH-ions.
8. The carbohydrate-radicals are fully dealt with because of their biological importance, and, for the same reason, throughout the book special attention has been paid to sulphur.
9. In continuation of my book on *Physiological Histology*, theoretical considerations bearing on the salts of albumins have again specially interested me. There is still far too little attention paid to the salts of albumins, for people will not realise that albuminous compounds in the absence of salts are, as I put it in my first book, in the true sense of the word, dead. Only in the presence of salts will amino-acids

and their higher derivatives either pass out of the pseudo-basic–pseudo-acid state, or will they be prevented from entering into it. In other words, only in the presence of salts will amino-acids and their higher derivatives interact.

A new view has been advanced, namely, that so-called neutral salts are, as a matter of fact, not neutral, for it is their very want of neutrality which allows them to dissolve in water, and which also enables them to dissolve globulins or to keep these compounds in solution.

10. Colloids are dealt with on lines similar to those adopted in my book on *Physiological Histology*, with the inclusion of certain additional matter, such as the papers of Posternak, which had escaped my notice when writing my first book. The more recent literature has also been added. The view first advanced by me in 1902, that the action of any given metal is the function of its electro-affinity, seems to have received complete confirmation through Galeotti and Pauli, whose researches are so important that they are given very fully. The conception that colloids are electrolytes, first put forward by me in my *Physiological Histology*, has since then also been advanced in papers coming from Ostwald's and Nernst's laboratories, and is once more insisted upon in this book. In regard to the question of mechanical conglutination or coagulation, Dr. Ramsden has been good enough to give me in his own words such information as he has obtained by his recent work.

11. The study of the autodigestion of the nucleo-proteids seems destined in the near future to throw more light on the question of metabolism than almost any other study, and hence the matter has also been specially gone into.

12. In the chapter on blood I have enjoyed the great privilege of extensive help by our first authority on blood, namely, Dr. John Haldane, F.R.S., to whom I wish to express my sincere thanks, especially as he has allowed me to make known several as yet unpublished observations.

My first book I dedicated to a botanist who aroused my interest in histological research and taught me patience; and this my second book I also dedicate to a botanist, namely, to my father, who, trained at Kew, was appointed in November 1859 Government-Botanist to the Niger Expedition, and who, according to Sir Joseph Dalton Hooker, was the most successful of all botanical explorers of western tropical Africa. During his arduous and perilous expeditions on the Cameroons, Fernando Po, and St. Thomas Mountains, my father made the first contributions regarding the botanical geography of these regions in 1860-1863. Subsequently he entered the services of the Government of India in the Forest Department; becoming the first forest officer appointed in British Sikkim and Assam, he administered with unremitting care the Forest Department in the latter province for more than twenty-one years.

DEDICATED

TO

MY FATHER

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LIST OF PREVIOUS PUBLICATIONS BY THE AUTHOR

1. 1887. On the Mechanism for Fertilisation in the Flowers of Bolbophyllum Lobbii. Transact. and Proc. Bot. Soc. Edinburgh, 1886-7, pp. 104-110 (1 plate).
2. 1889. A Comparative Study of Chlorophyll as occurring in some Algæ, Vascular Cryptogams and Phanerogams. Ibid. 1889-90, pp. 394-420 (1 plate). *Awarded the Ellis Prize in Physiology, Edinburgh*, 1900.
3. 1889. Some Observations on Spirogyra. Ibid. pp. 421-431 (1 plate).
4. 1890. On a Method of Preparing Vegetable and Animal Tissues for Paraffin-Imbedding. Ibid. pp. 432-435.
5. 1891. Methods of Differential Nucleolar Staining. Ibid. 1890-91, pp. 46-48.
6. 1891. Development of the Macrosporangium in Myosurus minimus, Linn. Ibid. p. 67.
7. 1891. Criticism of the Views with regard to the Embryosac of Angiosperms. Ibid. 136-148.
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   (*b*) The Origin of Sex. Ibid. p. 756.
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17. 1896. Relative Development of functionally similar areas in the Cerebrum of Different Animals. Journ. of the Oxford University Junior Scientific Club, vol. ii. May 1896.
18. 1896. Note on Structure of Nerve Cells as shown by Wax Models. Report Brit. Ass. for Adv. of Science, 1896, p. 981.
19. 1898. Die Fibrilläre Struktur der Nervenzellen. Verh. d. Anat. Gesellsch. 1898, pp. 39-40.
20. 1899. Report on the Comparative Histology of the Cerebral Cortex (Retina). Report Brit. Ass. for Adv. of Science, 1899, p. 603.
21. 1900. The Histology of Vaccination. Part II. Local Government Board Report, 1900, pp. 508-532 (13 plates).
22. 1902. Physiological Histology (Methods and Theory). Book, pp. 488. Clarendon Press, 1902.
23. 1904. The Thalamus. Opening Remarks in the Discussion on this subject before the Conjoined Sections of Anatomy and Physiology, at the Oxford Meeting of the British Medical Association. Journ. of the Brit. Med. Assoc. February 11, 1905.

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1. What part do Salts play in our Economy?
2. The Evolution of the Brain from Monkey to Man.

These two papers are being published in the Journal of the Oxford University Junior Scientific Club.

3. The Chemistry and Structure of Protoplasm. *Opening Address for the International Medical Congress at Lisbon*, 1906.

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1. Thesis for the B.Sc. Degree of Oxford: The Structure of Nerve Cells as revealed by the Modelling Method.
2. The Thalamic Nuclei of the Frog, constructed from Serial Sections. *Awarded the Rolleston Prize, Oxford* (1900).

Papers under the Author's Direction

Lily H. Huie:

1. On some Protein Crystalloids and their probable relation to the nutrition of the pollen-tube. La Cellule, **11.** 83 (1895).
2. Changes in the Cell-organs of Drosera rotundifolia, produced by feeding with egg-albumin. Quart. Journ. of Micr. Sci. **39.** 387 (1896-97).
3. Further Study of Cytological Changes produced in Drosera. (Feeding with paraffin, white of egg, amphopeptone, albumoses, ammonium sulphate, fibrin, fibrin-peptone, globulin, casein, nuclein, nucleic acid, milk, calcium phosphate, gelatine, leucin, creatin, urea.) *Ibid.* **42.** 203 (1899).

ERRATA

Page 270, line 11, *insert* acid *after* orthophosphoric.
,, 284, ,, 17 from foot, *for* abumins *read* albumins.
,, 301, ,, 14 ,, *for* radium *read* sodium.

GENERAL PART

INTRODUCTION

WHAT position do proteids and do we, who are built up of proteids, occupy in nature? How are we related to other chemical compounds?

Biologists of the present day may be divided into those who believe all animal and vegetable existence to be endowed with some special unexplainable force, which is the real essence of life, which causes all those phenomena, characteristic of individuals who feed, propagate, and die. This class of observers holds that man will never fathom the vital principle.

On the other hand we find the physico-chemical school who endeavour to expound organic life by only those laws which hold good for the lower inorganic compounds. This school forgets that because of the very evolution of so-called inorganic compounds into organic ones, which, for example, we see daily take place around us in connection with the growth of plants, we must have in addition to the old simple laws which govern the inorganic world, additional laws which regulate organic existence.[1]

The view the author holds, he trusts, will bridge over the gulf existing between the two schools mentioned above, namely, those of the vitalists and non-vitalists.

We have to distinguish between the origin of organic compounds and that of life. To be able to make marsh-gas, alcohols, aldehydes, acids, amino-acids, peptids, peptones, and albumin, however great an achievement in itself, is not the same as making life. To many people a living cell consists of 'protoplasm,' a substance they imagine to be one exceedingly complex body. They do not realise that in a cell we have a not very large number of comparatively simple compounds which only collectively form the protoplasm. What constitutes life, is the presence of a number of such 'organic' compounds, capable of mutually reacting upon one another, and hereby giving rise to new

[1] Such new laws, for example, as are created by the asymmetric carbon atom, by stereoisomers, etc.

compounds, which cannot react chemically with the mother-substances from which they are derived, but which by interacting with new radicals give rise to a cycle of events. In the following diagram the author has endeavoured to make his meaning clear. From the nucleus two arrows pass outwards: the one on the right represents the formation of 'extra-cellular' zymogen granules, their disruptive enzymes having the function of acting on extraneous chemical compounds in such a way as to make them available to the cell individual. These enzymes change, for example, albumin into albumoses, peptones and amino-acids. The arrow on the left of the figure represents 'intra-cellular' zymogens, the function of which is a constructive one; they bring about an aggregation of those amino-acids and peptone-like bodies which have been liberated from proteid food by the extra-cellular enzymes. The aggregates so formed constitute the main bulk of the cell-plasm, and they are subsequently transformed by the activity of the nucleus[1] into the extra-cellular and intra-cellular zymogens already alluded to. The cycle of events just described is what we call life. Cessation of life, or death, will be produced either by the inability to procure food, which is necessary to counterbalance the wear and tear necessitated by the conversion of one chemical compound into another—this amounts to death by starvation, or secondly, by the inability of the nucleus to digest the food and so make it available to the individual cell, as seen in old age. In addition to these two kinds of physiological death, we have another form due to violence, as, for example, by the application of excessive heat or cold or inorganic (corrosive sublimate, etc.) or organic (bacteria) poisons.

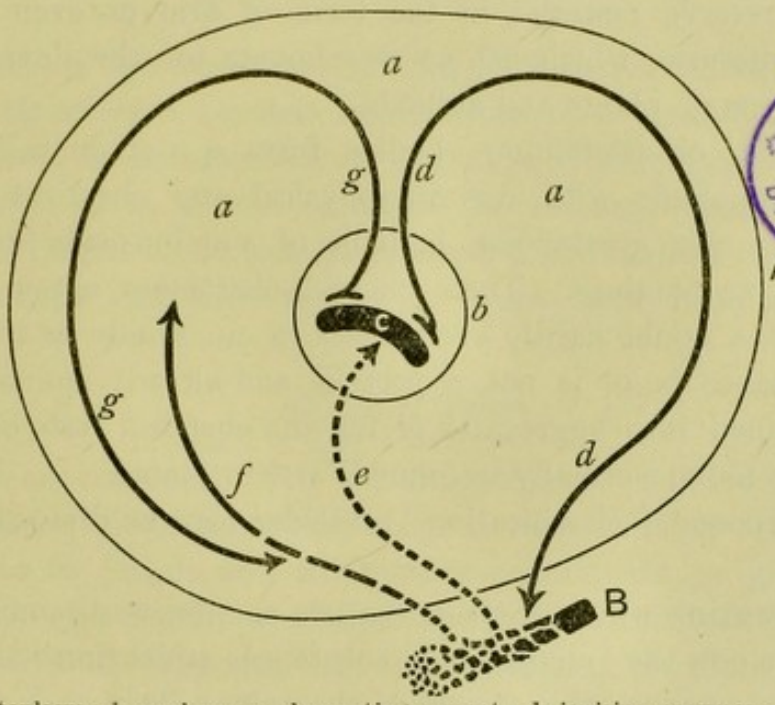
Those who take an interest in the actual phenomena exhibited by cells fed on proteids and derivatives of proteids, and who wish to see the histological appearances which cells assume during the various stages in a 'life-cycle' will find a detailed account in the papers of Lily Huie.[2] This work, done under the author's care, is now being continued by the author, for there is no other way of gaining an insight into the working of the different organs of a cell. It is only by histological research based on a sound knowledge of chemistry and physics that we will be able to understand and to modify the events in the life-cycle, that we will be able to accelerate and to slow down nuclear and cytoplasmic activities. The importance of such research in connection with cancer and all fevers cannot be overestimated.

[1] Gustav Mann: 'What is Life?' *Trans. of the Oxford University Junior Scientif. Club.* Feb. 1899. Read Nov. 1898.

[2] Lily Huie, *Quarterly Journ. of Micr. Science*, **39**. 387 (1896-97), and *ibid.* **42**. 203 (1899).

What must be the ultimate aim of chemical biology is to establish the sequence of events in the cycle from simple to more complex substances and the disintegration of the latter for the purposes of liberating energy and of so acting on other chemical compounds as to make these available to each individual cell.

An arrangement of compounds on such a physiological basis is as yet beyond us, for although we know a certain amount about the normal disintegration of albumins into amino-acids, we know nothing as to the physiological processes by which amino-acids are built up into albumins. That the nuclear compounds are the active agents in



A. Cell: *a*, cytoplasm; *b*, nucleus; *c*, chromatin segment; *d*, ionising enzymes; *e*, food entering nucleus; *f*, food being stored by de-ionising enzymes.
B. Food which is being digested or ionised.

this building up of higher compounds the author knows from his histological research, and he is at present engaged in adducing chemical proof, apart from experiments on plants and animals.

In the following chapters a purely chemical classification has been adopted by the author; the individual substances being arranged in such a way as to lead from the lower members of a series to the higher ones, from the less to the more highly oxidised forms, and from open chain- to ring-compounds.

Due stress has also been laid in the eighth chapter on the importance of salts in converting dead pseudo-amino-compounds into living ones.

Proteids are met with in nature in one of the following states:—

1. In the form of solution in the fluids of animals or plants, as, for example, in blood, lymph, and cell sap.

2. As so-called 'protoplasm' in the cells and tissues of plants and animals. This plasm is a mixture of albuminous substances and other organic and inorganic compounds; it possesses a definite structural arrangement and occupies a middle position between the solid and the fluid states. The animal supporting-structures built up of the so-called albuminoids are in a somewhat firmer state than is the ordinary cell-plasm. It is possible to extract these albuminous substances from the organs in which they occur either by the simple process of dissolving them out or by employing stronger measures.
3. As reserve material in the form of firm or even crystalline structures, which act as storehouses for the developing embryos of plants and animals.

'Proteids' or albuminous bodies form a well-defined group of organic compounds with definite physical and chemical properties. They are for the greater part built up of α-amino-acids linked to one another as acid-amines. Their general characters agree to such an extent that a doubt hardly ever arises in our minds as to whether a given substance is, or is not, a proteid, and already amino-acids have been combined into aggregates giving the chemical tests of such proteids as are found normally in animals and in plants.

For purposes of classification 'proteids' may be divided into three groups:

1. **Albumins** which occur in nature as 'native albumins.' They include the 'albuminoid' substances which form the supporting or connective tissues of the animal body.
2. **Proteids proper,** which are combinations of the native albumins with such other organic compounds as sugars or radicals containing phosphorus or iron.
3. **Derivatives** of the natural albumins and proteids, which retain in their chemical configuration the characteristics of albuminous substances, and are represented by the albumoses, peptones, peptids, and other compounds. These bodies are met with in nature as products of digestion and metabolism, but they may also be obtained artificially by hydrolysis of the more complex albuminous substances.

It is customary in England to use the term 'proteid' for all albuminous substances, while in France the term 'substances albuminoides' is used similarly. Of late there has been a tendency in Germany to use the English phraseology and to speak of 'Proteinsubstanzen.' The author has thought it best to follow Cohnheim in restricting the terms—albumin and proteid.

CHAPTER I

Reactions of Albuminous Substances

ALL albuminous substances are built up on the same chemical principle, and have therefore a number of reactions in common. Not one of these reactions is characteristic of albumins, if taken by itself; but if any substance gives several or all of the reactions described below, then we are permitted to class it amongst the albumins.[1]

The author has endeavoured to trace each test back to its originator.

I. COLOUR TESTS

No colour reactions, with the exception of the biuret-test, are characteristic of albuminous substances as such, for they all depend on the presence of certain non-albuminous compounds, or groupings of atoms occurring normally in albumins in such a form as to allow of interaction with the reagents. All tests, excepting the biuret-test, prove simply the presence or absence of certain radicals, thereby allowing us to differentiate between various kinds of albumins.

1. *The Biuret-Reaction of Rose and Wiedemann*

Add to a watery solution of an albumin a sufficiently large amount of soda or potash solution, and then add a few drops of a dilute solution of copper sulphate, when with the native albumins a blue or violet colour, and with dissociation-products such as albumoses or peptones, and also with certain vitellines and histones, a pure red colour is obtained. In performing the test an excess of copper sulphate must be avoided, as it is apt to obscure the reaction because of its own blue colour; and the solution should not be heated, because many peptones are decomposed by heat.

[1] The older literature up to 1893 is fully dealt with in J. W. Pickering's paper, *Journal of Physiology*, **14**. 347 (1893), while the more recent literature is given more fully than here in Mann's *Physiological Histology* (Clarendon Press, 1902).

Rose[1] in 1833 was the first to observe this reaction with albumin. It was discovered quite independently and investigated more fully by Piotrowski[2] in 1857. Wiedemann[3] had previously, in 1849, found biuret to give a rose-red reaction, and since his time this reaction has been known as 'the biuret-reaction.' Gnezda[4] in 1889 showed albuminous substances to give yellow and orange reactions if nickel was substituted for copper; and Pickering[5] in 1893 found cobalt to be an even more delicate test than copper, while no definite reactions were obtained with the salts of iron, manganese, and zinc. The first explanation of Rose's reaction we owe to Schiff, as is more fully explained on p. 141.

The biuret-reaction is of special interest because it differs from all other reactions in not being obtainable with any but the albuminous dissociation-products of 'proteids.' It is therefore generally used if we wish to distinguish between albumins and their simpler or secondary decomposition-products. A sharp line of demarcation between these compounds cannot, however, always be drawn. (See pp. 118, 144, 149.)

Stokvis[6] and Salkowski[7] draw attention to the fact that urobilin produces with sodium hydrate and copper sulphate a colour which is undistinguishable from that of the biuret-reaction.

2. *The Xanthoproteic-Reaction of Fourcroy and Vauquelin*

On adding a strong solution of nitric acid to a watery solution of an albumin or to a solid albumin, as contained, for example, in a piece of bread, there is produced either in the cold, as in the case of bread, but usually only after heating, a deep yellow coloration, which on the addition of soda solution becomes reddish brown, while with ammonia it turns a vivid orange colour.

This reaction was first noticed by Fourcroy and Vauquelin,[8] who called the product 'the yellow acid'; they also noted its very bitter taste. Fürth[9] has shown the reaction to depend on the formation of

[1] F. Rose, *Poggendorff's Ann.* **28**. 132 (1833).

[2] G. v. Piotrowski, *Sitzb. Akad. d. Wiss. Wien, math.-naturw. Classe*, **24**. 335 (1857).

[3] G. Wiedemann, *Poggendorff's Ann.* **74**. 67 (1849).

[4] J. Gnezda, *Proc. Roy. Soc.* **47**. 208 (1889).

[5] J. W. Pickering, *Journ. of Physiol.* **14**. 347 (1893).

[6] H. B. J. Stokvis, *Zeitschr. f. Biol.* **34**. 466 (1896).

[7] E. Salkowski, *Berl. klin. Wochensch.* 1897, No. 17.

[8] Fourcroy and Vauquelin, *Ann. Chim.* **56**. p. 37 [30 vendémiaire an XIV. *i.e.* 1805]. (See also Berzelius, *Medico-Chirurg. Trans.* **3**. 205 (1812).)

[9] O. v. Fürth, *Einwirkung von Salpetersäure auf Eiweissstoffe*, Habilitationsschrift (Strassburg, 1899).

nitro-derivatives, and Salkowski[1] that the reaction indicates the presence of aromatic radicals in the substance under investigation. Rohde[2] points out that a very intense reaction is obtained with tryptophane. In addition to albumins this reaction is also obtained with many other substances, such as the humins. (See p. 89.)

3. *The Reaction of Millon*

On boiling mercury with strong nitric acid until a portion of the liquid no longer gives a precipitate with common salt; adding strong nitric acid to the concentrated solution of mercuric nitrate, $Hg(NO_3)_2$; separating off the crystalline magma consisting of $2\,Hg(NO_3)_2 + H_2O$, there remains a thick mother liquor having the constant composition $Hg(NO_3)_2 + 2H_2O$. This solution possesses the power, first noticed by Libavius, of colouring the skin a dark-red tint. Mercurous nitrate, $Hg(NO_3)$, is formed by the action of dilute nitric acid, in the cold, on mercury. It is readily soluble in dilute nitric acid, and this solution brought on to the skin colours it, first purple and then of a black tint.

Millon's reagent is a solution of mercurous nitrate in nitric acid.[3] If it be added either to a watery solution of an albumin or to a suspension of solid albumin in water, or be poured, for example, over a piece of bread, there is obtained either in the cold (as with bread), or after boiling, a pink coloration of the fluid or a pink to blackish-red coloration of the precipitated albumin. The reaction is given by all benzene derivatives in which one hydrogen atom has been replaced by the hydroxyl group OH;[4] and as tyrosin is the only oxyphenyl compound in the proteid,[5] the reaction shows the presence of tyrosin. The latter is contained in all albumins with the exception of gelatine and certain albumoses and peptones. Inorganic salts in higher concentrations prevent the reaction. See also under tyrosin, p. 50. This reaction has been very fully investigated by Vaubel[6] and by Nasse,[7] whose papers are abstracted in the author's *Physiological Histology*, pp. 321-323.

[1] E. Salkowski, *Zeitschr. f. physiol. Chem.* **12**. 215 (1887).

[2] E. Rohde, *ibid.* **44**. 161 (1905).

[3] Millon, *Compt. Rend.* **28**. 40 (1849).

[4] A much fuller account, including the exact researches of Vaubel and Nasse, is given in Mann's *Physiological Histology*, 1902, pp. 321-323 (Clarendon Press).

[5] E. Salkowski, *Zeitschr. f. physiol. Chem.* **12**. 215 (1887); O. Nasse, *Pflüger's Arch. f. d. ges. Physiol.* **83**. 361 (1901).

[6] Vaubel, *Zeit. f. angew. Chem.* (1900), p. 1125.

[7] Nasse, *Pflüger's Arch.* **83**. 361 (1901).

4. *The Lead Sulphide Reaction of Vogel*

If albumins are boiled with a lead salt and soda solution there is formed either a black precipitate or at least a brown or blackish discoloration of the fluid. The reaction depends on the splitting off of the sulpho-hydryl group SH, derived from the cystein radical (Hofmeister), and the subsequent formation of lead sulphide. Instead of a lead salt, the salt of any other metal may be employed, the sulphide of which is black or darkly coloured. All albumins except protamin, peptones, and perhaps histone, contain sulphur, and therefore give this reaction. Vogel[1] first noticed that decomposing serum exhales a gas which has the property of blackening lead acetate.

5. *The Reaction of Molisch*

By the addition of a few drops of an alcoholic solution of α-naphthol to an albumin solution, followed by the addition of some strong sulphuric acid, there is produced a violet colour, which on the addition of alcohol, ether, or potash solution turns yellow. If thymol be taken instead of α-naphthol, a carmine-red colour results, which by the addition of water is turned green.

Molisch[2] considered this reaction to be characteristic of carbohydrates, but Seegen[3] showed later that albumins also give it. The reaction depends on the strong sulphuric acid converting carbohydrate radicals, wherever met with, into furfurol, which latter gives the colour reactions with α-naphthol or thymol. This reaction is therefore identical with Pettenkofer's bile acid reaction and belongs to the so-called furfurol reactions,[4] and is a sure index as to whether carbohydrate groups are present or absent in any given proteid.

6. *The Reaction of Adamkiewicz, Hopkins and Cole*

Adamkiewicz[5] described the following reaction: On dissolving dry, defatted albumin in glacial acetic acid and subsequently adding concentrated sulphuric acid, there are formed at the junction of the two fluids red, green, and violet rings. On shaking, the whole solution becomes coloured. On spectroscopic examination a broad band is seen stretching from the blue to the yellow. The explanation of

[1] Vogel, *Ann. Chim.* **87**. 215 (1813).

[2] H. Molisch, *Monatshefte f. Chem.* **7**. 198 (1888).

[3] J. Seegen, *Zentralbl. f. d. medizin. Wiss.* 1886, pp. 785 and 801.

[4] F. Mylius, *Zeitschr. f. physiol. Chem.* **11**. 492 (1887); L. v. Udránszky, *ibid.* **12** 389 (1888).

[5] A. Adamkiewicz, *Pflüger's Arch. f. d. ges. Physiol.* **9**. 156 (1874), and *Ber. d. deutsch. chem. Ges.* **8**. I. 161 (1875).

this reaction we owe to Hopkins and Cole,[1] who showed that the reaction is not due to acetic acid, but to glyoxylic acid, COOH—CHO, which is usually present in acetic acid. To get the best results they proceed thus: Glyoxylic acid is prepared by throwing some sodium amalgam into a strong solution of oxalic acid, and then filtering after the formation of gas has ceased. On adding some of this glyoxylic acid to an albumin solution, shaking up, and then adding strong sulphuric acid, a beautiful bluish-violet colour is seen. The reaction depends, as Hopkins and Cole[2] have also shown, on tryptophane or indol-amino-propionic acid. Tryptophane possesses two other colour reactions, which are, however, only given when it is in the free state, and not as long as it forms part of the albumin molecule. These two reactions are, firstly, a violet colour with chlorine water or bromine water in a solution of acetic acid, and secondly, the pyrrol reaction. (See p. 54.) [That tryptophane also gives the xanthoproteic reaction has been pointed out above.]

7. *The Reaction of Liebermann and Cole*

Liebermann[3] found albumins which had been purified and defatted by four alternate changes of alcohol and ether and then dried, to exhibit a deep blue or bluish-violet colour on being boiled with fuming hydrochloric acid.

This reaction Hofmeister[4] considered to be a furfurol reaction in which both the carbohydrate radical of the albumin molecule—changed into furfurol by the action of the acid—and the aromatic oxyphenyl radical took part. Cole,[5] however, has shown that Liebermann's reaction is due "to an interaction between the glyoxylic acid which is present in the ether used for washing the albumin, and the tryptophane which is split off from the albumin by the action of concentrated hydrochloric acid."

8. *The Diazo-Reaction of Ehrlich and Pauly*

Ehrlich[6] showed in 1882 that urine gives a very distinct red colour during certain pathological changes, for example during typhoid. If one litre of urine is mixed with 50 ccm. of hydrochloric acid and 1 grm. of sulphanilic acid, and if then to 250 ccm. of this mixture 5 ccm. of a half per cent solution of sodium nitrite are added, there is formed,

[1] F. G. Hopkins and S. W. Cole, *Proc. of the Royal Soc.* **68**. 21 (1901).

[2] F. G. Hopkins and S. W. Cole, *Journ. of Physiol.* **27**. 418 (1901).

[3] L. Liebermann, *Zentralbl. f. d. medizin. Wiss.* 1887, p. 371.

[4] F. Hofmeister, *Leitfaden f. d. praktisch-chemischen Unterricht d. Mediz.* p. 80. Braunschweig, 1899.

[5] Sydney W. Cole, *Journal of Physiology*, **30**. 311 (1904).

[6] P. Ehrlich, *Zeitschr. f. klinische Med.* **5**. 285 (1882).

hereby, the substance diazobenzene-sulphanilic acid, which with the unknown substance in the urine produces the red colour.

Pauly [1] prepares the diazobenzene-sulphanilic acid in the following way: 2 grams finely pulverised sulphanilic acid are shaken up with 3 ccm. water and 2 ccm. concentrated hydrochloric acid, and this mixture is added in small quantities, within one minute, to a solution of 1 gram of newly prepared potassium nitrite in 1 to 2 ccm. water; after each addition the mixture is cooled. The sulphanilic acid passes rapidly into solution and is soon replaced by a dense, white, crystalline deposit of diazobenzene-sulphanilic acid; after a few minutes the fluid part is sucked off and the crystals are then washed with a little water.

In testing for histidin, ascertain the absence of tyrosin by means of Millon's reagent; add sodium carbonate solution to the fluid under examination till the reaction is alkaline, and then add 3 to 5 ccm. of a freshly-prepared solution of a few centigrams of diazobenzene-sulphanilic acid in soda solution. The red colour of histidin appears usually at once, and at the latest after three minutes. On diluting with distilled water the red colour does not disappear (histidin) or becomes somewhat more yellow (tyrosin). All other albuminous derivatives, such as glycocoll, alanin, leucin, α-amino-valerianic acid, serin, lysin, ornithin, asparagin, glutaminic acid, cystin, and hippuric acid give in sodium carbonate solutions lemon yellow to deep yellow colours, which disappear on dilution and on acidification. α-Pyrrolidin-carboxylic acid and tryptophane give negative results.

Histidin is recognisable in dilutions of 1 : 100,000 (pale pink) to 1 : 20,000, in which case the colour is already a deep cherry-red in thicker solutions.—The diazo-reaction is also a more delicate test for tyrosin than is Millon's reaction.

9. *The Glucosamin-Test of Ehrlich*

Ehrlich [2] made in 1901 the discovery that urine turns a more or less pronounced carmine-red colour by the addition of a few drops of the pale yellow *p*-dimethyl-amino-benzaldehyde dissolved in normal hydrochloric acid. His pupil Pröscher [3] obtained a substance in sufficient quantity to determine its formula as $C_{16}H_{24}O_6N_2$, from which Ehrlich calculated that the substance was either formyl-

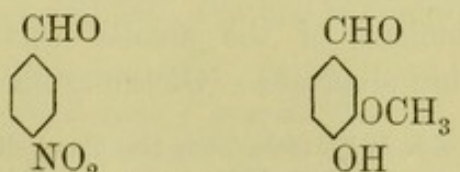
[1] Herm. Pauly, *Zeitschr. f. physiol. Chem.* **42**. 508 (1904). In a second paper, *ibid.* **44**. 159 (1905), it is pointed out that the diazo-reaction does not allow us to distinguish between imido-azols and the pyrimidin-nucleus, because the 4-methyl uracil, which is an oxygen-containing pyrimidin, also gives the diazo-reaction.

[2] Paul Ehrlich, *Die medic. Wochenschr.*, April 1901, No. 15.

[3] Pröscher, *Zeitschr. f. physiol. Chem.* **31**. 520 (1900-1), and *Deutsche med. Wochensch.* 1903, p. 927.

glucosamin, or an acetyl derivative of the still unknown pentosamin. Friedrich Müller confirms Ehrlich's statement that pure mucin and mucoid substances do not give the red reaction, if their watery solutions are treated directly with the reagent, or even after long treatment with boiling mineral acids. Positive results are, however, always obtained if the mucinoid substances are first rendered alkaline with a little alkali or baryta, and if they are then warmed. On adding to these alkaline solutions 2 to 5 per cent solutions of *p*-dimethyl-amino-benzaldehyde dissolved in normal hydrochloric acid till the reaction has become acid, a red colour is obtained, especially on heating. Ehrlich showed, microscopically, that in a section of cartilage the perichondrium stains an intense reddish violet, while the hyaline cartilage and the surrounding connective tissue or fat remain colourless, with the exception of a few peri-vascular strands containing elastic fibres, and Mann[1] demonstrated the distribution of the glucosamin histologically by fixing tissues in 0·5 per cent KOH in 90 per cent methyl-alcohol for 24 to 48 hours, or longer at 30 to 40°, and then transferring the tissues to a 2·5 per cent solution of *p*-dimethyl-amino benzaldehyde in 1 per cent HCl.

O. Neubauer believes Ehrlich's benzaldehyde reaction to depend on 'urobilinogen,' but also to be obtainable with albuminous substances in the presence of stronger acids.[2] His pupil Rohde[3] confirms the observation of Ehrlich that the benzaldehyde is linked up to proteids by its aldehydic radical, for in addition to the observation of Ehrlich, that formaldehyde, when added to urine, prevents the formation of the red colour, Rohde found the addition of form- and acet-aldehyde to casein to prevent the latter from giving colour-reactions with 'Ehrlich's benzaldehyde.' Rhode also showed that the aldehydes of the aliphatic series (form-, acet-, propyl-, butyl-aldehyde) do not give colour reactions; citral and furfurol give yellow colours, while all aromatic aldehydes show either a red colour (para-dimethyl-amino benzaldehyde, vanillin, salicylic, and cinnamic aldehydes, hadromal) or a green colour (para-nitrobenzaldehyde, amino-benzaldehyde), or a blue colour (gentisinaldehyde). The only brilliant colours are obtained with



p-nitro-benzaldehyde vanillin p-dimethyl-amino-benzaldehyde.

[1] Gustav Mann, *Physiological Histology*, 1902, p. 299.

[2] Neubauer, *Sitzber. d. Gesell. f. Morphol. u. Physiol. in München*, 1903, p. 32.

[3] Erwin Rohde, *Zeit. f. physiol. Chem.* **44**. 161 (1905).

The last of these, recommended by Ehrlich, is more delicate than the other two, for it will allow tryptophane to be recognised in dilutions of 0.003 per cent.

According to Rhode tryptophane is the only radical present in albumins which gives colour-reactions with the aromatic aldehydes.

To obtain colour-reactions with albuminous substances proceed thus :—Add to the albuminous solution 10 drops of a 5 per cent solution of p-dimethyl-amino-benzaldehyde in 10 per cent sulphuric acid ; then add strong sulphuric acid, shaking constantly, till the colour is obtained. Vanillin is used similarly in a 5 per cent alcoholic solution, while p-nitro-benzaldehyde is added in the solid form as it is insoluble in acids, alkalies, and alcohols.

When given to rabbits in the food, p-dimethyl-amino-benzaldehyde appears in the urine[1] as a paired glycuronic acid, having probably the formula—

$$(CH_3)_2N—C_6H_4—CO—CH—(CHOH)_2—CH—CHOH—COOH,$$
$$\qquad\qquad\qquad\qquad\qquad\quad |\!\!\_\_\_\_O\_\_\_\_|\!\!|$$

and also as p-monomethyl-amino-benzoic acid and as free p-dimethyl-amino-benzoic acid.

II. Precipitation Tests

Generally speaking, albumins are only soluble in water, and therefore are precipitated by most other fluids. Amongst the latter the most important is alcohol. In absolute alcohol all albumins are insoluble, but the percentage strength of alcohol required for precipitating different albumins varies greatly with the individual albuminous substances and may serve to distinguish between albumins (Hofmeister,[2] Mann,[3] Tebb[4]). That an albumin such as egg-white may show different states of precipitation according to the strength of ethyl and methyl alcohols and of acetone has been fully described by Mann.[3]

The chlorides and the sodium salts, especially of the denaturalised albumins, are much more soluble in alcohol than are the albumins themselves. Urea and the alcohol soluble salts behave, according to Spiro,[5] as bases, for they increase the solubility in alcohol. Spiro found, further, that the higher members of the alcohol series have an increasing power of precipitating albumins. Of the aromatic alcohols,

[1] M. Jaffé, *Zeit. f. physiol. Chem.* **43.** 374 (1905). See also about dimethyl-amino-antipyrin in *Ber. d. deutsch. Chem. Ges.* **34.** 2737 (1901).

[2] F. Hofmeister. See p. 179 and table on p. 180.

[3] Mann, *Physiological Histology*, 1902, pp. 103-104.

[4] M. Christine Tebb, *Journal of Physiology*, **30.** 25 (1904).

[5] K. Spiro, *Hofmeister's Beitr.* IV. 300 (1903).

phenol is the best precipitant, and the higher a member in this series, the less does it precipitate, and stronger solutions of all aromatic alcohols redissolve the originally formed precipitate (Spiro [1]). Acetone and chloroform precipitate as does alcohol.[2]

The processes of salting out and of heat precipitation will be discussed in Chapter VII.

With a number of acids and bases albuminous bodies form quite or almost insoluble bodies, and are therefore precipitated from their watery solutions. This reaction is characteristic of all natural albuminous substances, the complex albumins, and, for the most part, all derivatives which still resemble albumins, such as the albumoses. The further a derivative of an albumin is removed from its natural state, the more difficult does it become to precipitate it, as will be more fully described in Chapter IV.

1. *Precipitation of Albuminous Substances by Salts of the Heavy Metals*

Albumins, being potential acids because of their amino-acid nature, are precipitated by the salts of the heavy metals as insoluble metallic albuminates from acid, neutral, or alkaline solutions. The precipitation is always a complete one, and the precipitate is as a rule insoluble in an excess of the reagent when we are dealing with the albumins proper, while some of the proteid derivatives, such as albumoses, may redissolve. The myogen of muscle is an exception, as it is not precipitated by the heavy metals in the absence of alkali salts (Fürth [3]). The same holds good for hæmoglobin,[4] and may hold good for other albuminous substances (Cohnheim).

Nearly all the heavy metals precipitate, but those commonly employed are :

1. Ferric chloride and ferric acetate; they were used by P. Müller,[5] Schmidt-Mülheim,[6] Siegfried,[7] and others. With excess of ferric chloride the precipitates are apt to redissolve. Rose [8] was the first to describe a jelly-like compound formed by the union of albumin with ferric chloride.
2. Copper sulphate and the still more sensitive copper acetate.

[1] K. Spiro, *Hofmeister's Beitr.* IV. 300 (1903).
[2] E. Salkowski, *Zeitschr. f. physiol. Chem.* **31**. 329 (1901).
[3] O. v. Fürth, *Archiv f. experiment. Patholog. und Pharmak.* **36**. 231 (1895).
[4] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **29**. 86 (1899).
[5] P. Müller, *ibid.* **26**. 48 (1898).
[6] Schmidt-Mülheim, *Arch. f.* (*Anat. u.*) *Physiol.* 1880, p. 33.
[7] M. Siegfried, *Zeitschr. f. physiol. Chem.* **21**. 360 (1895), and *Arch. f.* (*Anat. u.*) *Physiol.* 1894, p. 401.
[8] Ferdinand Rose, *Poggendorff's Ann.* **28**. 140 (1833).

3. Mercuric chloride. It precipitates, according to Kühne,[1] Neumeister,[2] and Siegfried,[3] even the peptones.
4. Lead acetate, basic and neutral; it has been recommended by Hofmeister[4] as a very perfect precipitant.
5. Zinc acetate, introduced by Abeles.[5]
6. Uranyl acetate was employed by Jacoby[6] and Glässner[7] for purifying ferments.
7. Salts of platinum, cobalt, and many other heavy metals were investigated by Chittenden and Whitehouse.[8]

In addition to the heavy metals, albumins are also precipitated by a number of organic colour-bases. Mathews[9] and Heidenhain[10] obtained marked precipitation with malachite green, brilliant green, new fuchsin, auramin, phenosafranin, and rosaniline acetate; more feeble precipitation with Nile blue, vesuvin, thionin blue, toluidin blue, methyl green, methyl violet, chrysoidin, neutral red, and neutral violet. For a fuller account see p. 225, and especially Mann's *Physiological Histology*, 1902, pp. 452-459 (Clarendon Press).

A behaviour analogous to that of the colour-bases is shown by some basic albuminous bodies, namely, the histones and the protamins, which precipitate other albumins from alkaline solutions.

2. *Precipitation by means of Acids*

The Alkaloidal Reagents

Being amino-acids, albumins are potential bases. They are precipitated by a series of complex organic acids, the so-called 'alkaloidal reagents' (Mylius[11]). As albumins are very feeble bases, the salts formed by their union with the alkaloidal reagents undergo a hydrolytic dissociation when they are dissolved in water. Thus phosphotungstate of albumin is dissociated secondarily by the ions of the water, and can therefore be rendered permanent only in the presence of an excess of phosphotungstic acid. As a rule the precipitate is dissolved as soon

[1] W. Kühne, *Zeitschr. f. Biolog.* **22**. 423 (1885).
[2] R. Neumeister, *ibid.* **26**. 234 (1890).
[3] M. Siegfried, *Zeitschr. f. physiol. Chem.* **35**. 164 (1902).
[4] F. Hofmeister, *ibid.* **2**. 288 (1878).
[5] M. Abeles, *ibid* **15**. 495 (1891).
[6] M. Jacoby, *ibid.* **30**. 135 (1900).
[7] K. Glässner, *Hofmeister's Beitr.* I. 1 (1901).
[8] R. H. Chittenden and H. H. Whitehouse, *Maly's Jahresber. f. Tierchemie*, **17**. 11 (1887).
[9] Albert Mathews, *Amer. Journ. of Physiol.* p. 445, July, 1878.
[10] M. Heidenhain, *Pflüger's Arch. f. d. ges. Physiol.* **90**. 115 (1902).
[11] F. Mylius, *Ber. d. deutsch. chem. Ges.* **36**. I. 775 (1903).

as the reaction becomes alkaline, but the more strongly basic albumins—the histones, and especially the protamins—are precipitated even when the reaction is slightly alkaline, or at least neutral. With an excess of the reagent, the albumins proper remain precipitated, but peptones and some of the albumoses pass again into solution. (See Chapter V.)

The most important alkaloidal reagents are:

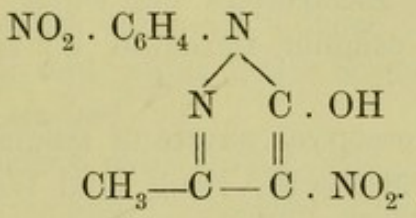
Phosphotungstic acid, Phosphomolybdic acid, Tannic acid } These three precipitate peptones and are used frequently.

Ferrocyanic acid, usually employed in the form of acetic acid + potassium ferrocyanide. It is used clinically as a test for albumin.

Trichloracetic acid.

Picric acid.

Picrolonic acid is 1 p-nitrophenyl–3 methyl–4 nitro–5 pyrazolon, $C_{10}H_8N_4O_5$ or



It was first recommended by Knorr [1] for the isolation of hexone-bases, in particular for arginin and histidin, as the lysin compound is soluble. To free the bases from picrolonic acid, H_2SO_4 is added to the hot watery solution of the bases, when on cooling the picrolonic acid separates out. The last traces of it are then removed with ether.

Iodine-hydriodic acid; iodide of mercury, iodide of bismuth, iodide of cadmium in hydriodic acid, employed usually in the form of iodine-potassium iodide, iodide of mercury-potassium iodide, etc. + hydrochloric acid. Iodide of mercury + iodide of potassium in hydrochloric acid is known as Brücke's reagent.

Platinum chloride, metaphosphoric acid, tungstic acid, and allotelluric acid precipitate also according to Mylius,[2] and Heidenhain [3] has shown that most of the acid aniline-dyes precipitate, and that some of these, *e.g.* violet black, ponceau, palatin red, and neucoccin, are the most sensitive of all precipitating agents, if the reaction be acid. Some of the complex organic acids of unknown constitution, such as nucleic,

[1] Knorr, *Ber. d. deutsch. Chem. Ges.* **30**. 909 (1897). See also M. Schenck, *Zeit. f. physiol. Chem.* **44**. 427 (1905).

[2] F. Mylius, *ibid.* **36**. I. 775 (1903).

[3] M. Heidenhain, *Pflüger's Arch. f. d. ges. Physiol.* **90**. 115 (1902).

taurocholic, and chondro-sulphuric acids [1] also precipitate, provided the reaction be acid.

The precipitation of albumins, and partly also that of albumoses, by means of strong mineral acids such as hydrochloric, nitric, sulphuric, and phosphoric acids, differs somewhat from the precipitation described above. The reaction with nitric acid is so sensitive that it is used clinically for detecting the presence of albumin in urine. True albumins are insoluble in an excess of acid and when heated, but not so the albumoses. With the latter the precipitate disappears on heating and reappears on cooling. On heating we see in addition to the precipitation also the xanthoproteic-reaction.

Microscopic Investigations

The behaviour of albuminous substances, under different physical and chemical conditions, is fully discussed in the author's *Physiological Histology*,[2] whose chief result, as far as structure is concerned, may be summed up thus: Electrolytes do, while non-electrolytes, such as formaldehyde and osmium tetroxide, do not, produce artefacts in 'fixing' tissues.

The ultra microscopical nature of albumins has been studied by F. Raehlmann,[3] by Michaelis,[4] Pauli,[5] and Waymouth Reid.[6]

[1] See under respective acids.

[2] Gustav Mann, *Physiological Histology: Theory and Methods.* Clarendon Press, 1902.

[3] F. Raehlmann, *Berliner klin. Wochensch.* 1904, p. 186.

[4] L. Michaelis, *Virchow's Arch.* **179**. 195 (1905).

[5] W. Pauli, *Hofmeister's Beiträge*, **6**. 258 (1905).

[6] E. Waymouth Reid, *Journ. of Physiol.* **33**. 12 (1905).

CHAPTER II

Dissociation-Products

With the view of throwing light on the constitution of albumins, albuminous substances have been dissociated up to a point where they lose their chemical character, and the various dissociation-products so obtained have been carefully investigated. Albumins have been boiled with acids and alkalies ; fused with potash ; acted upon by superheated steam ; by ferments derived from animals and plants and subjected to bacterial action. The changes which are induced in albumins during the metabolism of animals and plants under normal and pathological conditions have also been studied.

As the result of dissociation there are obtained, primarily, a number of substances which still resemble the mother substance because both possess, more or less, the same chemical constitution. The primary dissociation-products include such bodies as the albumoses, the peptones, and the peptids. These by further dissociation break up into entirely different groups of bodies, the so-called crystalline or abiuretic decomposition-products. Neither of the two names just mentioned is, however, still correct, since we now know of both crystalline albumins and peptones, and of substances which, when placed in series, form a complete chain, one end of which is formed by the peptones giving the biuret-reaction, while the other end is represented by simple non-albuminous dissociation-products. It is therefore best to call the non-albuminous dissociation-products the 'simple dissociation-products.'

A. Primary Dissociation-Products

Amongst the dissociation-products a special position is occupied by those which are obtained by boiling albuminous substances with hydrochloric or sulphuric acids or subjecting albumins to the action of such ferments as trypsin. The products formed hereby are the 'primary dissociation-products,' in which the carbon chain remains intact, and in which, as far as we know, the nitrogen does not change its position

either, while the imide-groups by which the different carbon-chains are kept together are broken, as first suggested as a possibility by Kossel :[1, 2]

$$CO - NH - C \text{ becomes } COOH, NH_2 - C.$$

In arginin, according to E. Schulze,[3] a guanidin remainder unites two carbon-chains, and is on the one side next to the carbonyl-group CO. The change produced by acids in guanidin is as follows :

$$CO - NH - CNH - NH - C \text{ becomes } COOH, NH_2 - CNH - NH - C.$$

Dissociation by acids resembles that produced by certain ferments. The best method for dissociating albumins by acids is that of Hlasiwetz and Habermann,[4] who use hydrochloric acid with the addition of stannous chloride. F. Bopp[5] and R. Cohn[6] omit the use of a reducing substance such as stannous chloride, but this is, according to Otori,[7] a mistake. The best general account of the hydrolysing action of acids is given by Kossel and Kutscher.[8]

The effect produced by ferments is discussed on pp. 187-199.

While it is thus possible, on the one hand, to dissociate albumins, E. Fischer,[9] on the other hand, has been successful in linking up two or more of these primary dissociation-products, and has thereby formed imino-compounds, which bear a great resemblance to the simplest of all albumins, namely, the peptones. (Compare Chapter VII.)

All the other dissociation-products are not formed directly from the albumin molecule, but only secondarily out of the primary dissociation-products, and are therefore only of subordinate importance in all investigations into the constitution of albuminous matter. These secondary products helped us, however, at one time in filling up gaps in our knowledge regarding the primary products, although of late their importance has been greatly diminished.

Historical Account

The oldest known dissociation-products of albumins are probably leucin, discovered in 1818 by Proust in cheese and called 'oxide

[1] A. Kossel, *Zeitschr. f. physiol. Chem.* **25**. 188 (1898).
[2] A. Kossel, *ibid.* **41**. 321 (1904).
[3] E. Schulze, *ibid.* **11**. 43 (1886).
[4] Hlasiwetz and Habermann, *Liebig's Ann.* **159**. 304 (1871) and **169**. 150 (1873).
[5] F. Bopp, *ibid.* **69**. 16 (1849).
[6] R. Cohn, *Zeitsch. f. physiol. Chem.* **22**. 153 (1896), and **26**. 395 (1899).
[7] J. Otori, *ibid.* **43**. 74 (1904).
[8] Kossel and Kutscher, *ibid.* **31**. 165 (1900).
[9] E. Fischer and E. Fourneau, *Ber. d. deutsch. chem. Ges.* **34**. II. 2868 (1901) ; E. Fischer, *ibid.* **35**. I. 1095 (1902) ; E. Fischer, *Chemikerzeitung*, 1902, II. p. 939 ; E. Fischer, *Ber. d. deutsch. chem. Ges.* **36**. II. 2094 (1903) ; E. Fischer and E. Otto, *ibid.* **36**. II. 2106 (1903). **36**. III. 2993 (1903) ; E. Fischer and P. Bergell, *ibid.* **36**. II. 2592 (1903) ; E. Fischer, *ibid.* **36**. III. 2982 (1903).

caséeux,'[1] and glycocoll (or glycin), which was obtained by Braconnot[2] in 1820, when he boiled gelatine and meat with sulphuric acid. Braconnot gave to Proust's substance the name leucin in 1820. In 1849 Liebig and Hinterberger[3] discovered tyrosin, which they prepared by treating hair with boiling sulphuric acid. In 1865 Cramer[4] added serin, which he obtained from silk glue. In 1867 Kühne[5] demonstrated the presence of leucin and tyrosin in fibrin by means of tryptic digestion, and since then up till now these substances have remained the most popular of dissociation-products. In 1868-69 Ritthausen[6] obtained aspartic and glutaminic acids from vegetable albumins, and Kreussler[7] (1869) and Hlasiwetz and Habermann[8] (1873) showed that they also occur in animal albumins. The just mentioned mono-amino-acids, along with alanin found by Weyl[9] in 1888 in the fibroin of silk, were the only known bodies till E. Schulze[10] in 1892 added amino-valerianic acid. E. and H. Salkowski in 1884[11] and Nencki[12] in 1889 investigated the aromatic products which are formed during the bacterial decomposition of albumins, and arrived at the conclusion that tyrosin could not possibly be the only aromatic compound in the albumin molecule, and that phenyl-amino-propionic acid and skatol-amino-acetic acid must also be preformed. Their prophecy has been brilliantly confirmed by the discovery of phenylalanin by E. Schulze,[13] and of tryptophane by Hopkins and Cole,[14] this tryptophane, according to Ellinger,[15] being indol-amino-propionic acid.

Mörner[16] showed further in 1901 that cystin, which previously had been found only occasionally, was a constant decomposition-product of albumin.

[1] M. Proust, *Ann. de Chimie et de Physique*, **10**. 40 (1818).

[2] H. Braconnot, *ibid.* (von Gay-Lussac and Arago), **13**. 113 (1820).

[3] F. Hinterberger, *Liebig's Annalen*, **71**. 70 (1849).

[4] E. Cramer, *Journ. f. prakt. Chem.* (1) **96**. 76 (1865).

[5] W. Kühne, *Virchow's Arch.* **39**. 130 (1867), and *Verhandl. des Heidelberger nat.-med. Vereins* (N.F.), I. 236, III. 463 (1886).

[6] H. Ritthausen, *Journ. f. prakt. Chem.* **103**. 213 (1868), **106**. 445 (1869), **107**. 218 (1869). [7] W. Kreusler, *ibid.* **107**. 240 (1869).

[8] Hlasiwetz and J. Habermann, *Liebig's Annalen*, **169**. 150 (1873).

[9] Th. Weyl, *Ber. d. deutsch. chem. Ges.* **21**. II. 1407 and 1529 (1888).

[10] E. Schulze, *Zeitschr. f. physiol. Chem.* **17**. 193 (1892).

[11] E. and H. Salkowski, *ibid.* **8**. 417 (1884), **9**. 8 (1884), **9**. 491 (1885); E. Salkowski, *Die Lehre vom Harn*, 1882, and in *Ber. d. deutsch. chem. Ges.* **34**. iii. 3884 (1901).

[12] M. Nencki, *Monatshefte f. Chem.* **10**. 506, 526, 862, 864, 908 (1889).

[13] E. Schulze, *Zeitschr. f. physiol. Chem.* **9**. 63 (1884), **17**. 193 (1892).

[14] F. G. Hopkins and S. W. Cole, *Journ. of Physiology*, **27**. 418 (1901).

[15] A. Ellinger, *Berichte d. deutsch. chem. Ges.* **37**. 1801 (1904); and *Zeitschr. f. physiol. Chem.* **43**. 325 (1903).

[16] K. A. H. Mörner, *ibid.* **28**. 595 (1899), **34**. 207 (1901).

A great advance was made when Drechsel[1] discovered that, in addition to mono-amino-acids, there occur also basic compounds amongst the dissociation-products of albumins. He found lysin, a diamino-caproic acid, and lysatinin. From the latter Hedin[2] prepared arginin, which E. Schulze[3] had previously already discovered in germinating lupine. Kossel[4] added to the two bases lysin and arginin a third one, namely, histidin, the constitution of which has recently been fully cleared up (see p. 41).

Lysin, arginin, and histidin Kossel[5] calls the three hexone bases. These substances formed, and still form, the centre of interest, especially since Kossel[6] succeeded in working out methods for their quantitative estimation, and so made it possible to determine, in a systematic manner, the relative amounts in which they occur in different albumins. Arginin is present in every albumin;[7] lysin and histidin are only absent in some of the protamins, and lysin also in some vegetable albumins. (See Tables on pp. 70-75, and also p. 65.)

Of very great importance also is the discovery by Skraup of the diamino-polycarboxylic acids (see p. 44), as these represent the connecting link between the amino-acids and the sugars.[8]

Through E. Fischer[9] interesting himself in the mono-amino-acids,

[1] E. Drechsel, *Arch. f.* (*Anat. u.*) *Physiol.* 1891, p. 248; *Ber. der Sächs. Ges. d. Wiss.* 1889, 1890.

[2] S. G. Hedin, *Zeitschr. f. physiol. Chem.* **21**. 155 and 297 (1895).

[3] E. Schulze, *ibid.* **11**. 43 (1886); *Ber. d. deutsch. chem. Ges.* **19**. I. 1177 (1886).

[4] A. Kossel, *Zeitschr. f. physiol. Chem.* **22**. 176 (1896).

[5] A. Kossel, *Deutsche med. Wochenschr.* 1898, S. 581.

[6] A. Kossel and Fr. Kutscher, *Zeitschr. f. physiol. Chem.* **31**. 165 (1900).

[7] A. Kossel, *Ber. d. deutsch. chem. Ges.* **34**. III. 3214 (1901).

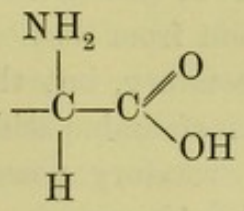
[8] C. Neuberg, Synthese von "Oxy- and Diamino-säuren," *Zeit. f. physiol. Chem.* **45**. 92 (1905).

[9] E. Fischer, 'Dissociation of Racemic Amino-Acids,' *Ber. d. deutsch. chem. Ges.* **32**. II. 2451 and 3638 (1899); E. Fischer, *ibid.* **33**. II. 2370 (1900); E. Fischer and A. Mouneyrat, *ibid.* **33**. II. 2383 (1900); E. Fischer and R. Hagenbach, *ibid.* **34**. III. 3764 (1901); E. Fischer, 'Esters of Amino-Acids,' *ibid.* **34**. I. 433 (1901); 'Synthesis of α-δ-Diamino-valerianic Acid,' **34**. I. 454 (1901); 'Synthesis of α-γ-Diamino-butyric Acid,' **34**. II. 2900 (1901); 'Synthesis of α-ε-Diamino-caproic Acid,' **35**. III. 3772 (1902); E. Fischer, 'Hydrolysis of Casein by means of Hydrochloric Acid,' *Zeitschr. f. physiol. Chem.* **33**. 151 (1901); E. Fischer and A. Skita, 'Fibroin of Silk,' *ibid.* **33**. 177 (1901); E. Fischer, 'Phenylalanin and α-Pyrrolidin-Carboxylic Acid from Egg Albumin,' *ibid.* **33**. 412 (1901); E. Fischer, P. A. Levene, and R. H. Aders, 'Hydrolysis of Gelatine,' *ibid.* **35**. 70 (1902); E. Fischer and A. Skita, 'Fibroin and Gelatine of Silk' *ibid.* **35**. 221 (1902); E. Fischer, 'Formation of α-Pyrrolidin-Carboxylic Acid during Hydrolysis of Casein by means of Alkali,' *ibid.* **35**. 227 (1902); E. Fischer, 'Quantitative Determination of Glycocoll,' *ibid.* **35**. 229 (1902); E. Fischer and E. Abderhalden, 'Oxyhæmoglobin,' *ibid.* **36**. 268 (1902); E. Fischer and T. Dörpinghaus, 'Hydrolysis of Horn,' *ibid.* **36**. 462 (1902); E. Fischer, 'Oxy-α-Pyrrolidin-

great progress has also been made with this class of substances. He first of all set himself the task of preparing, by synthesis, all those decomposition-products of albumins which had not yet been synthetised, and then he worked out a method for the separation of the mono-amino-acids from one another. With the help of his pupils he succeeded:

1. In obtaining two new amino-acids from albuminous substances, namely, α-pyrrolidin-carboxylic acid and oxy-α-pyrrolidin-carboxylic acid.
2. In demonstrating that alanin, serin, phenylalanin, and amino-valerianic acid form more or less constant dissociation-products of all the albumins and not merely of a few.
3. In estimating, at least approximately, the amount of amino-acids occurring in different albuminous bodies.

With the possible exception of tryptophane[1] and some substances isolated by Levene,[2]—who finds that the amino-valerianic acid, which is formed during the autolysis or autodigestion of the pancreas and of the liver, has a bitter taste, while all α-amino-acids have a sweet taste, —all the dissociation-products of albumins are α-amino-acids, which means the aminogen group, NH_2, is attached to the first carbon atom next the carboxyl group, COOH.



This configuration determines the chemical behaviour of amino-acids, and thereby of the whole albumin molecule. All the substances obtainable from albumin by its dissociation with acids or with trypsin are optically active with the exception of glycocoll and serin, while

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Carboxylic Acid,' *Ber. d. deutsch. chem. Ges.* **35**. III. 2660 (1902); E. Langstein, 'Hydrolysis of Zein,' *Zeitschr. f. physiol. Chem.* **37**. 508 (1903); E. Abderhalden, 'Oxyhæmoglobin,' *ibid.* **37**. 484 (1903); 'Serumalbumin,' *ibid.* **37**. 495 (1903); 'Edestin,' *ibid.* **37**. 499 (1903); 'Cystindiathesis,' *ibid.* **38**. 557 (1903); E. Fischer, 'Casein and Silk Fibroin,' *ibid.* **39**. 155 (1903); E. Fischer and P. Bergell, 'β-Napthalinsulfoderivates,' *Ber. d. deutsch. chem. Ges.* **35**. III. 3779 (1902); E. Fischer and H. Leuchs, 'Synthesis of Serin, of *l*-Glucosamic Acid and other Oxyamino Acids,' *ibid.* **35**. III. 3787 (1902); 'Synthesis of *d*-Glucosamin,' *ibid.* **36**. I. 24 (1903); E. Fischer and E. Abderhalden, 'Trypsin Digestion,' *Zeitsch. f. physiol. Chem.* **39**. 81 (1903); E. Fischer, *Ber. d. deutsch. chem. Ges.* **36**. III. 2982 (1903).

[1] See p. 53, under Tryptophane.

[2] Levene, *Zeitschr. f. physiol. Chem.* **41**. 100 (1904).

the corresponding inactive compounds are obtained, if the albumin be dissociated by means of boiling alkalies, especially if it be boiled under pressure.[1] The reason for this behaviour has been discovered by E. Schulze,[2] who showed that active amino-acids are racemised by being boiled with barium hydrate. Siegfried[3] has confirmed this especially for arginin and lysin. According to Kutscher,[4] arginin is racemised by being boiled for a short time with concentrated sulphuric acid, or by being heated for 15 to 20 minutes in an incubator to 210–220°. As, however, mere boiling with acids also racemises a larger or smaller portion of the amino-acids, the ultimate product will always contain a certain percentage of raceme-bodies.[5] This fact explains the discrepancies which arise in estimating the amount of polarisation exhibited by some mono-amino-acids,[6] and also partly explains why various observers have described several isomeric leucines[7] differing from one another in their properties.[8]

E. Fischer[9] has benzolysed the inactive, synthetically prepared amino-acids, and then dissociated the benzoyl-products by means of strychnine, brucine, or cinchonin salts into their active components, and finally prepared from these latter the pure amino-acids. He has therefore accomplished the synthesis of the dissociation-products of albumins. Another simple method of synthetising α-amino-acids by phtalimidmalonic ester has been described by Sörensen.[10]

The rotatory power of the salts which amino-acids form with acids and bases is different from that of the free amino-acids. Leucin and histidin are lævo-rotatory, but their hydrochlorides are dextro-rotatory. As the salts of amino-acids undergo great hydrolysis in watery solutions, their rotatory power is found to alter with the amount of hydrochloric acid present, and the rotation becomes only fairly constant if a large excess of hydrochloric acid be present.[11]

[1] E. Schulze and E. Bosshard, *Zeitschr. f. physiol. Chem.* **9**. 63 (1884).

[2] E. Schulze and E. Bosshard, *ibid.* **10**. 134 (1885).

[3] M. Siegfried, *Ber. d. deutsch. chem. Ges.* **24**. I. 418 (1891).

[4] F. Kutscher, *Zeitschr. f. physiol. Chem.* **32**. 476 (1901).

[5] E. Fischer, P. A. Levene, and R. H. Aders, *ibid.* **35**. 70 (1902); E. Abderhalden, *ibid.* **37**. 499 (1903); E. Fischer and P. Bergell, *Ber. d. deutsch. chem. Ges.* **36**. II. 2592 (1902).

[6] E. Schulze and E. Winterstein, *Zeitschr. f. physiol. Chem.* **35**. 299 (1902).

[7] R. Cohn, *ibid.* **20**. 203 (1894).

[8] E. Fischer, *Ber. d. deutsch. chem. Ges.* **33**. II. 2370 (1900).

[9] E. Fischer, *ibid.* **32**. II. 2451 (1899), **32**. III. 3638 (1899), **33**. II. 2370 (1900); E. Fischer and A. Mouneyrat, *ibid.* **33**. II. 2383; E. Fischer and R. Hagenbach, *ibid.* **34**. III. 3764 (1901).

[10] S. P. L. Sörensen, *Zeit. f. physiol. Chem.* **44**. 448 (1905).

[11] A. Kossel and F. Kutscher, *ibid.* **28**. 182 (1899); W. Gulewitsch, *ibid.* **27**. 178 and 368 (1899).

Therefore 21 per cent hydrochloric acid is used whenever the amount of polarisation of different amino-acids has to be determined.

Glycocoll,[1] as its name indicates, as well as other α-amino-acids, possess a sweet taste, while β- and γ-amino-acids are tasteless.[2] The two stereoisomers have the same taste.[2]

Methods of preparing and estimating Mono-amino-Acids

To prepare and estimate mono-amino-acids the following plan used to be adopted : The mixture of albuminous dissociation-products was inspissated, after the removal of the hydrochloric and sulphuric acids by means of cupro-oxide Cu_2O and barium hydrate, when leucin and tyrosin crystallised out because of their slight solubility. Although the solubility of these acids is greatly increased through the admixture of other dissociation-products, yet tyrosin separates out in large quantities. In their impure state both tyrosin and lysin crystallise out in very characteristic forms, which on microscopical examination cannot be mistaken, for tyrosin appears as needle-like conglomerations, while leucin forms round somewhat dentate nodules. The presence of these nodules has been considered for a long time to be the most ready means of diagnosing the presence of primary crystalline dissociation-products.

To separate leucin from tyrosin, Habermann and Ehrenfeld[3] use boiling glacial acetic acid, which readily dissolves leucin, while it has hardly any effect on tyrosin. Tyrosin is obtained as a rule in fairly pure crystals, while leucin is always contaminated by many impurities.[4]

Till quite recently no generally applicable method was known for separating other amino-acids from one another. Glycocoll may separate out if it be prepared from gelatine, as the latter is very rich in glycocoll. Glutaminic acid,[5] or especially its hydrochloride,[6] if they be present in large amount, also separate out, because glutamin-hydrochloride is nearly insoluble in strong hydrochloric acid. But, even if the amino-acids which have been just enumerated could be obtained as fairly pure crystals, there remain other amino-acids which will not crystallise,[7] because all amino-acids, being both acid and basic in their

[1] H. Braconnot, *Ann. de Chim. et de Physique*, **13**. 113 (1820).

[2] E. Fischer, *Ber. d. deutsch. chem. Ges.* **35**. III. 2660 (1902).

[3] J. Habermann and R. Ehrenfeld, *Zeitschr. f. physiol. Chem.* **37**. 18 (1902).

[4] E. Fischer, *ibid.* **33**. 151 (1901) ; *Ber. d. deutsch. chem. Ges.* **34**. I. 433 (1901).

[5] H. Ritthausen, *Journ. f. prakt. Chem.* **107**. 218 (1869).

[6] H. Hlasiwetz and J. Habermann, *Liebig's Ann.* **169**. 150 (1873) ; R. Cohn *Zeitschr. f. physiol. Chem.* **26**. 395 (1899).

[7] H. Ritthausen, *Journ. f. prakt. Chem.* **103**. 236 (1868) ; F. Kutscher, *Endprodukte der Trypsinverdauung*, Habilitationsschrift. Marburg, 1899.

nature, combine to form salts and thereby keep one another mutually in solution.[1] The solubilities of isolated, individual amino-acids, of many of their salts, and of their derivatives are frequently but low, but as soon as other homologous compounds are present, their solubilities are increased enormously.[2]

Even crystallisation is no guarantee as to the purity of an amino-acid, because leucin and amino-valerianic acid and their cuprates have a great tendency to crystallise out together either as double salts or as mixed crystals.[2] Ritthausen[3] used in his researches the method of displacing the bases of the amino-acids with barium hydrate, and then precipitating the slightly soluble barium salts of, *e.g.*, asparagin and glutamin with alcohol. Kutscher[4] adopted the plan of precipitating the bases of the amino-acids with phospho-tungstic acid, but he could not get the acid residue to crystallise out. In the case of glutaminic acid Kutscher obtained a crystalline product by removing the bases; allowing the tyrosin and part of the leucin to crystallise out; precipitating the glutaminic acid as a zinc salt; removing the zinc and finally evaporating the remaining fluid till crystals appeared. Aspartic acid has been estimated by Hlasiwetz and Habermann as a silver salt, and by Ritthausen as a copper salt, but these methods are very uncertain and accompanied by great loss, and therefore only applicable if a substance is very rich in asparagin. Kutscher's[5] method of forming silversalts of the amino-acids has not yet been put sufficiently to the test. He adds to a solution of amino-acids a 20 per cent solution of silver nitrate, and subsequently barium hydrate, when the slightly soluble silversalts of leucin and of glycocoll separate out, while the salts of alanin and of amino-valerianic acid remain in solution.

The first practical method for the isolation of amino-acids we owe to Emil Fischer,[6] who converts the amino-acids into their respective ethyl-esters, according to the method of Curtius,[7] and then separates the individual esters, by means of fractional distillation, under the lowest attainable pressure. The only ester which is not distilled but is allowed to crystallise out is glycocoll-ester.[8] From the residue

[1] F. Kutscher, *Zeitschr. f. physiol. Chem.* **28**. 123 (1899); F. Hofmeister, *Liebig's Ann.* **189**. 6 (1877). [2] E. Fischer, *Zeitschr. f. physiol. Chem.* **33**. 151 (1901).

[3] H. Ritthausen, *Journ. f. prakt. Chem.* **107**. 218 (1869).

[4] F. Kutscher, *Zeitschr. f. physiol. Chem.* **38**. 111 (1903).

[5] F. Kutscher, *Sitz.-Ber. d. Berliner Akad. d. Wiss., phys.-math. Kl.*, 29 May 1902; *Zeitschr. f. physiol. Chem.* **38**. 111 (1903).

[6] E. Fischer, *Ber. d. deutsch. chem. Ges.* **34**. I. 433 (1901); *Zeitschr. f. physiol. Chem.* **33**. 151 (1901); E. Fischer and E. Abderhalden, *ibid.* **36**. 268 (1902).

[7] Th. Curtius and F. Göbel, *Journ. f. prakt. Chem.* (2), **37**. 150 (1888).

[8] E. Fischer, *Zeitschr. f. physiol. Chem.* **35**. 229 (1902).

which remains after the esters have been extracted with ether, oxy-α-pyrrolidin-carboxylic acid [1] is obtained, while the residue which is left after the distillation of the esters yields leucinimide.[2] Part of the glutaminic acid was obtained also directly as the hydrochloride,[2] while tyrosin was allowed to crystallise out according to the old plan.[3] The reconversion of the esters into the amino-acids is brought about by boiling with water if the esters have a low boiling-point, and by means of barium hydrate if the boiling-point is high. This method of Fischer is the one on which, so far, the quantitative determination of various amino-acids has been based. A second method introduced by E. Fischer and Bergell [4] depends on the conversion of amino-acids into β-naphthalin-sulpho-derivatives, which, because of their slight solubility, are especially suitable for the separation of amino-acids. This second method has been used successfully by Abderhalden,[5] Abderhalden and Bergell,[6] and Fischer and Bergell.[7] Serin in particular was studied as serin-β-naphthalin sulphonate.[8] Amino-acids may also be coupled with phenyliso-cyanate, and the resulting compounds be used for the identification of the amino-acids.[9]

Siegfried [10] employs for the isolation of the complex dissociation-products of albumins the substance 4-nitrotoluol-2-sulphoglucin

$$C_6H_3NO_2 \cdot CH_3 \cdot SO_2 \cdot NH \cdot CH_2COOH.$$

An ethereal solution of this substance shaken with an alkaline solution of amino-acids, *e.g.* glycocoll, alanin, glutaminic acid, yields after acidification with hydrochloric acid well-marked crystals, slightly soluble in water and possessing a well-defined melting-point. For purification the crystals are dissolved in hot water, and are then allowed to crystallise out by cooling the water.

Even E. Fischer's method gives, however, only minimal values, as 'in the isolation of individual dissociation-products losses are unavoidable; to obtain amino-acids quantitatively is impossible even after a triple esterification, for the residue still possesses a very strong smell

[1] E. Fischer, *Ber. d. deutsch. chem. Ges.* **35.** III. 2660 (1902).
[2] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **37.** 499 (1903).
[3] E. Abderhalden, *ibid.* **37.** 484 (1903).
[4] E. Fischer and P. Bergell, *Ber. d. deutsch. chem. Ges.* **35.** III. 3779 (1902).
[5] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **38.** 557 (1903).
[6] E. Abderhalden and P. Bergell, *ibid.* **39.** 9 (1903).
[7] E. Fischer and P. Bergell, *Ber. d. deutsch. chem. Ges.* **36.** II. 2592 (1902).
[8] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **37.** 484 (1903).
[9] C. Paal, *Ber. d. deutsch. chem. Ges.* **27.** II. 974 (1894); H. Steudel, *Zeitschr. f. physiol. Chem.* **34.** 353 (1901); E. Fischer and A. Skita, *ibid.* **35.** 221 (1902).
[10] M. Siegfried, *ibid.* **43.** 68 (1904).

of esters, after the last ether-extraction. An accurate investigation of the individual fractions shows that special attention has to be paid to the higher fractions.'[1] The formation of leucinimide proves further that secondary reactions are also taking place. E. Fischer and Abderhalden[2] estimate the loss of amino-acids as amounting to 33 per cent when the ester-method is used, but the loss varies greatly with each individual amino-acid.

Methods for Preparing and Estimating Diamino Acids

A more detailed account than that given here will be found in the paper by Schulze and Winterstein.[3]

The methods for estimating the hexone-bases: lysin, arginin, and histidin, have been worked out by Kossel and Kutscher.[4] After the removal of the ammonia by distillation with barium carbonate,[5] the mixture of dissociation-products is treated with an excess[6] of silver sulphate, and then saturated with barium hydrate. Histidin and arginin are hereby precipitated. They are now dissolved, the barium and the silver are removed, the solution is treated with silver nitrate, and barium hydrate is then added very carefully till the histidin is precipitated. The latter is now purified by precipitation with mercuric sulphate dissolved in sulphuric acid according to Kossel and Patten.[7] The filtrate after the precipitation of the histidin is saturated with barium hydrate, when arginin separates out, which then may be converted into the sulphate or nitrate. From the filtrate after the first silver precipitate, lysin is precipitated with phospho-tungstic acid, and then converted into the picrate. This method has the great merit of working quantitatively, if care be taken.

The three hexone bases are therefore, along with ammonia, the only dissociation-products of albumins, the amounts of which we can express in definite percentage figures, instead of having to give minimal values, as in the case of the mono-amino acids. For this reason the hexone bases have received a great deal of attention during the last few years, and we are more accurately informed regarding their distribution than we are as to that of leucin and tyrosin, although

[1] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **37**. 493 (1903).

[2] E. Fischer and E. Abderhalden, *ibid.* **36**. 268 (1902).

[3] E. Schulze and E. Winterstein, *Ergebnisse d. Physiol.* **1**, 1, pp. 37-42 (1902).

[4] A. Kossel and F. Kutscher, *Zeitschr. f. physiol. Chem.* **31**. 165 (1900).

[5] E. Hart, *ibid.* **33**. 347 (1901).

[6] A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900); F. Kutscher, *ibid.* **38**. 111 (1903).

[7] A. Kossel and A. J. Patten, *ibid.* **38**. 39 (1903).

these mono-amino acids have been known for so long a time, and are so readily demonstrated.

Methods for other Substances

The methods for the estimation of ammonia, cystin, tryptophane, etc., will be discussed when dealing with these substances.

Enumeration of the Primary Dissociation-Products

The primary dissociation-products have been arranged according to the following system. [The numbers before the individual compounds correspond to those in the text.]

A. Open-chain Amino-acids.

I. (*a*) mono-amino–mono-carboxylic acids.

1.	amino-acetic	$C_2H_5NO_2$.
2.	amino-propionic	$C_3H_7NO_2$.
	amino-butyric	$C_4H_9NO_2$.
3.	amino-valerianic	$C_5H_{11}NO_2$.
4.	amino-iso-butyl-acetic	$C_6H_{13}NO_2$.

(*b*) mono-amino–mono-carboxylic–hydroxy acids.

5.	amino-hydroxy-propionic	$C_3H_7NO_3$.
6.	amino–tetra-hydroxy–caproic	$C_6H_{13}NO_6$.

(*c*) mono-amino–di-carboxylic acids.

7.	amino-succinic	$C_4H_7NO_4$.
8.	amino-glutaminic	$C_5H_9NO_4$.

(*d*) mono-amino–di-carboxylic–hydroxy acids.

9.	amino-hydroxy-succinic	$C_4H_7NO_5$.
10.	amino-hydroxy-suberic	$C_8H_{15}NO_5$.

II. (*e*) diamino–mono-carboxylic acids.

11.	diamino-propionic	$C_3H_8NO_2$.
12.	diamino-caproic	$C_6H_{14}N_2O_2$.
13.	guanidin-amino-valerianic	$C_6H_{14}N_4O_2$.
14.	histidin	$C_6H_9N_3O_2$.

(*f*) diamino–mono-carboxylic–hydroxy acids.

15.	diamino-trihydroxy-dodecanoic	$C_{12}H_{26}N_2O_5$.

(*g*) diamino–di-carboxylic acids.

16.	diamino-glutaric	$C_5H_{12}N_2O_4$.
17.	diamino-adipic	$C_6H_{14}N_2O_4$.

(*h*) diamino–di-carboxylic–hydroxy acids.

18.	diamino–di-hydroxy–suberic	$C_8H_{16}N_2O_6$.
19.	diamino–hydroxy–sebacic	$C_{10}H_{20}N_2O_5$
20.	caseanic acid (?)	$C_9H_{16}N_2O_6$.
21.	caseinic acid (?)	$C_{12}H_{16}N_2O_5$.

B. RING-COMPOUNDS. (See Histidin under No. 14.)

(i) pyrrolidin compounds.

22. α-pyrrolidin-carboxylic acid $C_5H_9NO_2$.
23. hydroxy-pyrrolidin-carboxylic acid $C_5H_9NO_3$.

(k) aromatic amino-acids.

24. phenyl-amino-propionic $C_9H_{11}NO_2$.
25. phenyl-hydroxy-amino-propionic $C_9H_{11}NO_3$.
26. indol-amino-propionic $C_{11}H_{12}N_2O_2$.

C. AMMONIA.

(l) ammonia.

27. ammonia NH_3.

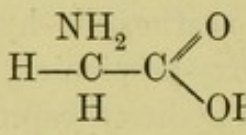
D. THIO-AMINO-ACIDS.

(m) diamino–di-thio–di-carboxylic acid.

28. cystin $C_6H_{12}N_2O_4S_2$.

The following substances have been definitely shown to be primary products :—

1. Glycocoll, or **Amino-acetic Acid,** $C_2H_5NO_2$



is normal α-amino-acetic acid or 'glycin.' Glycocoll is the simplest of all amino-acids, and its peculiar property of acting both as a base and as an acid, is typical of all amino-acids, and therefore also of the albumins (see Chapter V.). It was discovered by Braconnot[1] in gelatine, and by Städeler[2] in the fibroin of silk, while the first quantitive determinations for gelatine[3] and silk[4] were made by E. Fischer. Later Spiro[5] and E. Fischer[6] and Abderhalden[7] have found it also in serum-globulin, fibrin, edestin, and in horn. Spiro converted glycocoll into hippuric acid, while E. Fischer, in preparing it, made use of the insolubility of the glycocoll-ethyl-ester hydrochloride. Glycocoll can be determined quantitatively more accurately than any other mono-amino acid. In most proteids it is absent altogether, a fact of special interest as

[1] H. Braconnot, *Ann. de Chim. et de Physique* (Gay-Lussac and Arago), **13**. 113 (1820).

[2] G. Städeler, *Liebig's Ann.* **111**. 12 (1859).

[3] E. Fischer, *Zeitschr. f. physiol. Chem.* **35**. 229 (1902) ; E. Fischer, P. A. Levene, and R. H. Aders, *ibid.* **35**. 70 (1902).

[4] E. Fischer and A. Skita, *ibid.* **33**. 177 (1901).

[5] K. Spiro, *ibid.* **28**. 174 (1899).

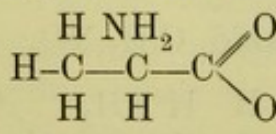
[6] E. Fischer and T. Dörpinghaus, *ibid.* **36**. 462 (1902).

[7] E. Abderhalden, *ibid.* **37**. 499 (1903) ; E. Abderhalden and W. Falta, *ibid.* **39**. 143 (1903).

glycocoll, according to Pick [1] and E. Fischer and Abderhalden,[2] is only contained in the 'anti-group' of albumins (see below). The occurrence of glycocoll in globin (asserted by Spiro,[3] denied by Abderhalden [4]) and in silk glue [5] is questionable. Glycocoll, as already mentioned, is also called glycin, and its derivatives, such as glycylglycin [6] and others, having become of the greatest importance for investigations bearing on the constitution of albumins, are dealt with more fully on pp. 115-137. On being oxidised with manganese dioxide and sulphuric acid it gives rise to prussic acid as first observed by Liebig in 1849.[7] Quite recently this question has been thoroughly investigated by Aders Plimmer (see p. 86).

The physiology of glycocoll is discussed on p. 108.

2. Alanin, or **Amino-propionic Acid,** $C_3H_7NO_2$



is α-amino-propionic acid. It used to be believed that alanin was only present in some albuminoids, as, *e.g.*, in the fibroin of silk,[8] but E. Fischer [9] has shown it to be widely distributed, and he attributes its former non-discovery to its great solubility. The aromatic derivatives of alanin, namely, phenylalanin, and especially tyrosin or oxyphenyl-amino-propionic acid, have been known for a long time, and the mother substance of the indol radical of albumins is also an alanin derivative. Serin and cystin are other alanin derivatives.

The alanin occurring in proteids is *d*-alanin.[10] Its specific rotation in strong hydrochloric acid, according to E. Fischer,[10] is

$$\alpha_D^{20} = +9{\cdot}68.$$

By treatment with nitrous acid it is converted into *d*-lactic acid,[11]

[1] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28.** 219 (1899).

[2] E. Fischer and E. Abderhalden, *ibid.* **39.** 81 (1903).

[3] K. Spiro, *ibid.* **28.** 174 (1899). [4] E. Abderhalden, *ibid.* **37.** 484 (1903).

[5] E. Fischer and A. Skita, *ibid.* **35.** 221 (1902).

[6] Fischer and Fourneau have given the term glycyl to the radical $NH_2 \cdot CH_2 \cdot CO$, glycin or glycocoll being $NH_2 \cdot CH_2 \cdot COOH$.

[7] J. Liebig, *Ann. Chem. Pharm.* **120,** 311 (1849).

[8] Th. Weyl, *Ber. d. deutsch. chem. Ges.* **21.** II. 1407 and 1529 (1888).

[9] E. Fischer, *Zeitschr. f. physiol. Chem.* **33.** 151 (1901); see also tables, pp. 70 to 75, and the other quoted papers by Fischer and his pupils, p. 20.

[10] E. Fischer, *Ber. d. deutsch. chem. Ges.* **32.** II. 2451 (1899); E. Fischer, P. A. Levene, and R. H. Aders, *Zeitschr. f. physiol. Chem.* **35.** 70 (1902).

[11] Lactic acid occurs in the form of the two isomers:

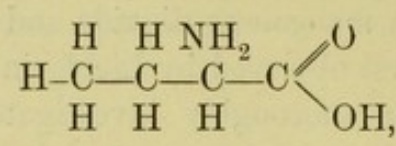
α-oxy-propionic acid or $CH_3—CHOH—COOH$

β-oxy-propionic acid or $CH_2(OH—CH_2—COOH$.

The α-oxy-propionic acid, if formed by ordinary fermentation, is racemised, *i.e.* it

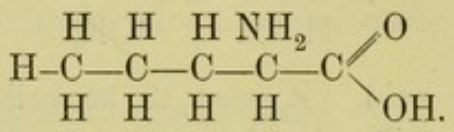
$CH_3 \cdot CH(OH) \cdot COOH$, which is identical with the sarcolactic acid found in muscle during *rigor mortis* and also in the living organism.[1] As carbohydrates readily pass into lactic acid, alanin acts also as a connecting link with these.[2] (See also p. 108, under Physiological siderations.)

Amino-butyric Acid, $C_4H_9NO_2$



is, according to Schützenberger, in all probability also a primary dissociation-product, but it has not been included here, as there is still some doubt (see p. 83).

3. Amino-valerianic Acid, $C_5H_{11}NO_2$



It was first found by E. Schulze[3] in germinating plants, and later by Kossel[4] in the protamin clupein, which is prepared from the milt of herrings. E. Fischer has been able to demonstrate its presence also in casein,[5] horn,[6] and gelatine,[7] while in all probability it also occurs in fibroin[8] and zein.[9] As this amino-acid greatly resembles leucin in its properties, it is very difficult to demonstrate its existence in the presence of leucin, as the latter is met with always in much larger quantities. E. Fischer for this reason has not as yet been able to obtain it in such purity as to be able to characterise it more definitely and to identify it with one of the different amino-valerianic acids prepared by him and Slimmer.[10] He believes it to be, however, α-amino-valerianic

---

is optically inactive, being composed of dextro- and lævo-rotatory lactic acids, while the lactic acid found in meat is dextro-rotatory, and has been called sarcolactic acid by Liebig. This α-oxy-propionic acid, when oxidised yields acetic acid and carbonic acid.

The β-oxy-propionic acid contains no asymmetric carbon atom, and is therefore optically inert. On oxidation it yields oxalic acid and carbonic acid.

[1] E. Fischer and A. Skita, *ibid.* **33**. 177 (1901).

[2] E. Fischer and Abderhalden, *ibid.* **36**. 268 (1902).

[3] E. Schulze, *Zeitschr. f. physiol. Chem.* **17**. 193 (1892), **28**. 465 (1899).

[4] A. Kossel, *ibid.* **26**. 588 (1899).

[5] E. Fischer, *ibid.* **33**. 151 (1901).

[6] E. Fischer and T. Dörpinghaus, *ibid.* **36**. 462 (1902).

[7] E. Fischer, P. A. Levene, and R. H. Aders, *ibid.* **35**. 70 (1902).

[8] E. Fischer and A. Skita, *ibid.* **33**. 177 (1901).

[9] L. Langstein, *ibid.* **37**. 508 (1903).

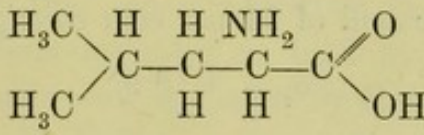
[10] M. D. Slimmer, *Ber. d. deutsch. chem. Ges.* **35**. I. 400 (1902).

acid. It has been examined more carefully by Schulze and Winterstein.[1] It is soluble in 11 parts of water, forms a readily soluble copper salt, which, according to E. Fischer,[2] has a great tendency to crystallise out with the leucin copper salt. It is dextro-rotatory.[1]

$$\alpha_D = +27{\cdot}9.$$

As guanidin-amino-valerianic acid, or arginin, is a constant dissociation-product of all albumins (see below), one might believe that amino-valerianic acid is obtained by the disintegration of arginin. This, however, is impossible as it is derived from an iso-valerianic acid, while arginin possesses a straight carbon chain. The amounts of arginin which can be obtained under varying conditions from the same albumin also correspond so closely, that we have to exclude the occasional further disintegration of arginin into amino-valerianic acid.[3] The reason that this acid was not discovered sooner and more frequently, E. Fischer[2] attributes to the great difficulties connected with its isolation. Schulze and Winterstein[1] recommend for its preparation germinating plants of *Lupinus luteus* and *Lupinus albus* two to three weeks old, as they contain relatively small amounts of leucin. A δ-amino-valerianic acid, which H. Salkowski[4] found once during putrefaction of gelatine, is a secondary dissociation-product, and must not be confounded with the α-acid under consideration.

4 (*a*). **Leucin,** $C_6H_{13}NO_2$, is not amino-normal-caproic acid, $CH_3 . (CH_2)_3CH(NH_2) . COOH$, but isobutyl-α-amino-acetic acid



As already mentioned, it is, along with glycocoll, the oldest and, along with tyrosin, the best known of all the dissociation-products of albumins. With the exception of the protamins, it has been found up till now in every one of the albumins whenever it was looked for. But whether this great distribution is characteristic of leucin is somewhat doubtful, especially if we consider that up to the time of Drechsel, Kossel, and E. Fischer, the only readily demonstrable dissociation-products were leucin, tyrosin, and perhaps glutaminic and aspartic acids. We know already that the hexone-bases have a wider distribution than has leucin, and Fischer has found in every

[1] E. Schulze and E. Winterstein, *Zeitschr. f. physiol. Chem.* **35**. 299 (1902).

[2] E. Fischer, *ibid.* **33**. 151 (1901).

[3] A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900).

[4] H. Salkowski, *Ber. d. deutsch. chem. Ges.* **16**. I. 1191 and **16**. II. 1802 (1883), **31**. II. 776 (1898).

one of the albumins he examined the substances alanin, phenylalanin, α-pyrrolidin-carboxylic acid, glutaminic and aspartic acids. As regards quantity, however, in most albumins leucin exceeds by far the other dissociation-products.[1] The older figures of Cohn,[2] Pröscher,[3] and Erlenmeyer and Schöffer,[4] who found about 40 per cent of leucin in casein, globin, and elastin, are certainly too high, for they based their figures on the 'Rohfraction,' of which leucin only forms a part. From such an impure product E. Fischer[5] obtained by means of the ester method only about one-third of pure leucin, and he goes fully into the difficulties he experienced in separating, by means of recrystallisation,[6] leucin from other closely-related amino-acids, such as amino-valerianic acid. Most of the leucin obtained from albumin is said to be contaminated with a substance containing nitrogen and sulphur.[7] But even pure leucin, the preparation of which is accompanied by great loss, makes out a very large portion of the dissociation-products. Abderhalden found in globin[8] 30 per cent, in serum albumin[9] 20 per cent, in edestin[10] 20·9 per cent, all these figures representing minimal values, as explained above when describing the ester method.

As was pointed out above, leucin is α-amino-isobutyl-acetic acid,[11] and therefore contains a branched carbon chain. This fact distinguishes it sharply from lysin, the diamino-normal-caproic acid.

According to E. Fischer,[12] leucin obtained from albumins is *l*-leucin, as in watery solutions it is lævo-rotatory; in acid or alkaline solutions it is, however, dextro-rotatory. According to E. Schulze and Winterstein,[13] in hydrochloric acid of 24 per cent strength

$$\alpha_D = +18{\cdot}9.$$

Leucin is soluble in 46 parts of water.[14]

E. Fischer[12] has examined *d*, *l*, and *r*-leucin, as well as a series of

[1] For an exception see under glutaminic acid, p. 35.

[2] R. Cohn, *Zeitschr. f. physiol. Chem.* **22**. 153 (1896), **26**. 395 (1899).

[3] F. Pröscher, *ibid.* **27**. 114 (1899).

[4] Erlenmeyer and A. Schöffer, *Journ. f. prakt. Chem.* (1) **80**. 357 (1860).

[5] E. Fischer, *Ber. d. deutsch. chem. Ges.* **34**. I. 433 [p. 446] (1901).

[6] E. Fischer, *ibid.* **34**. I. 433 [p. 446] (1901); and *ibid.* **33**. II. 2370 (1900).

[7] E. Fischer, *ibid.* **33**. II. 2370 (1900).

[8] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **37**. 484 (1903).

[9] E. Abderhalden, *ibid.* **37**. 495 (1903).

[10] E. Abderhalden, *ibid.* **37**. 499 (1903).

[11] E. Schulze and Likiernik, *ibid.* **17**. 513 (1893); B. Gmelin, *ibid.* **18**. 21 (1893).

[12] E. Fischer, *Ber. d. deutsch. chem. Ges.* **33**. II. 2370 (1900).

[13] E. Schulze and E. Winterstein, *Zeitschr. f. physiol. Chem.* **35**. 299 (1902).

[14] B. Gmelin, *ibid.* **18**. 21 (1893).

their derivatives, and converted them into one another. Active leucin is very readily racemised,[1] and E. Fischer has therefore recommended to convert all the leucin straight away into the racemised variety, especially as the raceme-compounds are less soluble. The melting-point of all three leucins is 293-295 ; they undergo decomposition on melting. Leucin-imide (see below) is a derivative of leucin.

4 (*b*). **Iso-leucin,** $C_6H_{13}NO_2$ is probably a *β*-amino-caproic acid.

This naturally occurring isomer of leucin has been discovered by Felix Ehrlich.[2] While leucin in water is lævo-rotatory, iso-leucin in watery, acid, and alkaline solutions is dextro-rotatory ; in 20 per cent HCl compared with leucin, it is twice as strongly dextro-rotatory than in leucin, iso-leucin shows the following amounts of rotation, in

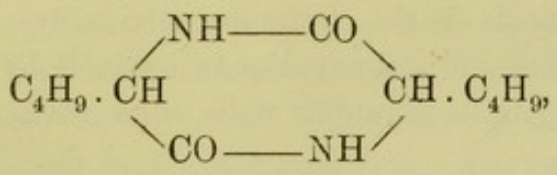
$[\alpha]_D^{20}$	water	20 p.c. HCl	in alkaline solution
	+ 9·74	+ 36·80	+ 11·1

Iso-leucin is very widely distributed throughout the animal and vegetable kingdom, occurring everywhere where leucin is found, *e.g.* in blood fibrin and in molasses. It has a bitter taste, and is therefore not an *α*-amino-acid ; it forms a well-defined compound with phenyl-hydantoin, and must therefore be a *β*-amino-acid, as phenylhydantoin only reacts with *α* and *β* acids.

Iso-leucin can only be separated from leucin by conversion into a coppersalt as fully explained by F. Ehrlich.

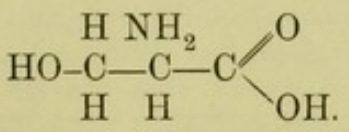
Cohnheim believes that leucin probably does not stand in direct relationship to the carbo-hydrates as held by Kossel,[3] Fr. Müller,[4] and E. Fischer,[5] and this view is shared by Halsey.[6]

Leucin-imide, $C_{12}H_{22}N_2O_2$



may be a primary product, but as there is some doubt it has been described amongst the secondary dissociation products on p. 85.

5. Serin, $C_3H_7NO_3$, is *α*-amino-*β*-hydroxy-propionic acid.

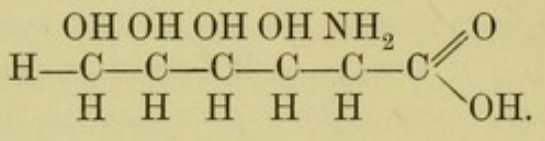


[1] E. Fischer, *Ber. d. deutsch. chem. Ges.* **33**. II. 2370 (1900).
[2] Felix Ehrlich, *ibid.* **37**. 1809 (1904). [3] A. Kossel, *ibid.* **25**. 165 (1898).
[4] Fr. Müller, *Zeitschr. f. Biolog.* **42**. 468 (1901).
[5] E. Fischer and E. Abderhalden, *Zeitschr. f. physiol. Chem.* **36**. 268 (1902).
[6] J. T. Halsey, *Amer. Journ. of Physiol.* **10**. 229 (1904).

Serin was discovered by Cramer[1] amongst the dissociation-products of silk, and hence its name. E. Fischer[2] first demonstrated its wide occurrence and also determined its composition.[3] Up till now it has not been missed in any albumin, although generally it is only present in small quantities, a circumstance which is partly due to the great difficulty of its preparation.[4] Serin occurs also in gelatine to the extent of, at least, 0·4 per cent.[5] While iso-serin is β-amino-α-oxypropionic acid, serin is α-amino-β-oxypropionic acid, and is therefore closely related to cystein, which is thio-serin.[6] A ready means of synthetising serin has been found by Erlenmeyer,[7] and Fischer and Leuchs.[8] Ellinger has also converted diamino-propionic acid into iso-serin.[9]

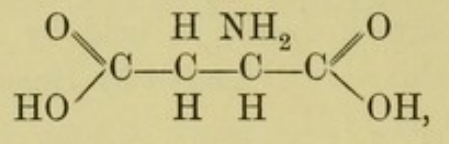
In watery solutions it does not rotate.[3] It tastes sweet.[10] E. Fischer[2] emphasises how important it is to find also in serin, *i.e.* amongst the simple amino-acids, an oxy-amino acid. He suspects some relationship to the carbohydrates, inasmuch as he regards glucosamin as a link between the hexoses and the oxy-amino acids.[11]

6. Amino-tetrahydroxy-caproic Acid, $C_6H_{13}NO_6$



This acid has been isolated by Neuberg and Orgler from cartilage[12] (see later). They have also synthetised it.

7. Aspartic Acid, $C_4H_7NO_4$



is amino-succinic acid, or the Aminobernsteinsäure of the Germans. It was first discovered and estimated quantitatively by von Ritthausen,[13] who dissociated vegetable albumins with sulphuric acid. By means of

[1] E. Cramer, *Journ. f. prakt. Chem.* [1] **96**. 76 (1865).

[2] E. Fischer and A. Skita, *Zeitschr. f. physiol. Chem.* **35**. 221 (1902).

[3] E. Fischer and H. Leuchs, *Ber. d. deutsch. chem. Ges.* **35**. III. 3787 (1902).

[4] E. Fischer and T. Dörpinghaus, *Zeitschr. f. physiol. Chem.* **36**. 462 (1902); E. Fischer, *ibid.* **39**. 155 (1903).

[5] E. Fischer and E. Abderhalden, *ibid.* **32**, 540 (1904).

[6] E. Friedmann, *Hofmeister's Beitr.* **3**. 1 (1902).

[7] E. Erlenmeyer, jun., *Ber. d. deutsch. chem. Ges.* **35**. III. 3769 (1902).

[8] E. Fischer and H. Leuchs, *ibid.* p. 3790. [9] A. Ellinger, *ibid.* **37**. 335 (1904).

[10] E. Fischer, *ibid.* **35**. III. 2660 (1902).

[11] E. Fischer and H. Leuchs, *ibid.* **36**. I. 24 (1903).

[12] Neuberg and Orgler, *Zeitschr. f. physiol. Chem.* **37**. 407 (1904).

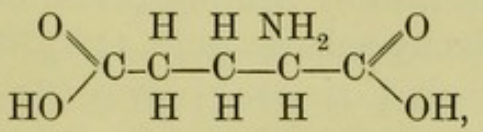
[13] H. Ritthausen, *Journ. f. prakt. Chem.* (1) **106**. 445 (1869); H. Ritthausen and U. Kreusler, *ibid.* (2) **3**. 314 (1871).

the same method Kreusler [1] obtained it from casein, egg-white, and the vitelline prepared from yolk; Hlasiwetz and Habermann [2] employed hydrochloric acid for its preparation from casein. Salkowski and Radziejewski [3] got it by the tryptic digestion of fibrin, and E. Schulze found it to represent one of the chief dissociation-products of many germinating seeds (Tables, pp. 70-75). E. Fischer and his pupils have obtained it from all albumins hitherto examined, except from fibroin, but it is present everywhere only in very small quantities. According to E. Fischer,[4] the acid occurring in albumins is *l*-aspartic acid. It is feebly lævo-rotatory in watery and alkaline solutions, while in strong hydrochloric acid it is dextro-rotatory:

$$\alpha_D = +25{\cdot}7.$$

Aspartic acid has not a sweet, but a strongly acid taste.[5]

8. Glutaminic Acid, $C_5H_9NO_4$



is α-amino-normal-glutaric acid, or α-Amino-normal-brenzweinsäure of the Germans. This acid, also, was discovered by Ritthausen [6] in vegetable albumins, and subsequently by Hlasiwetz and Habermann [2] on dissociating casein with hydrochloric acid; by Knieriem [7] and Kutscher [8] in tryptic digests of fibroin and in the albumins of the pancreas. Later Kutscher [9] and E. Fischer demonstrated the very wide distribution of this substance. With the exception of the protamins and of silk, glutaminic acid can be demonstrated in considerable quantities in all albumins. Osborne and Harris [10] on hydrolysing the alcohol-soluble wheat albumin gliadin with sulphuric acid obtained 25 per cent, and with hydrochloric acid an average minimal amount of 36 per cent of glutaminic acid. This is, so far, the record amount of any one amino-acid in a given albumin. Langstein [11] has demon-

[1] W. Kreusler, *Journ. f. prakt. Chem.* (1) **107.** 240 (1869).

[2] Hlasiwetz and J. Habermann, *Liebig's Annalen*, **169.** 150 (1873).

[3] E. Salkowski and J. Radziejewski, *Ber. d. deutsch. chem. Ges.* **7.** II. 1050 (1874).

[4] E. Fischer, *ibid.* **32.** II. 2451 (1899). [5] E. Fischer, *ibid.* **35.** III. 2660 (1902).

[6] H. Ritthausen, *Journ. f. prakt. Chem.* (1) **106.** 445 (1869); H. Ritthausen and U. Kreusler, *ibid.* (2) **3.** 314 (1871); H. Ritthausen, *Die Getreidearten usw.*, Bonn, M. Cohen and Co. (1872).

[7] Knieriem, *Zeitschr. f. Biolog.* **11.** 199 (1875).

[8] F. Kutscher, *Die Endprodukte der Trypsinverdauung*, Marburger Habilitationsschrift, Strasburg, Trübner, 1899.

[9] F. Kutscher, *Zeitschr. f. physiol. Chem.* **38.** 111 (1903).

[10] T. B. Osborne and I. F. Harris, *Amer. Journ. of Physiol.* **13.** 35 (1905).

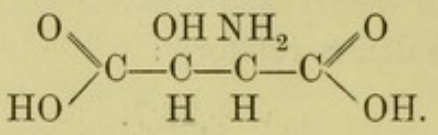
[11] L. Langstein, *Zeitschr. f. physiolog. Chem.* **37.** 508 (1903).

strated in zein 12 per cent. Glutaminic acid and leucin are thus the most abundant mono-amino acids. As to the occurrence of glutamins in plants, see p. 105. According to E. Fischer,[1] the naturally occurring acid is *d*-glutaminic acid. In hydrochloric acid solutions

$$\alpha_D = +30{\cdot}45.$$

Glutaminic acid does not taste sweet but stale, and only slightly acid. A minute description of glutaminic acid, its salts and crystals, is given by Habermann.[2]

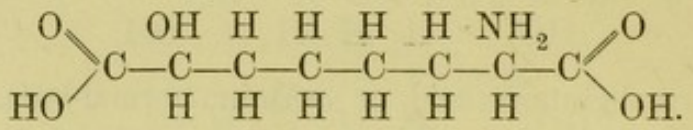
9. Amino-hydroxy-succinic Acid, $C_4H_7NO_5$



It was isolated by Skraup[3] as one of the products of casein-hydrolysis resulting from the action of hydrochloric acid.

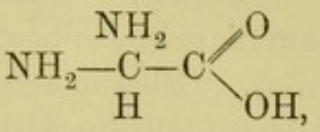
It has been synthetised by Neuberg and Silbermann.[4]

10. Amino-hydroxy-suberic Acid, $C_8H_{15}NO_5$



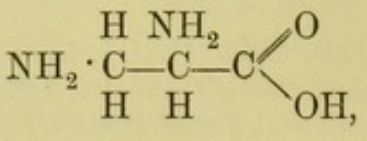
It is a derivative of octandoic acid $COOH[CH_2]_6COOH$, which is called suberic acid in England and Korksäure by the Germans. The amino-oxy-suberic acid was discovered by Wohlgemuth on hydrolysing liver-proteid.[5]

Diamino-acetic Acid, $C_2H_6N_2O_2$



according to Willstätter[6] and Sörensen,[7] is not a normal dissociation-product.

11. Diamino-propionic Acid, $C_3H_8NO_2$



is, according to Paul Mayer,[8] the simplest diamino-acid occurring in

[1] E. Fischer, *Ber. d. deutsch. chem. Ges.* **32**. II. 2451 (1899).
[2] J. Habermann, *Liebig's Ann.* **179**. 248 (1875).
[3] Zd. H. Skraup, *Sitzb. Akad. d. Wiss. Wien*, **113**. II.^{b} p. 263 (1904).
[4] C. Neuberg and M. Silbermann, *Zeitschr. f. physiol. Chem.* **44**. 147 (1905).
[5] J. Wohlgemuth, *Ber. d. deutsch. chem. Ges.* **37**. 4362 (1904).
[6] R. Willstätter, *ibid.* **35**. II. 1378 (1902).
[7] S. P. L. Sörensen, *C. r. des travaux du Laboratoire de Carlsberg*, **6**. 1 (1903).
[8] Paul Mayer, *Zeitschr. f. physiol. Chem.* **42**. 59 (1904).

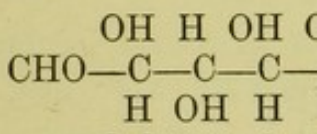
the body. It is related chemically to a whole series of physiologically very important substances :[1]—

$CH_2 \cdot NH_2—CH \cdot NH_2—COOH$	Diamino-propionic acid
$CH_2 \cdot OH —CH \cdot NH_2—COOH$	Serin
$CH_2 \cdot OH —CH \cdot OH —COOH$	Glyceric acid
$CH_3 —CH \cdot NH_2—COOH$	Alanin
$CH_3 —CH \cdot OH —COOH$	Lactic acid
$CH_2 \cdot NH_2—CH \cdot SH —COOH$	Stone-cystein
$CH_2 \cdot SH —CH \cdot NH_2—COOH$	Protein-cystein

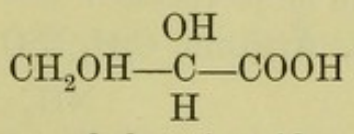
Diamino-propionic acid is of special interest in connection with tryptophane (see p. 51) and with the formation of carbohydrates out of proteids (see below, under the heading of "Physiological Considerations," p. 164).

The conversion of diamino-propionic acid into iso-serin has been accomplished by Ellinger[2] and by Neuberg and Silbermann.[1]

As Neuberg and Neimann have previously prepared *d*-glyceric acid by the action of lime on *d*-glycuronic acid, the formulæ for *d*-glycuronic acid and *d*-glyceric acid may resemble one another in their configuration. This conclusion Neuberg and Silbermann thought it best not to draw because of the complexity of the question :[3]—

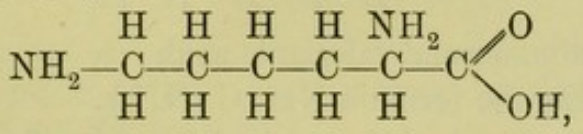


d-glycuronic acid.



d-glyceric acid.

12. Lysin, $C_6H_{14}N_2O_2$



is α-, ϵ-diamino-normal-caproic acid. It was the first base which Drechsel[4] discovered in casein. Later on Siegfried,[5] E. Schulze,[6] Kossel,[7] Kutscher,[8] and Abderhalden[9] showed that it is one of the

[1] C. Neuberg and M. Silbermann, *Ber. d. deutsch. chem. Ges.* **37**. 341 (1904).

[2] Alex. Ellinger, *ibid.* **37**. 335 (1904).

[3] E. Friedmann, *Centralbl. f. Physiol.* **18**. No. 3, p. 67 (1904).

[4] E. Drechsel, *Arch. f.* (*Anat. u.*) *Physiol.* 1891, p. 248.

[5] M. Siegfried, *Ber. d. deutsch. chem. Ges.* **24**. I. 418 (1891). See also *Zeitschr. f. physiol. Chem.* **43**. 363 (1905).

[6] E. Schulze and E. Winterstein, *ibid.* **28**. 459 (1899), **33**. 547 (1901).

[7] A. Kossel, *ibid.* **26**. 586 (1899) ; A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900); D. Lawrow, *ibid.* **28**. 388 (1899) ; E. Hart, *ibid.* **33**. 347 (1901) ; A. Kossel, *Ber. d. deutsch. chem. Ges.* **34**. III. 3214 (1901).

[8] F. Kutscher, *Zeitschr. f. physiol. Chem.* **25**. 195 (1898), **26**. 110 (1898) ; *Die Endprodukte der Trypsinverdauung*, Marburger Habilitationsschrift (1899).

[9] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **37**. 484, 495, 499 (1903).

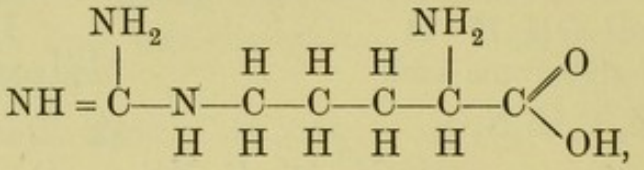
most widely-distributed disintegration-products of albumin. It is only absent in a few vegetable albumins,[1] and in some protamins.[2] (Compare tables on pp. 70-75.)

Lysin is, according to Ellinger[3] and E. Fischer,[4] α-ϵ-diamino-caproic acid. It is dextro-rotatory, and is converted, as are all other amino-acids, into an inactive form on being heated under pressure with barium hydrate.[5] It is, according to Ellinger, the mother-substance of pentamethylene-diamin or cadaverin: $NH_2(CH_2)_5NH_2$. A fuller description of its properties is given by Kossel,[6] Willdenow,[7] and Schulze and Winterstein.[8] For its isolation Kossel[9] makes use of the insolubility of the picrate, and for its identification Herzog[10] prepares the phenylhydantoin having a melting-point of 183-184°, as this compound results from the union of lysin with phenyl-isocyanate.

Amongst readily accessible albumins, lysin is found in greatest quantities in casein and in gelatine. According to Henderson,[11] Kutscher, and Steudel,[12] the nitrogen-determination by Kjeldahl's method does not always give correct values, probably because a part of the nitrogen is converted into hydrocyanic acid, as has been observed by Zickgraf[13] on oxidising lysin with barium permanganate.

Diamino-valerianic Acid, or **Ornithin,** is discussed under Arginin on p. 40.

13. Arginin, $C_6H_{14}N_4O_2$



is guanidin-α-amino-normal-valerianic acid.

Guanidin has the formula $NH:C(NH_2)_2$.

Besides lysin Drechsel[14] prepared from casein a second base, the

[1] A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900).

[2] A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900); A. Kossel, *ibid.* **26**. 588 (1899).

[3] A. Ellinger, *Ber. d. deutsch. chem. Ges.* **32**. III. 3544 (1899); *Zeitschr. f. physiol. Chem.* **29**. 334 (1900).

[4] E. Fischer and F. Weigert, *Ber. d. deutsch. chem. Ges.* **35**. III. 3772 (1902).

[5] M. Siegfried, *Ber. d. deutsch. chem. Ges.* **24**. I. 418 (1891). See also *Zeitschr. f. physiol. Chem.* **43**. 363 (1905).

[6] A. Kossel, *ibid.* **26**. 586 (1899). [7] Cl. Willdenow, *ibid.* **25**. 523 (1898).

[8] E. Schulze and E. Winterstein, 'Ergebnisse der Physiologie,' I. *ibid.* p. 57 (1902).

[9] A. Kossel, *Zeitschr. f. physiol. Chem.* **26**. 586 (1899); A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900).

[10] R. O. Herzog, *ibid.* **34**. 525 (1902). [11] Y. Henderson, *ibid.* **29**. 320 (1900).

[12] F. Kutscher and H. Steudel, *ibid.* **39**. 12 (1903).

[13] Zickgraf, *Ber. d. deutsch. chem. Ges.* **35**. III. 3401 (1902).

[14] E. Drechsel *Sitzungsber. d. Sächs. Ges. d. Wissensch., math.-nat. Kl.* 1889, 1890; *Arch. f. (Anat. u.) Physiol.* 1891, p. 248.

lysatinin. Later Hedin[1] prepared from horn arginin, which had previously been discovered by E. Schulze[2] in germinating lupine, and showed that lysatinin is only a mixture of arginin and lysin.[3] Since then, through the researches of E. Schulze,[4] and especially Kossel[5] and his pupils,[6] arginin has been shown to be the most widely distributed dissociation-product of albumin. An albumin, not containing arginin, is unknown. Salmin,[7] the protamin of the spermatic fluid of the salmon, and sturin, the protamin out of the spermatic fluid of the sturgeon, contain arginin to the extent of 80 per cent. In other protamins arginin is in excess of all other diamino-acids, and is very abundant also in the histones and some vegetable albumins, but in all other albumins it is quantitatively much less abundant than are the mono-amino acids. (See tables on pp. 70-75; and also p. 201.) Kossel's view that arginin is the nucleus of the albumin molecule is discussed on p. 154. As protamins are not readily accessible, edestin and thymus-histone may be recommended especially for the preparation of arginin. Schulze and Winterstein[8] recommend also germinating plants of *Lupinus luteus*. How to obtain arginin by Kossel's[9] method has already been described on p. 26. The properties of arginin and its salts is fully described in the *Ergebnisse der Physiologie*, I. i., p. 46 (1902) by Schulze and Winterstein.

Arginin, according to E. Schulze,[10] Ellinger,[11] Kutscher,[12] and E. Fischer,[13] has this structure—

[1] S. G. Hedin, *Zeitschr. f. physiol. Chem.* **20**. 186 (1894), **21**. 155 and 247 (1895).

[2] E. Schulze, *Ber. d. deutsch. chem. Ges.* **19**. I. 1177 (1886); *Zeitschr. f. physiol. Chem.* **11**. 43 (1886).

[3] S. G. Hedin, *ibid.* **21**. 297 (1895).

[4] E. Schulze, *ibid.* **24**. 276 (1897), **25**. 360 (1898); E. Schulze and E. Winterstein, *ibid.* **28**. 459 (1899), **33**. 547 (1901).

[5] A. Kossel, *ibid.* **22**. 176 (1896), **25**. 165 (1898), **26**. 588 (1899); A. Kossel and F. Kutscher, *ibid.* **25**. 551 (1898); **31**. 165 (1900); A. Kossel, *Ber. d. deutsch. chem. Ges.* **34**. III. 3214 (1901).

[6] F. Kutscher, *Zeitschr. f. physiol. Chem.* **25**. 195 (1898), **26**. 110 (1898); *Endprodukte der Trypsinverdauung*, Marburger Habilitationsschrift, 1899; D. Lawrow, *Zeitschr. f. physiol. Chem.* **28**. 388 (1899); E. Hart, *ibid.* **33**. 347 (1901).

[7] A. Kossel, *ibid.* **26**. 588 (1899).

[8] E. Schulze and E. Winterstein, *ibid.* **35**. 299 (1902).

[9] A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900).

[10] E. Schulze and E. Winterstein, *ibid.* **26**. 1 (1898); *Ber. d. deutsch. chem. Ges.* **30**. III. 2879 (1897), **32**. III. 3191 (1899).

[11] A. Ellinger, *Zeitschr. f. physiol. Chem.* **29**. 334 (1900); *Ber. d. deutsch. chem. Ges.* **31**. III. 3183 (1898).

[12] E. Benech and F. Kutscher, *Zeitschr. f. physiol. Chem.* **32**. 278 (1901); F. Kutscher, *ibid.* **32**. 413 (1901); and especially F. Kutscher and J. Otori, *ibid.* **43**. 93 (1904).

[13] E. Fischer, *Ber. d. deutsch. chem. Ges.* **34**. I. 454 (1901).

$$\begin{matrix} HN \\ NH_2 \end{matrix}\!\!\diagdown\!\!C\!-\!NH\!-\!CH_2\!-\!CH_2\!-\!CH_2\!-\!\overset{\displaystyle NH_2}{\overset{\cdot}{C}}H\!-\!COOH.$$

Therefore by a simple splitting both urea and ornithin (see below) may be derived from it. Thus boiling with baryta water gives rise to urea, because arginin belongs to the same class of bodies as does creatinin or methyl-guanidin-acetic acid.

$$\begin{matrix} NH \\ NH_2 \end{matrix}\!\!\diagdown\!\!C\!\vdots\!N(CH_3)CH_2COOH = \begin{matrix} NH_2 \\ NH_2 \end{matrix}\!\!\diagdown\!\!CO + NH(CH_3)CH_2COOH$$

Creatin = urea + sarcosin.

On the other hand, oxidation with barium permanganate[1] leads to the production of guanidin-butyric acid, and subsequently to that of guanidine, imido-urea, or carbamidine, $NH : C(NH_2)_2$, and succinic acid, $COOH . CH_2 . CH_2 . COOH$. The formation of urea from arginin, or, as he thought, lysatinin, is first mentioned by Drechsel, and later Kossel[2] laid stress on the different biological significance of that urea which is preformed in arginin, and of that which is formed synthetically. That arginin gives rise to urea during ordinary metabolism seems to be proved by the discovery of arginase by Kossel and Dakin, see p. 111.

Ornithin, $CH_2(NH_2) . CH_2 . CH_2 . CH(NH_2) . COOH$, or α-δ-diamino-valerianic acid, is of great importance, because E. Schulze and E. Winterstein[3] have shown that arginin may be regarded as a union of guanidin (see above) with ornithin. This latter has been found in the urine of birds as dibenzoyl-ornithin or ornithuric acid by Jaffé,[4] and has been synthetically prepared by E. Fischer.[5]

A full description of arginin, a number of its salts, and derivatives, is given by Gulewitsch;[6] Lawrow[7] describes a benzoyl derivative, and Herzog[8] the phenylhydantoin of ornithin. Arginin forms a very slightly soluble salt with picrolonic acid,[9] which may be employed for its isolation. Arginin is dextro-rotatory. In strong hydrochloric acid Gulewitsch found

$$\alpha_D = +21{\cdot}25.$$

[1] E. Benech and F. Kutscher, *Zeitschr. f. physiol. Chem.* **32.** 278 (1901); F. Kutscher, *ibid.* **32.** 413 (1901); and especially F. Kutscher and J. Otori, *ibid.* **43.** 93 (1984). [2] A. Kossel, *Ber. d. deutsch. chem. Ges.* **34.** III. 3214 (1901).

[3] E. Schulze and E. Winterstein, *ibid.* **30.** III. 2879 (1897); *Zeitschr. f. physiol. Chem.* **26.** 1 (1898).

[4] M. Jaffé, *Bericht d. deutsch. chem. Ges.* **10.** II. 1925 (1877), **11.** I. 406 (1878).

[5] E. Fischer, *ibid.* **34.** I. 454 (1901).

[6] W. Gulewitsch, *Zeitschr. f. physiol. Chem.* **27.** 178 and 368 (1899).

[7] D. Lawrow, *ibid.* **28.** 585 (1899). [8] R. O. Herzog, *ibid.* **34.** 525 (1902).

[9] H. Steudel, *ibid.* **37.** 219 (1902).

The ornithuric acid, according to E. Fischer, is also dextro-rotatory:

$$\alpha_D = +7{\cdot}85.$$

On being heated with barium hydrate arginin becomes racemised (see p. 91). The two arginins differ from one another as regards polarisation, solubility, and shape of crystal.[1] Why fibrin yields much larger quantities of *r*-arginin than do other albumins is not known.[1]

The occurrence of inactive arginin has also been observed by Cathcart,[2] who subjected fibrin to 'urotryptic' digestion,[3] and coagulated serum to Hedin's spleen-enzyme: 'lieno-α-protease.' Cathcart points out that the optically active arginine complex may become racemised through the agency of the enzyme either before or after liberation from the rest of the proteid molecule, analogous to the partial racemisation of such compounds as atropine and amygdalin under the katalytic action of weak alkali, or, that the arginin complex of the proteid molecule is normally symmetrical and is liberated as the inactive form by certain enzymes, while it is converted into the optically active variety by the action of acids during the hydrolysis of albuminous substances.

Seemann's view as to how arginin is linked up in the albumin molecule is given on pages 246 and 247.

The part played by arginin in the metabolism of plants has been investigated by E. Schulze and N. Castoro.[4]

14. Histidin, $C_6H_9N_3O_2$, was discovered by Kossel[5] as a dissociation-product of sturin, the protamin of the spermatic fluid of the sturgeon, and also found by Hedin[6] in casein, egg-white, etc. Since then it has been shown by Kossel[7] and his pupils, and by E. Schulze,[8] to be a widely distributed dissociation-product. Except in a few protamins, histidin has been found in all albumins hitherto investigated. It is most abundant in globin, the albumin of hæmoglobin. It was first prepared by Kossel,[9] and again recently by Fränkel,[10] who used mercuric chloride as the precipitating agent; later

[1] F. Kutscher, *Zeitschr. f. physiol. Chem.* **28.** 88 (1899); **32.** 476 (1901).

[2] E. P. Cathcart, *Journ. of Physiol.* **32.** *Proceedings XV.*; also p. 300 and *Proceedings XXXIX.* (1905).

[3] Urotrypsin is that proteolytic enzyme found in urine which digests in an alkaline medium. See Cathcart's paper in Salkowski's *Festschrift* (1904).

[4] E. Schulze and N. Castoro, *Zeitschr. f. physiol. Chem.* **43.** 170 (1904).

[5] A. Kossel, *ibid.* **22.** 176 (1896).

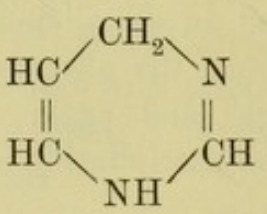
[6] S. G. Hedin, *ibid.* **22.** 191 (1896).

[7] See above under Arginin.

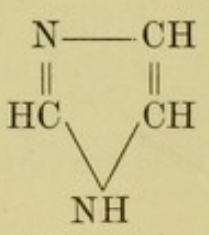
[8] S. Fränkel, *Sitzungsber. d. Akad. d. Wissensch. in Wien, mathem.-naturw. Kl.* **112.** Abt. II.ᵇ März 1903.

Kossel[1] described the method given above, namely, that of precipitating it with silver sulphate and barium hydrate, and then purifying it with mercuric sulphate.

The constitution of histidin has been studied by Herzog,[2] Fränkel,[3] Pauly,[4] and Knoop and Windaus.[5] Histidin possesses two hydrogen atoms replaceable by metals, one of which is in a carboxyl group, COOH, and the second attached to an imide radical, NH; it contains neither methoxyl nor methyl joined to nitrogen, nor a guanidin remainder; it is optically active, and therefore contains an asymmetric carbon atom; on being boiled with alkaline permanganate solution it yields hydrocyanic acid, carbonic acid, and ammonia; it resists oxidation by means of sulphuric acid + permanganate solution (Herzog), and by means of dilute nitric acid (Fränkel), and therefore it cannot be a dihydropyrimidin ring, as held by Fränkel (Pauly); on being boiled with barium hydrate solution it does not form ammonia; it gives a violet biuret-reaction (Herzog) and Ehrlich's diazo-reaction (Pauly) (see p. 10 and below); it replaces CO_2 from the carbonates of silver and copper, and gives off CO_2 on being heated above its melting-point, and therefore contains the carboxyl group, COOH (Fränkel); when acted upon by sodium hypobromite it liberates one atomic nitrogen atom, while sodium nitrite replaces 1 nitrogen by 1 oxygen (Fränkel); on being heated with lime it gives rise to ammonia and a substance which gives the pyrrol reaction; with ammonia and chlorine water it further gives Weidel's pyrimidin reaction. The firmness with which histidin resists oxidation by means of sulphuric acid + permanganate shows that two nitrogen atoms must be linked up in a ring-like form, a fact which led Fränkel to believe that histidin was dihydropyrimidin,



Dihydropyrimidin.



Imido-azol.

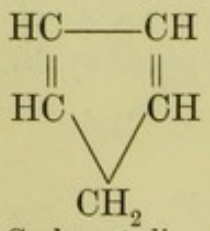
and Pauly to assume the existence of an imido-azol or glyoxal ring, because in such a ring the hydrogen atom of a CH_2 or of an NH-group may be replaced by a metal.

[1] A. Kossel and F. Kutscher, *Zeitschr. f. physiol. Chem.* **31**. 165 (1900); A. Kossel and A. J. Patten, *ibid.* **38**. 39 (1903). [2] R. O. Herzog, *ibid.* **37**. 248 (1902).

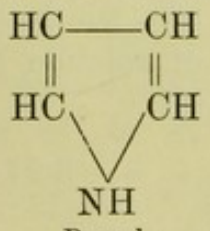
[3] S. Fränkel, *Sitzungsber. d. Akad. d. Wissensch. in Wien, mathem.-naturw. Kl.* **112**. Abt. II.[b] März 1903. [4] H. Pauly, *Zeitschr. f. physiol. Chem.* **42**. 508 (1904).

[5] F. Knoop and A. Windhaus, *Hofmeister's Beiträge*, **1**, 144 (1905).

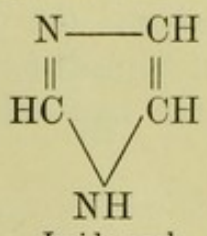
Pauly gives in support of his view the fact established by Pinner and Schwarz [1] that imido-azol derivatives are very susceptible to alkaline oxidising agents, while they resist acid oxidising media. The property possessed by the imide-group, of acting as a weak base as well as a weak acid, explains also its power of forming coloured compounds with diazonium salts, as do, *e.g.* cyclopentadien, pyrrol, and imido-azol.



Cyclopentadien.



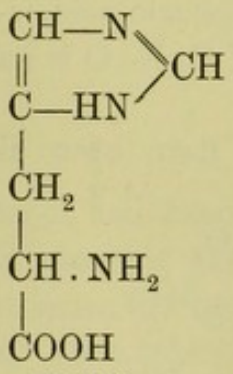
Pyrrol.



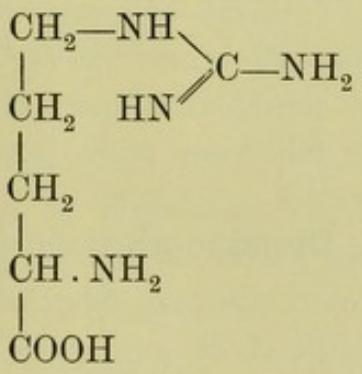
Imido-azol.

A solution of histidin in sodium carbonate gives with diazobenzene sulphanilic acid, in acid solutions, a pure orange, and in alkaline solutions a deep cherry-red colour. As no other tissue constituent gives this reaction, apart from tyrosin, the presence of histidin may always be readily ascertained in mixtures of albuminous dissociation-products and also in the native albumins, whenever by Millon's reaction the absence of tyrosin has been ascertained (see p. 10).

Pauly, taking into account the close relationship of arginin and histidin, gave, provisionally, the following formula for histidin :—



Histidin.



Arginin.

Pauly's view that histidin is α-amino-β-imido-azol propionic acid, Knoop and Windhaus have proved to be correct by synthesis, and therefore histidin should be placed amongst the ring compounds. The position of the NH_2-group is still uncertain. Herzog's discovery of a violet biuret reaction points to a terminal $.CONH_2$ group. Arginin does not give a biuret reaction. When albumins or peptones are digested tryptically or ereptically,[2] the originally intense biuret-reaction of the albumoses and peptones diminishes so much as to be only demonstrable on taking the greatest care in performing the

[1] Pinner and Schwarz, *Ber. d. deutsch. chem. Gesellsch.* **35**. 2448 (1902).

[2] O. Cohnheim, *Zeitschr. f. physiol. Chem.* **33**. 451 (1901), **35**. 134 (1902); K. Mays, *ibid.* **38**. 428 (1903).

test, and in many cases the reaction shows a different colour from the pure red of the peptone reaction. This feeble and impure biuret reaction was found frequently to resist strongly the dissociating action of ferments. The idea suggests itself that in these cases we are dealing with histidin derived from the peptones.

A full description of the salts of histidin is given by Kossel and Kutscher;[1] the picrolonic salt, which because of its slight solubility might be used for purposes of isolation, has been prepared by Steudel.[2] The free base is lævo-rotatory:

$$\alpha_D = -3{\cdot}974.$$

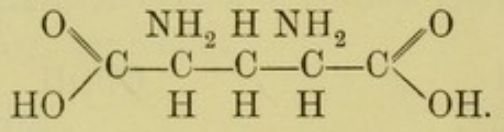
The salts are, however, dextro-rotatory. Bauer[3] has investigated the shape of the crystals. See, further, Pauly,[4] who also gives directions for the preparation of histidin.

The inter-relation of imido-azol compounds to grape sugar is referred to by the author in the chapter dealing with the carbohydrate radical of albumin.

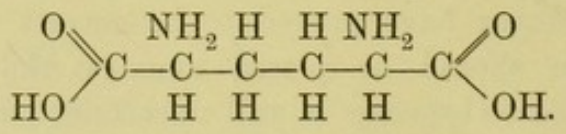
15. Diamino-trioxy-dodecanoic Acid, $C_{12}H_{26}N_2O_5$, is a saturated, aliphatic, oxy-amino-acid, reacting acid to litmus, with a feebly bitter taste and readily soluble in dilute acids. It has been prepared by Fischer and Abderhalden from the impure tyrosin-fraction obtained by the hydrolysis of casein with boiling sulphuric acid. It resembles Skraup's caseinic acid[5] as far as the ratio of C : N : O is concerned, but contains considerably more H.

The following acids (except No. 19) have been discovered by Skraup:[6]—

16. Diamino-glutaric Acid, $C_5H_{10}N_2O_4$



17. Diamino-adipic Acid, $C_6H_{12}N_2O_4$



Adipic acid is COOH—$(CH_2)_4$—COOH.

18. Diamino-dihydroxy-suberic Acid, $C_8H_{16}N_2O_6$.

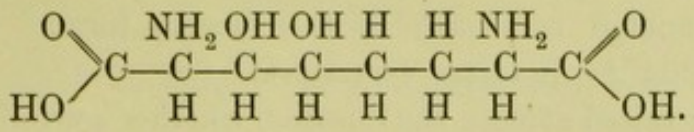
[1] A. Kossel and F. Kutscher, *Zeitschr. f. physiol. Chem.* **28.** 382 (1899).

[2] H. Steudel, *ibid.* **37.** 219 (1902). [3] M. Bauer, *ibid.* **22.** 285 (1896).

[4] Herm. Pauly, *ibid.* **42.** 508 (1904).

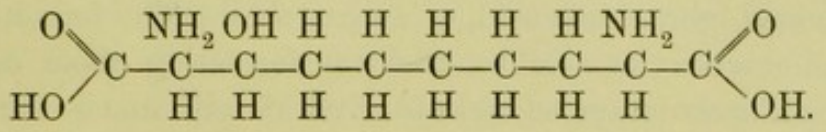
[5] Zd. H. Skraup, *Ber. d. deutsch. chem. Ges.* **37.** 1596 (1904).

[6] Zd. H. Skraup, *Sitzb. Akad. d. Wiss. Wien,* **113.** II.^{b} p. 263 (1904), and in *Zeitschr. f. physiol. Chem.* **42.** 276 (1904).



Suberic acid is $COOH(CH_2)_6 . COOH$.

19. Diamino-oxy-sebacic Acid, $C_{10}H_{20}N_2O_5$

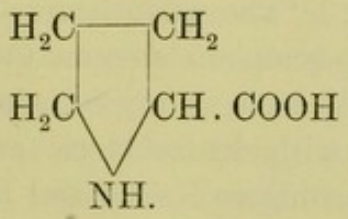


This acid was obtained by Wohlgemuth on hydrolysing liver-nucleo-proteid.[1]

20. Caseanic Acid, $C_9H_{16}N_2O_6$. This is a tribasic acid, and probably a diamino-oxy-compound. Its constitution is unknown.

21. Caseinic Acid, $C_{12}H_{16}N_2O_5$. This is a dibasic acid. It occurs in two modifications, of which the active, slightly dextro-rotatory fraction melts at 228°, while the other inactive fraction melts at 245°.

22. α-Pyrrolidin-carboxylic Acid, or **Prolin,**[2] $C_5H_9NO_2$



Soon after it had been first described by Willstätter,[3] E. Fischer[4] discovered it amongst the dissociation-products of casein, and since then its occurrence has been demonstrated in all proteids examined for its presence. (See tables, pp. 70-75.) According to E. Fischer and Abderhalden[5] it belongs to the anti-group.[6]

The acid occurring normally in albumins is, according to E. Fischer and Dörpinghaus,[7] *l*-α-pyrrolidin-carboxylic acid, having

$$\alpha_D^{20^\circ} = -47{\cdot}6.$$

[1] J. Wohlgemuth, *Ber. d. deutsch. chem. Ges.* **37**. 4362 (1904).

[2] E. Fischer has abbreviated α-pyrrolidin-carboxylic acid into "prolin." *Ibid.* **37**. 2843 (1904).

[3] R. Willstätter, *ibid.* **33**. I. 1160 (1900).

[4] E. Fischer, *Zeitschr. f. physiol. Chem.* **33**. 151 (1901).

[5] E. Fischer, and E. Abderhalden, *ibid.* **39**. 81 (1903).

[6] The evolution of the chemistry of pyrrol compounds during the last twenty-five years has been described by Giacomo Ciamician, *Ber. d. deutsch. chem. Ges.* **37**. 4200 (1904).

[7] E. Fischer and T. Dörpinghaus, *ibid.* **36**. 462 (1902).

A high percentage is usually in the raceme form. The melting-point is 203-204°.[1] It tastes very sweet.[2]

E. Fischer raised at once the question whether pyrrolidin-carboxylic acid is a primary dissociation-product, or whether the pyrrol-ring is liberated through the agency of the strong hydrochloric acid from another less dissociated disintegration product, such as an amino-valerianic acid, glutaminic acid, or an oxy-acid. The formation of the α-pyrrolidin-carboxylic acid by the transformation of an open chain into a ring-like structure he had observed himself under the action of hydrochloric acid.[3] On dissociating casein by means of 10 per cent soda solution, he obtained approximately the same amount of pyrrolidin-carboxylic acid as after the dissociation with hydrochloric acid,[4] but as during the esterification strong hydrochloric acid had to act on the dissociation-products, he does not as yet consider the question definitely settled.

The attempt to solve the question by tryptic digestion is not possible, as trypsin does not liberate the pyrrolidin-carboxylic acid at all.[5] But although the question is as yet an open one, E. Fischer[6] points out that the percentage-amounts of pyrrol-derivatives and of the diamino-acids agree. 'The quantitative relation between the cyclic acid and the diamino-compounds seems to be a general one, and makes it probable that both have an origin in common.'[7] On boiling, however, diamino-acids with hydrochloric acid no pyrrolidin-carboxylic acid is formed,[5] according to Kossel and Dakin.[8]

23. Hydroxy-α-pyrrolidin-carboxylic Acid, or **Hydroxyprolin,** $C_5H_9NO_3$.

This acid also was discovered by E. Fischer[9] amongst the dissociation-products of gelatine, but the position of the oxy-group is not yet settled. It crystallises from the residue after the esters of the amino-acids have been distilled off, and after the diamino-acids have been precipitated by means of phosphotungstic acid. It has also been found in casein,[7] globin, and edestin, and may be even more widely distributed. As to the possibility of this acid being formed secondarily, the same arguments hold good as were advanced for the previous acid. It is lævo-rotatory :

[1] E. Fischer, P. A. Levene, and R. H. Aders, *Ber. d. deutsch. chem. Ges.* **35**. 70 (1902).

[2] E. Fischer, *ibid.* **35**. III. 2660 (1902). [3] E. Fischer, *ibid.* **34**. I. 454 (1901).

[4] E. Fischer, *Zeitschr. f. physiol. Chem.* **35**. 227 (1902).

[5] E. Fischer and E. Abderhalden, *ibid.* **39**. 81 (1903)

[6] E. Fischer and E. Abderhalden, *ibid.* **36**. 268 (1902).

[7] E. Fischer, *ibid.* **39**. 155 (1903).

[8] Kossel, *Berliner klinische Wochensch.* No. 41, Oct. 1904, p. 1065.

[9] E. Fischer, *Ber. d. deutsch. chem. Ges.* **35**. III. 2660 (1902).

$$a_D = -81{\cdot}04.$$

It is very soluble in water; very slightly soluble in alcohol. It tastes sweet.

24. Phenylalanin, $C_9H_{11}NO_2$

$$C_6H_5.CH_2.CH(NH_2).COOH,$$

is phenyl-α-amino-propionic acid. E. Schulze[1] first succeeded in isolating it from germinating plants, although its occurrence in albumin had previously been assumed, because, when albumin is decomposed by putrefactive bacteria, there are formed,—in addition to phenol, cresol, oxyphenyl-acetic and oxyphenyl-propionic acids, which are derived from tyrosin,—also phenyl-ethylamin, phenyl-acetic, and phenyl-propionic acids. Nencki[2] and Salkowski[3] assumed the last mentioned substances to be derived from the mother substance phenyl-amino-propionic acid. Guckelberger,[4] Maly,[5] Bernert,[6] Pick,[7] Ducceschi,[8] and Spiro[9] also found derivatives of a non-hydroxylated amino-acid. E. Fischer and his pupils succeeded in demonstrating the occurrence of phenylalanin in all the proteids they investigated, and indeed, as Nencki[10] had already assumed, in such quantities that it was at least equal to, and in many cases in excess of, the other aromatic body present, namely, tyrosin (compare tables on pp. 70-75). In the albumin-molecule, phenylalanin occurs, along with glycocoll and α-pyrrolidin-carboxylic acid, only in the so-called anti-group,[11] and is therefore characteristic of this group, in which the tyrosin radical is absent.

The naturally occurring phenylalanin is the *l*-variety.[12] E. Schulze and Winterstein[13] determined

$$a_D = -38{\cdot}1 \text{ to } 40{\cdot}2.$$

[1] E. Schulze and E. Bosshard, *Zeitschr. f. physiol. Chem.* **9**. 63 (1884); E. Schulze, *ibid.* **17**. 193 (1892).

[2] M. Nencki, *Monatshefte für Chemie*, **10**. 506, 526, 862, 864, 908 (1889).

[3] E. and H. Salkowski, *Zeitschr. f. physiol. Chem.* **8**. 417 (1884), **9**. 8 (1884), **9**. 491 (1885); E. Salkowski, *Die Lehre vom Harn*, p. 26, 1872; *Ber. d. deutsch. chem. Ges.* **34**. III. 3884 (1901).

[4] Guckelberger, *Liebig's Annalen*, **64**. 39 (1848).

[5] R. Maly, *Monatshefte f. Chemie*, **10**. 26 (1889).

[6] R. Bernert, *Zeitschr. f. physiol. Chem.* **26**. 272 (1898).

[7] E. P. Pick, *ibid.* **28**. 219 (1899).

[8] V. Ducceschi, *Hofmeister's Beiträge*, **1**. 338 (1901).

[9] K. Spiro, *ibid.* **1**. 347 (1901).

[10] M. Nencki, *Monatsh. f. Chem.* **10**. 506 (1889).

[11] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899); E. Fischer and E. Abderhalden, *ibid.* **39**. 81 (1903).

[12] E. Schulze and E. Winterstein, *ibid.* **35**. 299 (1902); E. Fischer and A. Mouneyrat, *Ber. d. deutsch. chem. Ges.* **33**. II. 2383 (1900).

[13] E. Schulze and E. Winterstein, *Zeitschr. f. physiol. Chem.* **35**. 299 (1902).

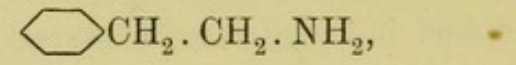
E. Fischer,[1] who did not succeed in preparing a perfectly pure *l*-phenylalanin, found for the *d*-phenylalanin

$$a_D = +35{\cdot}08.$$

A ready means of preparing racemic phenylalanin by the action of ammonia on phenyl-brom-propionic acid is also described by E. Fischer.[2]

Phenylalanin is not readily soluble, as one part requires, according to E. Fischer,[3] 35·3; according to Schulze and Winterstein,[4] 39·5 parts of water. Phenylalanin has a sweet taste.[5] On the oxidation of phenylalanin with sulphuric acid and bichromate, the characteristic smell of phenyl-acet-aldehyde is developed.[6] In contrast to the other mono-amino acids, which are either not precipitated at all or only by very strong solutions of phosphotungstic acid, phenylalanin is precipitated by 0·25 per cent solutions,[7] a fact which Schulze and Winterstein[7] made use of for its isolation. They recommend germinating *Lupinus luteus* and *L. albus*, two to three weeks old, as most suitable for the preparation of phenylalanin.[8]

Phenyl-ethylamin, $C_8H_{11}N$



is a derivative of phenylalanin and is basic in character. It was first discovered by Nencki[9] in putrefying gelatine, and identified by him as phenyl-ethylamine[10] after E. Schulze[11] had demonstrated the occurrence of phenylalanin in germinating lupine. Phenylethylamine is obtained from phenylalanin by dry distillation, and is formed by a CO_2 group being split off from the phenylalanin. Spiro[12] has shown that the phenylethylamine obtained by putrefaction is identical with that prepared synthetically.

[1] E. Fischer and A. Mouneyrat, *Ber. d. deutsch. chem. Ges.* **33**. II. 2383 (1900).

[2] E. Fischer, *ibid.* **37**. 3064 (1904).

[3] E. Fischer and A. Mouneyrat, *ibid.* **33**. II. 2383 (1900).

[4] E. Schulze and E. Winterstein, *Zeitschr. f. physiol. Chem.* **35**. 299 (1902).

[5] E. Fischer, *Ber. d. deutsch. chem. Ges.* **35**. III. 2660 (1902).

[6] E. Fischer, *Zeitschr. f. physiol. Chem.* **33**. 151 (1901).

[7] E. Schulze and E. Winterstein, *ibid.* **33**. 574 (1901), **35**. 210 (1902). Hausmann has denied the precipitability (*ibid.* **29**. 138), but he is in the wrong, for Kutscher and Lohmann, *ibid.* **44**. 384 (1905), have confirmed Schulze and Winterstein.

[8] E. Schulze and E. Winterstein, *ibid.* **35**. 299 (1902).

[9] M. Nencki, *Über d. Zersetzung der Gelatine und des Eiweisses bei der Fäulniss*, Bern, 1876; Verlag Dalp.

[10] M. Nencki, *Sitzb. d. kais. Akad. d. Wiss. in Wien*, 1889.

[11] E. Schulze and Barbieri, *Journ. f. prakt. Chem.* **29**. 331 (1888), and **14**. 1785 (1881).

[12] K. Spiro, *Hofmeister's Beit.* **1**. 347 (1901).

25. Tyrosin, $C_9H_{11}NO_3$

$$HO\langle\bigcirc\rangle CH_2 . CH(NH_2) . COOH$$

is phenyl-*p*-hydroxy-*a*-amino-propionic acid. It was one of the first discovered dissociation-products, and was estimated quantitatively at an early period because of its slight solubility. Even now the best method of estimating it consists in freeing the mixture of dissociation-products from sulphuric acid, barium hydrate, or hydrochloric acid, and then inspissating the remainder, when tyrosin crystallises out fairly completely and approximately pure (for its separation from leucin, see p. 23). In contrast to phenylalanin, it is characteristic of the hemi-group of the albumin molecule,[1] and is therefore absent in gelatine and in hetero-albumose, also in some protamins.

The occurrence of tyrosin in plants has been definitely established by Sack and Tollens,[2] who found it in the berries of the elder (*Sambucus nigra*, L.); by Steudel,[3] and by Schulze and Winterstein,[4] who discovered it in potatoes and in germinating cucumber.

The naturally occurring variety is *l*-tyrosin.[5] E. Fischer[5] found for a solution of tyrosin in 21 per cent hydrochloric acid

$$a_D = -8{\cdot}64.$$

Schulze and Winterstein discovered, however, that tyrosin differs from other amino-acids in showing the greatest amount of rotation in 4 per cent hydrochloric acid, for which strength

$$a_D = -14{\cdot}6 \text{ to } 16{\cdot}1.$$

By splitting racemic tyrosin into its optically active components E. Fischer obtained in 4 per cent HCl

$$a_D = +16{\cdot}4,$$

while Schulze and Winterstein have prepared from germinating lupine a tyrosin having

$$a_D = -16{\cdot}2.$$

[1] W. Kühne, *Verh. d. Heidelberger naturhist. med. Vereins*, N.F. III. 286 (1885); W. Kühne and R. H. Chittenden, *Zeitschr. f. Biologie*, **22**. 423 (1886); E. P. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899); E. Fischer and P. Bergell, *Chemikerzeitung*, 1902, II. p. 939; E. Fischer and E. Abderhalden, *Zeitschr. f. physiol. Chem.* **39**. 81 (1903).

[2] J. Sack, Dissertation, Göttingen, 1901; and J. Sack and B. Tollens, *Ber. d. deutsch. chem. Ges.* **37**. 4115 (1904).

[3] H. Steudel, *Deutsche med. Wochenschr.* 1900, p. 273.

[4] E. Schulze and E. Winterstein, *Zeitschr. f. physiol. Chem.* **35**. 299 (1902), and **45**. 79 (1905).

[5] E. Fischer, *Ber. d. deutsch. chem. Ges.* **32**. III. 3638 (1899).

They suggest that figures lying between these extremes are obtained owing to an admixture of racemic tyrosin.

Tyrosin is very slightly soluble in water. It is nearly tasteless like chalk, and in this respect resembles phenyl-amino-acetic acid ($C_6H_5 . CH(NH_2) . COOH$), (Fischer).

Tyrosin gives a number of characteristic colour reactions :—

(*a*) *Millon's Reaction.*—If one boil a fluid containing tyrosin with Millon's reagent, a solution of mercurous nitrate in dilute nitric acid (see p. 7), the fluid turns red, and the depth of colour, from pink to a reddish black, depends on the amount of tyrosin present. This reaction is given by all benzene-derivatives in which one hydrogen atom has been replaced by a hydroxyl group. Nasse [1] has made a special study of the behaviour of different benzene derivatives towards Millon's reagent. Substitution of the other benzene hydrogen atoms by halogens destroys Millon's reaction.[2] Not only tyrosin, but albumin itself gives the reaction (see p. 7).

(*b*) *Piria's Reaction.*[3]—Tyrosin is added in a solid form to a small quantity of sulphuric acid ; this mixture is then neutralised with barium carbonate, and is filtered. On adding to the solution of barium-tyrosin sulphate a very dilute solution of ferric chloride, a deep violet colour results, owing to the formation of iron-tyrosin sulphate.

(*c*) *Mörner's Reaction.*[4]—Mix formaldehyde 1 part, water 45 parts, and concentrated sulphuric acid 55 parts. When this solution, which is permanent, is boiled with tyrosin, a green colour is obtained.

(*d*) *Bertrand's Reaction.*[5]—Very characteristic is also the darkening of tyrosin which is produced by vegetable oxydases, the so-called tyrosinases. Extract mushrooms (*Russula nigricans*) with water and precipitate with alcohol. The precipitate dissolved in water colours tyrosin in a few hours first red, then black (see also p. 89).

(*e*) Ehrlich-Pauly's diazo-reaction has already been described on p. 9.

The largest amount of tyrosin is obtainable from the fibroin of silk [6] and from zein,[7] next from keratin, casein, and protalbumose (compare tables on pp. 70-75).

[1] O. Nasse, *Pflüger's Arch. f. d. ges. Physiol.* **83**. 361 (1901).

[2] F. Blum and W. Vaubel. *Journ. f. prakt. Chem.* (2) **56**. 393 (1897) ; (2) **57**. 365 (1898) ; A. Oswald, *Hofmeister's Beitr.* **3**. 391 (1903).

[3] R. Neumeister, *Lehrbuch d. physiol. Chem.* 2. Aufl. Jena, G. Fischer, 1897, p. 250.

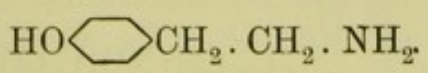
[4] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **37**. 86 (1902).

[5] G. Bertrand, *Compt. rend.* **123**. 463 (1896).

[6] E. Fischer and A. Skita, *Zeitschr. f. physiol. Chem.* **33**. 177 (1901).

[7] F. Kutscher, *ibid.* **38**. 111 (1903).

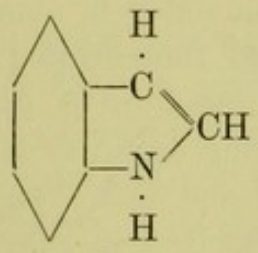
Oxyphenyl ethylamin, $C_8H_{11}ON$



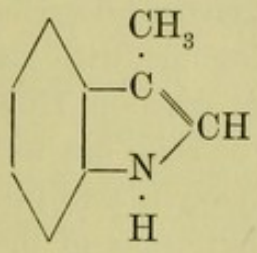
It is derived from tyrosin by a splitting off of a CO_2 group.[1] It has been found by Emerson[2] amongst the products of autodigestion of the pancreas, and by Langstein[3] amongst the final products of peptic digestion.

26. Tryptophane, or **Indol-amino-propionic Acid,** $C_{11}H_{12}N_2O_2$. (The constitutional formula is given below.)

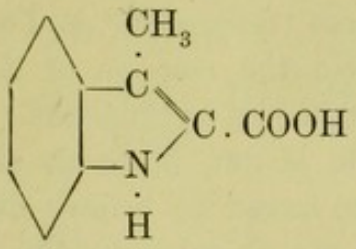
Nencki,[4] and Kühne,[5] and, later on, particularly E. and H. Salkowski,[6] found amongst the products of putrefying albumin the substances indol and skatol, and their derivatives skatol-carboxylic[7] and skatol-acetic acids.[4 8]



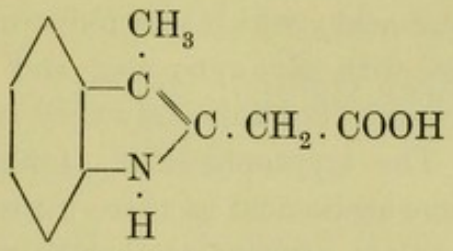
Indol.



Skatol
(or β-methyl-indol).



Skatol-carboxylic acid.



Skatol-acetic acid.

The mother-substance of these bodies Nencki and Salkowski[9] believed to be skatol-amino-acetic acid.

[1] R. Schmitt and O. Nasse, *Ann. d. Chem. u. Pharm.* **133**. 311 (1865).

[2] R. L. Emerson, *Hofmeister's Beitr.* **1**. 501 (1902).

[3] L. Langstein, *ibid.* **1**. 507 (1901).

[4] M. Nencki, *Ber. d. deutsch. chem. Ges.* **7**. II. 1593 (1874), **8**. I. 336 (1875), **10**. I. 1032 (1877); *Monatshefte f. Chem.* **10**. 506, 526, 862, 864, 908 (1889).

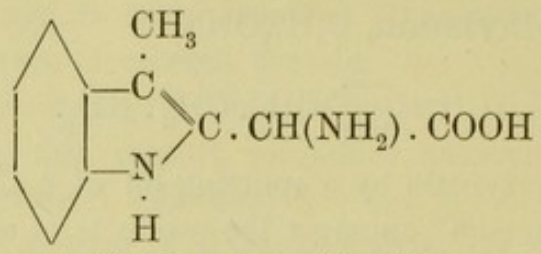
[5] W. Kühne, *Ber. d. deutsch. chem. Ges.* **8**. I. 206 (1875).

[6] E. and H. Salkowski, *Zeitschr. f. physiol. Chem.* **8**. 417 (1884), **9**. 8 (1884), **9**. 491 (1885).

[7] E. and H. Salkowski, *Ber. d. deutsch. chem. Ges.* **13**. I. 189 (1880); **13**. II. 2217 (1880).

[8] E. Salkowski, *Zeitschr. f. physiol. Chem.* **27**. 309 (1899).

[9] E. Salkowski, *Die Lehre vom Harn*, p. 26 (1882); *Ber. d. deutsch. chem. Ges.* **34**. III. 3884 (1901).



Skatol-amino-acetic acid.

In addition to these substances, Tiedemann and Gmelin, then Kühne,[1] described a peculiar chromogen amongst the tryptic disintegration-products of albumin which, in an acid solution, gives a violet colour with bromine or chlorine water. Stadelmann[2] calls the substance proteinochrome, while Neumeister[3] gave it the name of tryptophane. Winternitz,[4] Nencki,[5] Beitler,[6] and Kurajeff[7] have attempted to isolate it.

Adamkiewicz[8] described a colour reaction which is obtainable by treating albumins with glacial acetic acid and concentrated sulphuric acid. This reaction Hopkins and Cole[9] showed to be due, not to glacial acetic acid, but to a commonly occurring impurity in this acid, namely, glyoxylic acid (see p. 9). Hopkins and Cole[10] succeeded in isolating, by means of mercuric sulphate dissolved in sulphuric acid, from the mixture of substances set free by tryptic digestion, or by the action of acids, a body which they believed to be skatol-amino-acetic acid; which was indistinguishable from tryptophane, and which gave with glyoxylic acid and sulphuric acid the reaction of Adamkiewicz. For this body they retained the name of tryptophane.

The tryptophane of Hopkins and Cole is not, however, skatol-amino-acetic acid as these investigators were forced to believe on the prevalent assumption that Salkowski's acid was 'skatol-carboxylic acid.' Gentzen[11] has shown that skatol administered subcutaneously, or by the mouth, or injected into the large intestine, never leads to the

[1] W. Kühne, *Verhandl. d. Heidelberger naturh.-med. Vereins*, N.F. I. 236, III. 467 (1886).

[2] E. Stadelmann, *Zeitschr. f. Biol.* **26**. 491 (1890).

[3] R. Neumeister, *ibid.* **26**. 324 [Anm. p. 329 ff.] (1890).

[4] H. Winternitz, *Zeitschr. f. physiol. Chem.* **16**. 460 (1892).

[5] M. Nencki, *Ber. d. deutsch. chem. Ges.* **28**. I. 560 (1895).

[6] C. Beitler, *ibid.* **31**. II. 1604 (1898).

[7] D. Kurajeff, *Zeitschr. f. physiol. Chem.* **26**. 501 (1891).

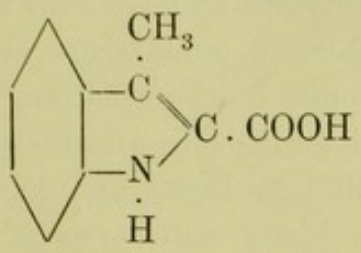
[8] A. Adamkiewicz, *Pflüger's Arch. f. d. ges. Physiol.* **9**. 156 (1874); *Ber. d. deutsch. chem. Ges.* **8**. I. 161 (1875).

[9] F. G. Hopkins and S. W. Cole, *Proc. Roy. Soc.* **68**. 21 (1901).

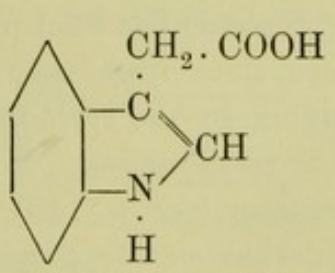
[10] F. G. Hopkins and S. W. Cole, *Journ. of Physiol.* **27**. 418 (1901), **29**. 451 (1903).

[11] P. Gentzen, *Über die Vorstufen des Indols bei der Eiweissfäulnis im Tierkörper*, Inaugural Dissertation, Königsberg, 1904.

formation of indican, while tryptophane prepared by the method of Hopkins and Cole on being injected into the larger intestine invariably led to the appearance of indican in the urine. He therefore comes to the conclusion that tryptophane cannot be a skatol-compound, but must be an indol-derivative, and Ellinger [1] has proved by synthetic work that Salkowski's acid is really not a skatol-carboxylic acid, but an indol-acetic acid.

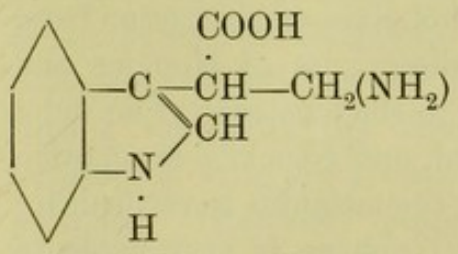


Skatol-carboxylic acid.

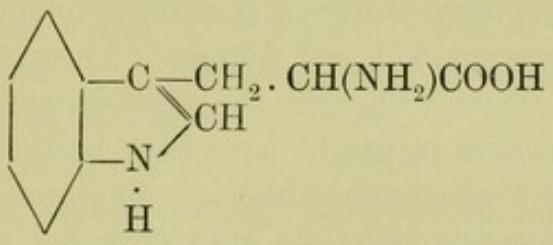


Indol-acetic acid.

Either of these alternative constitutions will explain the yield of skatol and CO_2 on putrefaction. Now, skatol-carboxylic acid being recognised as indol-acetic acid, it is clear that 'skatol-acetic' must be indol-propionic acid, and therefore tryptophane an indol-amino-propionic acid. Ellinger [2] has also found that Hopkin's tryptophane, fed to dogs, gives kynurenic acid in the urine, a fact which inclines him to ascribe to it the constitution of indol-β-amino-propionic acid.



Tryptophane (Ellinger).



Tryptophane (Hopkins).

Ellinger's formula would further explain the tendency towards the closure of the pyridin-ring in tryptophane, and would make the assumption of a pyridin-nucleus in the albumin-molecule [3] superfluous, a view which was based on the fact that melanoidins form pyridin on being reduced (Samuely).[4] According to Ellinger a genetic relationship exists between tryptophane and many of the pyridin and quinoline derivatives in plants.

Hopkins [5] thinks it unlikely that Ellinger's view is correct, because

[1] Alexander Ellinger, *Ber. d. deutsch. chem. Gesellsch.* **37**. 1801 (1904).

[2] Alexander Ellinger, *Zeitschr. f. physiol. Chem.* **43**. 325 (1904).

[3] Hofmeister, *Ergebnisse d. Physiol.* **1**. 768 (1902).

[4] Samuely, *Hofmeister's Beitr.* **2**. 355 (1902).

[5] Hopkins, private communication, January 1905.

tryptophane would then not be an α-amino-acid.[1] Only a synthesis can, however, clear up this matter. See also under Kynurenic Acid, p. 84.

Tryptophane, as prepared by the method of Hopkins and Cole, has very strong reducing powers, as the chlorides of gold, platinum, and palladium are reduced in a few seconds, the metals passing through a soluble colloidal state into an insoluble form (Mann).[2]

Tryptophane has been prepared by Hopkins and Cole up till now in a pure state from casein, fibrin, egg-white, and serum albumin; but, judging by the tryptophane reaction and indol formation, it must exist in most albumins. If one divide albumins into a hemi- and into an anti-group (see p. 148) then, according to Pick[3] and E. Fischer and Abderhalden,[4] tryptophane belongs, along with tyrosin, to the hemi-group. It is, therefore, absent from gelatine[5] and from hetero-albumose.[3]

In the older works of Nencki, Beitler, and Kurajeff mention is made of several colouring matters, but it is difficult to say whether one is dealing simply with impurities, or whether tryptophane may be bromated to different degrees, or whether, in addition to tryptophane, still other chromogens are present in albumins. The bromo-tryptophane is insoluble in water, chloroform, benzene, ether, and petroleum ether, very slightly soluble in alcohol, but readily soluble in acetic ester and amyl-alcohol. A solution in amyl-alcohol gives an absorption band between 571 and 540 $\mu\mu$. Through the discoveries of Hopkins and Cole, and Ellinger, the older statements as to the existence of an indol nucleus in tryptophane have been confirmed, and Nencki's assumption of a relationship between tryptophane and the melanins is verified, for Hopkins and Cole have observed that tryptophane is very liable to change into brown-coloured substances on being boiled with acids or merely with water. For this reason tryptophane is usually absent amongst the dissociation-products, which are obtained by treating albumins with acids.

The derivatives of tryptophane are carriers of still another colour reaction—the so-called pyrrol reaction. If one immerse a chip of pinewood, *e.g.* a wooden match, into strong hydrochloric acid, and then place it in a watery solution of indol, it will gradually become of

[1] Levene has described amino-acids which are not α-amino-acids (Levene, *Zeitschr. f. physiol. Chem.* **41**. 100 (1904).

[2] Gustav Mann, *Physiological Histology*, 1902, p. 269.

[3] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899).

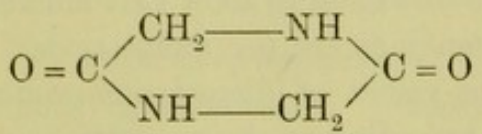
[4] E. Fischer and E. Abderhalden, *ibid.* **39**. 81 (1903).

[5] R. Maly, *Monatsh. f. Chem.* **10**. 26 (1889); M. Nencki, *Ber. d. deutsch. chem. Ges.* **7**. II. 1593 (1874).

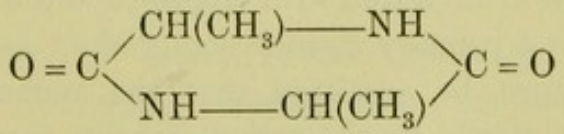
a cherry-red colour. Colour reactions of skatol-carboxylic acid have been described by Salkowski.[1]

Diacipiperazin-Compounds.

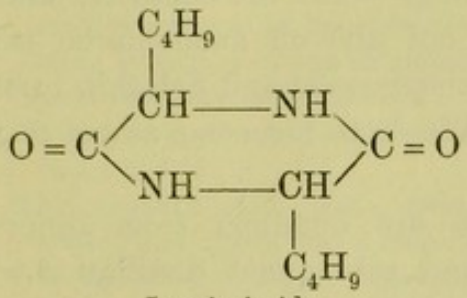
According to Fischer, see p. 132, diacipiperazins occur in all probability in the albumin-molecule. He first discovered a glycocoll-alanin compound amongst the products of partially digested silk-fibrin, and subsequently manufactured a similar, if not identical compound, see p. 127. The four diacipiperazins which Fischer has synthetised are :—



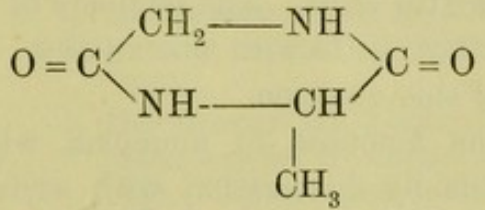
Diglycocoll anhydride.



Di-alanin-anhydride.



Leucin-imide.



Glycin-alanin-anhydride.

27. Ammonia, NH_3. Nasse[2] was the first to accurately study the splitting off of ammonia from albuminous substances. He as well as subsequent investigators—Hlasiwetz and Habermann,[3] Kossel and Kutscher,[4] E. Fischer and his pupils,[5] and various others—agree in stating that after the dissociation of albumins by means of acids, ammonium salts are always met with in the mixture of dissociation-products. Hirschler,[6] Stadelmann,[7] and Kutscher[8] observed its formation also after tryptic digestion. Ammonia ought to be regarded, therefore, as a primary dissociation-product, were it not for the possibility that it may be formed secondarily out of some primary dissociation-product, as soon as endeavours are made to isolate it by distillation with fixed alkalies. For Nasse has shown that a great deal more ammonia is obtained if the dissociation of albumin is

[1] E. Salkowski, *Zeitschr. f. physiol. Chem.* **9**. 8 and 23 (1884).

[2] O. Nasse, *Pflüger's Arch. f. d. ges. Physiol.* **6**. 598 (1872), **7**. 139 (1872), **8**. 381 (1874).

[3] H. Hlasiwetz and J. Habermann, *Liebig's Ann.* **169**. 150 (1873).

[4] A. Kossel and F. Kutscher, *Zeitschr. f. physiol. Chem.* **31**. 165 (1900).

[5] See p. 20, footnote 9.

[6] A. Hirschler, *ibid.* **10**. 302 (1886).

[7] E. Stadelmann, *Zeitschr. f. Biol.* **24**. 261 (1888).

[8] F. Kutscher, *Endprodukte der Trypsinverdauung*, Marburger Habilitationsschrift, Strasburg, 1899.

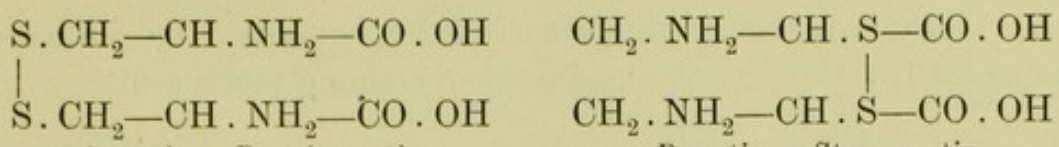
brought about with alkalies instead of with acids, and there cannot be any doubt that the usual method of distilling with magnesia does not only yield preformed ammonia. For this reason Hart[1] distils the mixture of dissociation-products resulting from the action of sulphuric acid, with barium carbonate, and obtains ammonia in smaller quantities. Dzierzgowski and Salaskin[2] have studied the formation of ammonia according to the method of Nencki and Zaleski,[3] after peptic and tryptic digestion, and Cohnheim[4] has done the same after digestion with erepsin, and the results seem to show that ammonia is a primary dissociation-product as much as are the amino-acids. It must be admitted that the ammonia values of dissociation-products obtained by the action of acids vary according to the concentration of the acid and the duration of the boiling,[5] while the definitely known dissociation-products of albumins do not give off ammonia on being boiled with acids. The numbers of Dzierzgowski and Salaskin further agree but little with one another. We have not come as yet to the end of this question.

The amounts of ammonia which are obtained from different proteids by dissociation with acids and subsequent distillation with magnesia, have been determined quantitatively by Hausmann,[6] Kossel and Kutscher,[7] Friedmann,[8] Henderson,[5] Osborne and Harris,[9] Schulze,[10] Pick,[11] and others. Most of these numbers will be found in the tables on pp. 70-75. (See also p. 77.) The amount of ammonia varies from 4 per cent in some vegetable proteids to 0·4 per cent in gelatine. Ammonia seems to be absent only in some of the protamins.

28. Cystin, $C_6H_{12}O_4N_2S_2$.

Cystin occurs in nature in two distinct forms, in the one the amino-group is in the α-position = A-cystin or Protein-cystin, while in the other the amino group is in the β-position = B-cystin or Stone-cystin.[12]

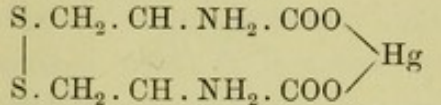
[1] E. Hart, *Zeitschr. f. physiol. Chem.* **33**. 347 (1901).
[2] S. Dzierzgowski and S. Salaskin, *Zentralbl. f. Phys.* **15**. 249 (1901).
[3] M. Nencki and J. Zaleski, *ibid.* **33**. 193 (1901).
[4] O. Cohnheim, *Zeitschr. f. physiol. Chem.* **35**. 134 (1902).
[5] Y. Henderson, *ibid.* **29**. 47 (1899).
[6] W. Hausmann, *ibid.* **27**. 75 (1899), **29**. 136 (1900).
[7] A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900).
[8] E. Friedmann, *ibid.* **29**. 50 (1899).
[9] T. B. Osborne and J. F. Harris, *Journ. Amer. Chem. Soc.* **25**. 323 (1903).
[10] E. Schulze, *Zeitschr. f. physiol. Chem.* **25**. 360 (1898).
[11] E. P. Pick, *ibid.* **28**. 219 (1899).
[12] The author proposes to distinguish the two cystins as A and B, for both are met with in proteids and in urinary calculi.



A-cystin or Protein-cystin
(α-amino-β-thioglyceric acid 'disulphide.')

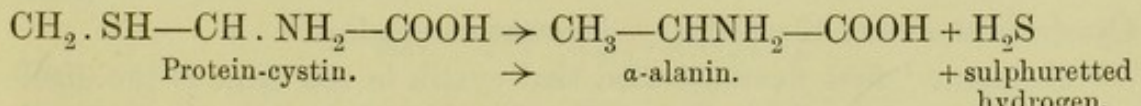
B-cystin or Stone-cystin
(α-thio-β-amino-glyceric acid 'disulphide.')

Both cystins may be regarded as sulphuretted serins (see p. 33); being isomers they resemble one another in certain respects, but differ markedly in the following points, according to Neuberg and Mayer [1]

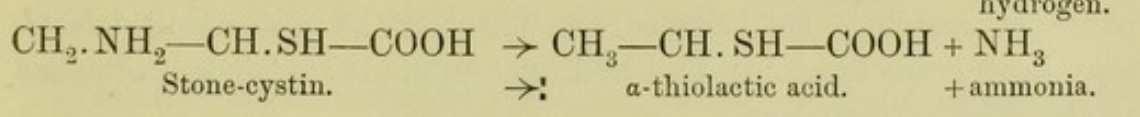
A-cystin or Protein-cystin.	B-cystin or Stone-cystin.
1. Pure, optically active compound crystallises in six-sided plates.	1. Pure, optically active compound crystallises in needles.
2. Racemic compound crystallises in needles arranged in bunches resembling tyrosin, and also in globules.	2. Racemic cystin is amorphous, and cannot be made to crystallise by means of protein-cystin.
3. Specific rotatory power is – 224°.	3. Specific rotary power is – 206.
4. No melting point; decomposes between 258-261°.	4. Melts at 190-192°, giving rise to a foam.
5. The mercury salt, made from 1 molecule of cystin and 2 molecules of NaOH and $HgCl_2$ corresponds exactly to the formula  It is very stable and pure white.	5. The mercury salt prepared in the same way has no constant composition; it becomes reddish brown on drying, and then black.

In addition to these characteristics Neuberg and Mayer give six other points of difference, so that there cannot be any doubt as to real existence of two isomeric cystins.

Protein-cystin when dissociated with HCl under pressure gives rise in addition to H_2S and NH_3 to α-alanin, while stone-cystin gives rise to α-thiolactic acid, according to K. A. H. Mörner,[2] who was the first to point out the existence of two kinds of sulphur atoms in albumin.



Protein-cystin. → α-alanin. + sulphuretted hydrogen.

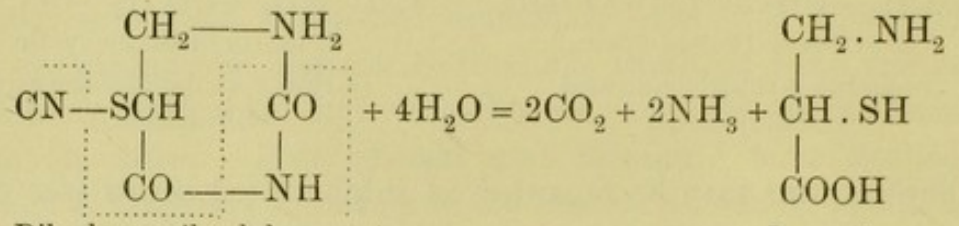


Stone-cystin. → α-thiolactic acid. + ammonia.

An inactive modification of what Neuberg and Mayer call 'stone-cystein' has been prepared by Gabriel,[3] who calls his compound iso-

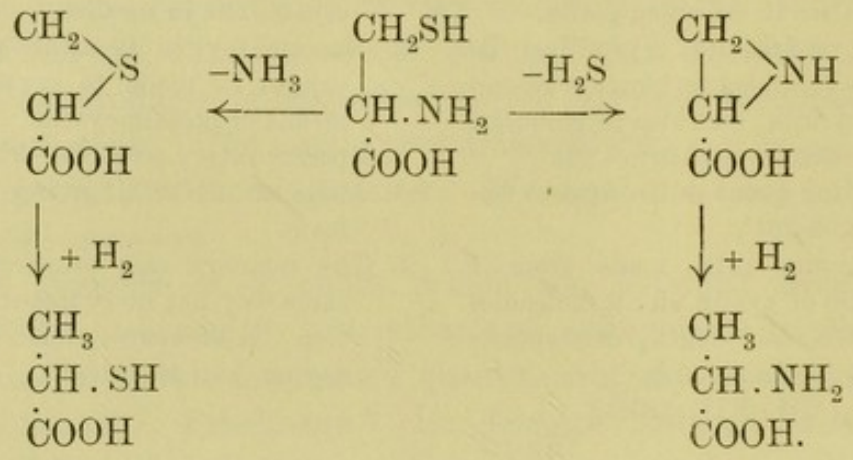
[1] Carl Neuberg and Paul Mayer, *Zeitschr. f. physiol. Chem.* **44**. 472 (1905).
[2] K. A. H. Mörner, *ibid.* **34**. 295 (1902).
[3] S. Gabriel *Ber. d. deutsch. chem. Gesell.* **38**. 637 (1905).

cystin. By dissociating dihydrouracil sulphocyanate by means of fuming HCl at 170°, he obtained α-thio-β-amino glyceric acid :



Dihydrouracil sulphocyanate. Isocystin.

Gabriel derives both α-alanin and α-thio-lactic acid (see above) from protein-cystin or α-amino-β-thioglyceric acid "disulphide" according to the following scheme :

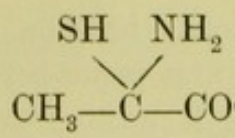


Historical Account.—Cystin was first discovered in 1810 by Wollaston[1] in a urinary calculus. Külz[2] found it occasionally and in small quantities on digesting fibrin with trypsin ; Emmerling[3] on dissociating horny substances with acids, but subsequently it was shown by Mörner[4] and Embden[5] to be a constant and abundant dissociation-product of most albumins.

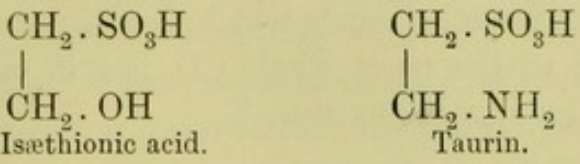
Mörner and Embden have also found cystein, along with, and in place of, cystin. Embden took cystein to be the primary dissociation-product ; Patten,[6] however, has shown that in making cystin, a part of it is converted into cystein, while the converse does not hold good. Cystin is therefore the primary dissociation-product.

Baumann[7] first demonstrated that cystin in the urine is the disulphide of cystein. He believed the latter to be α-α-amino-thiolactic acid, having the ammonia- and hydrogen-sulphide remainders attached to the same carbon atom.

[1] W. H. Wollaston, *Phil. Trans.* 1810, pp. 223-330.
[2] E. Külz, *Zeitschrift für Biologie*, **27**. 415 (1890).
[3] A. Emmerling, *Verhandl. d. Ges. deutsch. Naturforscher u. Ärzte*, 1894, II. **2**. 391.
[4] K. A. H. Mörner, *Zeitschr. f. physiol. Chem.* **28**. 595 (1899), **4**. 207 (1901).
[5] G. Embden, *ibid.* **32**. 94 (1900). [6] A. J. Patten, *ibid.* **39**. 350 (1903).
[7] E. Baumann, *ibid.* **8**. 299 (1884).

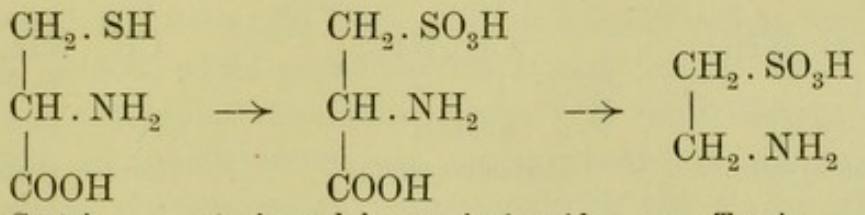


That the SH and the NH_2 radicals are not attached to the same C atom was first shown by C. Neuberg[1] and E. Friedmann.[2] Neuberg prepared isæthionic acid by oxidising cystein directly with nitric acid. Isæthionic acid bears to taurin the same relation as does an oxy-acid to an amino-acid.



Isæthionic acid. Taurin.

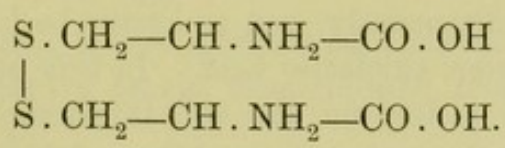
Friedmann oxidised cystein to amino-sulpho-propionic acid, and the latter by removal of CO_2 into taurin:



Cystein. Amino-sulpho-propionic acid. Taurin.

Cystin is lævo-rotatory, but being an amino-acid it becomes partly racemised when it is acted upon by acids; these two cystins Mörner showed to differ from one another also in other properties, for the active cystin crystallised in six-sided plates, while the inactive cystin formed needles resembling tyrosin, which substance also frequently accompanies cystin.[3] Mörner prepared cystin by decomposing horn filings or human hair with hydrochloric acid, removing the acid, crystallising out the cystin and tyrosin, and finally separating these by means of fractional crystallisation. Patten precipitated it with mercuric sulphate dissolved in sulphuric acid.

In his second paper Friedmann[2] stated cystin to be α-diamino-β-dithio-dilactylic acid.



This formula corresponds, therefore, to the one given subsequently by Neuberg and Mayer[4] to their 'protein-cystin.' Cystin would,

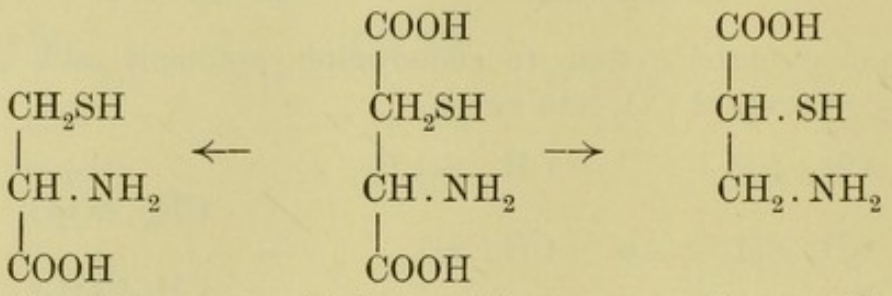
[1] C. Neuberg, *Ber. d. deutsch. chem. Ges.* **35**. 3161 (1902).

[2] That optically active stone-cystin also crystallises in needles has already been pointed out when comparing protein- and stone-cystins on p. 57.

[3] E. Friedmann, *Hofmeister's Beitr.* **2**. 433, and **3**. 1 (1902).

[4] C. Neuberg and P. Mayer, *Vortrag in d. d. chem. Gesellsch. zu Berlin*, May 25,

therefore, have the constitutional formula given on p. 57. These authors distinguished in their first paper between two distinct cystins: one occurring in cystin-stones is α-thio-β-amino-propionic acid, while the other found in proteids is α-amino-β-thiopropionic acid. That these two cystins really exist is clearly shown by the researches of Loewy and Neuberg,[1] who find that the cystin occurring in cystinuria is protein- or horn-cystin, and is not stone-cystin. They suggest, in following Baumann's view,[2] that in the albumin-molecule a carboxy-cystein (or carboxy-cystin) exists which is a thio-amino-succinic acid, which, according to the place where CO_2 is split off, gives rise either to proteid-cystin or to stone-cystin.



Protein-cystein. Carboxy-cystein. Stone-cystein.

E. Erlenmeyer,[3] jun., then succeeded in preparing, synthetically, α-amino-β-thiopropionic acid, and from it cystin. Mörner[4] found thiolactic acids to be converted into the disulphides by gentle oxidation; treating, *e.g.*, a watery solution of α-thiolactic acid with ferric chloride or iodine, yields α-dithiodilactylic acid, which is very soluble in water, while β-dithiodilactylic acid is much less soluble. Mörner was the first to state that there exist not only stereo-isomeric, but also structurally isomeric cystins and cysteins. In cystin prepared from human hair and filings of cows' horns both β-amino-α-thiolactic acid and α-amino-β-thiolactic acid exist, perhaps even in equal quantities. On being decomposed the α-amino-β-thiolactic acid gave rise to sulphuretted hydrogen and alanin, while β-amino-α-thiolactic acid gave rise to ammonia and α-thiolactic acid. Oxidation may also take place simultaneously, and so cystin be formed from cystein, or the disulphide from thiolactic acid. In this paper Mörner gives a full account of how to test for the two thiolactic acids and also the best methods for their preparation. Mörner[5] does not believe α-thio-

1903. Quoted by J. Wohlgemuth in *Zeitschr. f. physiol. Chem.* **40**. 32 (1903), and *ibid.* **44**. 472 (1905).

[1] A. Loewy and C. Neuberg, *ibid.* **43**. 338 (1904).

[2] Baumann, *ibid.* **20**. 583 (1895).

[3] E. Erlenmeyer, jun., *Ber. d. deutsch. chem. Ges.* **36**. 2720 (1903).

[4] K. A. H. Mörner, *Zeitschr. f. physiol. Chem.* **52**. 349 (1904).

[5] K. A. H. Mörner, *ibid.* **52**. 365 (1904).

lactic acid to be a primary dissociation-product of albumin (see p. 83).

Keratines are richest in sulphur and cystin. From horn filings Mörner obtained 6·8 per cent, from human hair 13·92 per cent, and these figures are certainly too low, as they only express minimal estimates. Other albumin substances contain much less: serum-albumin 2·5 per cent, and casein and egg-white only traces.

Patten [1] and Rothera,[2] on using Hopkins' and Cole's acid mercuric sulphate reagent,[3] obtained from hair only 4 per cent of cystin in the shape of hexagonal crystals. Rothera states that there is no difference between stone or calculus cystin and that prepared by hydrolysis of hair. As he noticed a violet colour-reaction by the simultaneous use of ferric chloride and ammonia, which Mörner believes to be characteristic of α-thiolactic acid, and as the latter is a derivative of α-thio-β-amino-glyceric acid, it follows that stone-cystin is decomposed by the mercuric-sulphate method, while protein-cystin is not, for the latter Rothera obtained in the typical hexagonal plates. It must also be borne in mind that all cystin stones are not composed of stone-cystin, for which reason the expression 'stone-cystin' is not a very appropriate one. It is for this reason that the author suggests to call the protein-cystin, A-cystin; and the stone-cystin, B-cystin.

The second paper by Neuberg and Mayer and that of Gabriel were alluded to at the beginning.

Physiological Considerations.—Cystin, according to Wohlgemuth,[4] is the principal, if not the only, mother substance of the sulphates, the unoxidised sulphur and the salts of dithionic acid ($H_2S_2O_6$)[5] in the urine; further, of the taurin of bile, and also of the products of intestinal decomposition, namely, hydrogen sulphide, H_2S; methyl-mercaptane, $CH_3 . SH$, and ethyl-sulphide, $(C_2H_5)_2 . S$. Wohlgemuth[6] has shown experimentally that feeding rabbits with cystin leads to an increased production of taurin, and partly also to the formation of taurocholic acid in the bile.

During cystinuria, which depends on a disturbance of the amino-acid-metabolism, tyrosin, leucin, aspartic acid, and cystin are not dis-

[1] A. J. Patten, *Zeitschr. f. physiol. Chem.* **39**. 350 (1903).

[2] C. H. Rothera, *Journ. of Physiol.* **32**. 175 (1905).

[3] Hopkins and Cole, *ibid.* **27**. 421 (1902).

[4] J. Wohlgemuth, *Verhand. d. Gesellsch. deutsch. Naturf. u. Ärzte*, 1903, p. 423, and *Zeitschr. f. physiol. Chem.* **40**. 81 (1903); *ibid.* **43**. 469 (1905). Here also the older litteration is dealt with.

[5] Dithionic acid decomposes according to the equation $H_2S_2O_6 + H_2O = H_2SO_3 + H_2SO_4$. No sulphur separates out.

[6] J. Wohlgemuth, *ibid.* **40**. 81 (1903).

sociated in the body as they are normally, but appear almost quantitatively and completely unaltered in the urine. The behaviour of the two cystins when administered to a person suffering from cystinuria is especially interesting, because such a person reacts to the isomeric stone-cystein, as does a healthy individual to protein-cystin, *i.e.* the cystin disappears completely as such, there being eliminated a corresponding amount of sulphates and thiosulphates. If protein-cystin be given to a patient suffering from cystinuria, then this cystin is excreted as such in addition to the amount of protein-cystin normally excreted.

While mono-amino-acids appear in the urine when given to a cystinuric person, di-amino-acids behave quite differently, thus lysin gives rise to cadaverin and arginin gives rise to putrescin. We are dealing here with the first known fermentative process by which CO_2 is split off.

Lysin $CH_2 . NH_2—(CH_2)_3—CH . NH_2 . COOH =$
$CH_2 . NH_2—(CH_2)_3—CH_2 . NH_2$ or pentamethylene-diamine.

In the case of arginin there is also liberated cyanamide or a urea-remainder:

$$NH_2 . C(NH) . NH—CH_2—(CH_2)_2—CH . NH_2—COOH =$$
$$NH_2—CN + CO_2 + NH_2CH_2—(CH_2)_2—CH_2 . NH_2.$$

These observations of Loewy and Neuberg[1] are a complete confirmation of the results which Ellinger[2] obtained in the test tube.

The ratio of the sulphur taken in the food to that excreted in the bile has been especially investigated by Kunkel,[3] Spiro,[4] and Bergmann.[5] The last mentioned determined in the dog the amount of sulphur eliminated in the bile as taurin after the administration of cystin mixed with a diet in other respects constant. As long as only cystin was given no increase in the amount of taurin could be observed, but as soon as sodium glycocholate as well as cystin was fed, then an increase in taurocholate took place. He explains this result by assuming that cystin is converted into taurin in the body, but that the dog could not form the requisite amount of cholalic acid to enable it to synthetise taurocholates.

Blum[6] administered cystin to dogs and rabbits by the mouth, and found it to become oxidised into sulphates. Cystein injected sub-

[1] A. Loewy and C. Neuberg, *Zeitschr. f. physiol. Chem.* **43**. 338 (1904).
[2] Ellinger, *Bericht. d. deutsch. chem. Ges.* **31**. 3183 (1899).
[3] A. Kunkel, *Verh. königl. sächs. Akad. d. Wiss.* **27**. 344 (1875).
[4] P. Spiro, *Arch. f. Physiol.* **22**. 714 (1890).
[5] G. v. Bergmann, *Hofmeister's Beitr.* **4**. 192 (1903). [6] Blum, *ibid.* **5**. 1 (1903).

cutaneously behaved similarly. If large quantities of cystin, dissolved in soda, are injected rapidly into a peripheral vein, some thiosulphate but mostly cystin was found in the urine. By injecting cystin into a mesenteric vein and so forcing it to pass through the liver, he showed that the generally accepted theory as to taurocholates resulting from the union of taurin and cholalic acid need not, of necessity, be the only possible one, by bringing forward chemical proof that direct compounds of cystin and cholalic acid are possible, and that cystin-cholates may be oxidised secondarily into taurocholates. Starting with this idea, Simon and Campbell [1] made experiments on a person suffering from cystinuria, to see whether this complaint could not be cured by the administration of cholalic acid. Their results were negative, and hence they arrive at the conclusion that in cases of cystinuria either no synthesis of cholalic acid and cystin takes place with subsequent conversion into taurocholate, or that cystin is not converted into taurin. Rothera,[2] experimenting on himself, found that both calculus-cystin and hair-cystin, taken in doses of 1 gramme per day, were completely recovered from the urine as sulphates. "Larger doses were purposely avoided in order that bacterial action in the intestine might not complicate the issue. Thus cystin, dissolved in nutrient gelatine and inoculated with Bacillus coli communis, is broken up with liberation of sulphuretted hydrogen even under anaerobic conditions. This . . . explains the different results obtained by Blum and by Wohlgemuth with rabbits . . . for Wohlgemuth used large quantities of cystin, only portions of which were absorbed; the rest undergoing decomposition in the intestine might well account for the thiosulphate in the urine."

That the mercapturic acid which is found in dog's urine after feeding with benzene chloride and benzene bromide is derived from the same cystein which is found in proteid-cystin, and that the mercapturic acid is not a derivative of α-thiolactic acid, Friedmann has shown in a third paper.[3] The mother substance of mercapturic acid has the acetamide in the α- and the mercaptane in the β-position.

$$CH_2 . (SX)—CH . NHCOCH_3—COOH.$$

The occurrence of taurin in lower animals has been investigated by Agnes Kelly.[4]

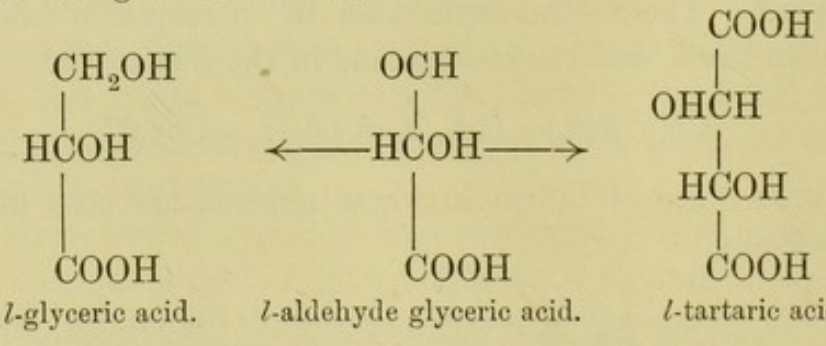
[1] C. E. Simon and D. G. Campbell, *Hofmeister's Beitr.* **5**. 401 (1904).
[2] C. H. Rothera, *Journ. of Physiol.* **32**. 175 (1905).
[3] E. Friedmann, *Hofmeister's Beitr.* **4**. 486 (1904).
[4] Agnes Kelly, *ibid.* **5**. 377 (1904).

The Stereo-Chemistry of Amino-Acids

The first investigations into the stereo-chemistry of the 3-carbon compounds have been made by Neuberg and Silbermann,[1] who have endeavoured to throw light on the inter-relationship of amino-acids and glucose. The following 3-carbon compounds are of especial interest, as the first four are primary dissociation products of albumins, while lactic acid is also probably a derivative of alanin :—

1. Alanin	CH_3 $CHNH_2$ $COOH$	lactic acid	CH_3 $CHOH$ $COOH$
2. Serin	CH_2OH $CHNH_2$ $COOH$	diamino - propionic acid	CH_2NH_2 $CH.NH_2$ $COOH$
3. Stone-cystein	CH_2NH_2 $CHSH$ $COOH$	isoserin	CH_2NH_2 $CHOH$ $COOH$
4. Protein-cystein	CH_2SH $CHNH_2$ $COOH$	glyceric acid	CH_2OH $CHOH$ $COOH$

The configurative inter-relationship of the lævo-rotatory aldehyde-glyceric acid to the lævo-rotatory glyceric and tartaric acids Neuberg and Silbermann give as follows :—



l-glyceric acid. *l*-aldehyde glyceric acid. *l*-tartaric acid.

[1] C. Neuberg and M. Silbermann, *Zeitschr. f. physiol. Chem.* **44**. 134 (1905).

The Quantitative Composition of Albumins

When estimating the percentage-amounts of the different dissociation-products found in individual albumins, we must exclude all substances which are formed secondarily. Of the substances enumerated in the list on p. 27 we may assume that they are preformed in the albumin-molecule.

As to whether this list includes all the substances existing primarily in the albumin-molecule cannot be decided as yet, because up till now none of the higher albumins has been dissociated quantitatively. We cannot account for more than 72 per cent of globin, 73 per cent of fibroin, and 58 per cent of edestin, while Kossel and Dakin [1] can account for the whole of the protamin salmin.

The composition of the protamins, which Kossel [1] regards as the simplest albumins, has been put into tabular form by him.

TABLE SHOWING THE COMPOSITION OF THE PROTAMINS

	Scombrin.	Salmin.	Clupein.	Sturin.	Cyclopterin.	α-Cyprinin.	β-Cyprinin.
Alanin	+	0	+	+	?	?	?
Serin	0	+	+	0	?	?	?
Amino-valerianic acid	0	+	+	0	?	+	+
Leucin	0	0	0	+	?	?	?
Diamino-valerianic acid (ornithin)	+	+	+	+	+	+	+
Diamino-caproic acid (lysin)	0	0	0	+	0	+	+
Histidin	0	0	0	+	0	0	0
α-Pyrrolidin-carboxylic acid	+	+	+	0	?	?	?
Tyrosin	0	0	0	0	+	0	+
Urea	+	+	+	+	+	+	+
Tryptophane	0	0	0	0	+	0	0

The ordinary albumins differ from the protamins simply in possessing larger accumulations of mono-amino-acids. Substances, such as leucin, tyrosin, alanin, serin, and diamino-caproic acids, which occur only sporadically in the protamins, are always present in albumins, which in itself renders the albumin molecule more complex than a protamin. This complexity becomes, however, still greater by acids occurring in the albumin, which are altogether absent in the protamins, namely, the dibasic amino-acids: glutaminic acid and aspartic acid.

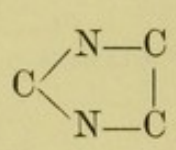
[1] Paper read at Brit. Ass. for Adv. of Science, Cambridge, 1904, and in *Berliner klin. Wochensch.* No. 41, Oct. 1904, p. 1065; also in *Zeitschr. f. physiol. Chem.* **44**. 342 (1905).

Miescher and Schmiedeberg[1] were the first to suggest that protamin is formed from histone or 'Kernalbumose,' as they called it, by a splitting off leucin and tyrosin. Kossel and Kutscher,[2] Ehrström,[3] and Kossel[4] have evolved the conception, that while in the salmon, the albumin is converted into histone and the latter into protamin, the change in the cod stops short at the histone-stage.

By this change of albumin into protamin the mono-amino-acids are removed, while reversely during poisoning with phosphorus, the hexone-bases or di-amino-acids are eliminated.

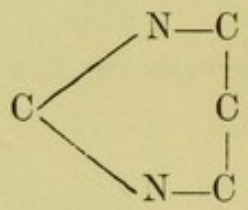
The nuclear proteids will be dealt with later, but attention may be drawn here to the fact pointed out by Kossel, that the cell-nucleus is especially rich in atomic chains, built up of alternate carbon and nitrogen atoms, as, for example, in the

Imido-azol ring.



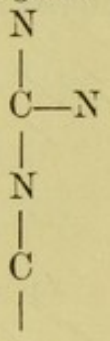
[In the alloxur bases; probably also in histidin.[5]]

Pyrimidin ring.



[In the alloxur bases, in uracil thymin, cytosin.]

Arginin.



Because of the part which nuclei play in the new-formation of organic tissues, Kossel believes these nitrogen-carbon chains to be concerned in synthetic processes. He holds that they are either the machinery which makes the amino-acids, or that they are intermediate products on the road towards amino-acids. The histological proof that the nucleus is concerned in synthetising albumins was brought forward six years ago in the papers of L. H. Huie, working under the author's care in the Physiological Laboratory, Oxford.[6] (See also p. 2.)

If we take into account that the isolation of the mono-amino-acids —which form the main bulk of the albumin-molecule—is accompanied by considerable loss, we must not overlook the possibility of new undiscovered groups existing in the albumin-molecule. Amino-butyric acid has already been mentioned, and E. Fischer throws out occasional hints as to the existence of unknown substances. Great surprises are,

[1] Miescher and Schmiedeberg, *Arch. f. exp. Pathol. u. Pharmak.* **37**. (1896); Miescher's *Histochemische und physiologische Arbeiten*, pp. 412-413 (1897).

[2] Kossel and Kutscher, *Zeitschr. f. physiol. Chem.* **31**. 165 (1900).

[3] Ehrström, *ibid.* **32**. 351 (1901). [4] Kossel, *ibid.* **44**. 342 (1905).

[5] Pauly, *Zeitschr. f. physiol. Chem.* **42**. 508 (1904).

[6] Lily H. Huie, *Quart. Journ. of Microsc. Science*, **39**. 387 (1896-97), and **42**. 203 (1899).

however, hardly in store for us, because the discovery of indol-amino-propionic acid, of phenylalanin, and of cystin allows us to refer all the secondary dissociation-products directly to the known primary ones. The only possibility which suggests itself is that, in addition to serin or amino-oxy-propionic acid, tetra-oxy-amino-caproic acid, and diamino-trioxy-dodecanoic acid, yet other oxy-acids may be discovered. If we take globin, which has been dissociated more than any other albumin, as an example, we find, on adding together all the known dissociation-products (after deducting the corresponding amounts of water), that the sum-total shows a deficiency in oxygen and an excess of carbon and nitrogen. Therefore certain compounds must exist in the albumin-molecule, such as oxy-amino-acids or carbohydrate radicals, which are richer in oxygen.

We are as yet imperfectly informed as to how the known dissociation-products are distributed over the individual albumins. Kossel and Dakin[1] found, for example, that arginin in certain albumins amounts to more than 83 per cent of the total amount, while in others, such as maize-albumin, it is present only to the extent of 1·8 per cent. Kossel and Soare, in the same paper, point out further that the amount of arginin varies even for the different albumins of one and the same muscle. The arginin fraction amounts to 2·49 per cent in the albumins which are readily precipitable by ammonium sulphate, while in the non-precipitable albumins the fraction only amounts to 0·64 per cent. Lysin is even completely absent in the alcohol-soluble albumins of wheat and maize. In the protamins only 4 to 5 out of a possible of 17 to 18 primary dissociation-products are found, as has already been expressed in Kossel's table given above on p. 65. Ammonia and the hexone bases have been determined most frequently, and their values have been ascertained most accurately. The systematic preparation of mono-amino-acids according to E. Fischer's method has been carried out, so far, only in the case of a few albumins, and even in these cases only minimal values have been obtained. All the older estimations of the mono-amino-acids, except those of aspartic and glutaminic acids and of tyrosin, are of but little value.

Siegfried[2] has further raised the question as to whether we have any right to assume the dissociation-products to be preformed. He believes if we were to add together the whole of the C and the N of all such dissociation-products as can be obtained by acting, for example, on any given albumin with hydrochloric acid, that the sum-total of

[1] A. Kossel, *Berliner klin. Wochensch.* No. 41, Oct. 1904, p. 1065.

[2] M. Siegfried, *Ber. d. Sächs. Ges. d. Wiss. zu Leipzig*, 1903, p. 85.

the C and the N of the dissociation-products would not correspond with the amount present in the native albumin we are examining. "It is more probable, judging by analogy, if we take well-known processes of dissociation of complex bodies into consideration, that, although not in every case, yet in many instances the albumin-molecule may at one time split in one direction, and at another time in a different direction, so that the sum of the C-atoms of all the dissociation-products may be greater than the number of C-atoms of the mother substance." Facts, however, do not seem to bear out this contention. Siegfried[1] himself found on dissociating kyrin by means of hydrochloric and sulphuric acids the same values for lysin, arginin, and the mono-amino-acids. Kossel and Kutscher[2] also found no difference when they employed either hydriodic acid or 33 per cent or 47 per cent sulphuric acid. E. Fischer[3] found on dissociating casein with sodium hydrate solution approximately the same amount of α-pyrrolidin-carboxylic acid as after a dissociation with hydrochloric acid; Cohnheim[4] obtained similarly on dissociating muscle-albumin by means of erepsin exactly the same amount of ammonia as did Hart[5] on boiling with sulphuric acid. Schulze and Winterstein,[6] Kossel and Patten,[7] and Abderhalden[8] give for the hexone-bases of edestin values which closely resemble one another, although some used hydrochloric acid while others used sulphuric acid. The yield of tyrosin, which Reach[9] obtained after tryptic digestion of casein, and Cohn[10] after treatment with acids, is in both cases identical. Within the errors of experimentation, the glutamin and asparagin values of zein agree perfectly, notwithstanding that Langstein[11] used hydrochloric acid, while Kreusler and Ritthausen[12] employed sulphuric acid. Schulze and Winterstein[13] have also pointed out that the dissociation of the reserve-material of germinating plants by means of ferments, yields qualitatively and quantitatively the same substances as when acids are used. Only Goto[14] found certain differences when working

[1] M. Siegfried, *Ber. d. Sächs. Ges. d. Wiss. zu Leipzig*, 1903, p. 85.
[2] A. Kossel and F. Kutscher, *Zeitschr. f. physiol. Chem.* **31**. 165 (1900).
[3] E. Fischer, *ibid.* **35**. 227 (1902).
[4] O. Cohnheim, *ibid.* **35**. 134 (1902).
[5] E. Hart, *ibid.* **33**. 347 (1901).
[6] E. Schulze and E. Winterstein, *ibid.* **33**. 547 (1901).
[7] A. Kossel and A. J. Patten, *ibid.* **38**. 39 (1903).
[8] E. Abderhalden, *ibid.* **37**. 499 (1903).
[9] F. Reach, *Virchow's Arch.* **158**. 288 (1899).
[10] R. Cohn, *Zeitschr. f. physiol. Chem.* **22**. 153 (1896).
[11] L. Langstein, *ibid.* **37**. 508 (1903).
[12] H. Ritthausen and U. Kreusler, *Journ. f. prakt. Chem.* (2) **3**. 314 (1871)
[13] E. Schulze and E. Winterstein, *Zeitschr. f. physiol. Chem.* **35**. 299 (1902).
[14] M. Goto, *ibid.* **37**. 94 (1903).

with clupein. The differences which used to be brought forward of old have rapidly become less and less marked with the improvements in our methods.

Quite apart from a certain unreliability in our methods, we have to deal with another much greater difficulty in making quantitative determinations, namely, the difficulty of obtaining a pure uniform material on which we can experiment. In organic chemistry the most important characteristic of a pure and uniform substance is its power of crystallisation. The only crystalline albumins we know of are hæmoglobin, serum-albumin, egg-albumin, ichthulin, and a number of vegetable albumins. It will be pointed out, however, in Chapter VIII. that we cannot consider crystalline albumins in every case to be pure substances, inasmuch as Wichmann[1] and Schulz and Zsigmondy[2] have shown how readily albumins absorb impurities, and with what tenacity they retain them. According to Schulz and Zsigmondy it is necessary to recrystallise egg-albumin five to seven times to obtain it in a pure state. The impurities in question are by no means small in amount, for Abderhalden[3] found that hæmoglobin which was crystallised twice contained no glycocoll, while after the first recrystallisation there was still present 0·62 per cent of glycocoll. This difference he explains as due to an admixture of serum-globulin. As serum-globulin contains 4 per cent of glycocoll, there must be present in oxyhæmaglobin after the first recrystallisation 15 per cent of serum-globulin.

Amongst non-crystalline albumins those precipitable by acids, namely, casein, mucins, etc., are the purest, while the others can only be classified according to differences in solubility and in precipitability by the salting-out method. For this reason serum-globulin is believed to contain 1, 2, 4, and 6, and wheat-glutin 1, 3, and 4 different substances.

What difficulties are met with in using the salting-out method is described on p. 184.

In Cohnheim's tables on pp. 70-75 only well-ascertained figures are given, and when these were not available, the occurrence (+) or absence (−) of a substance is indicated. For the mono-amino-acids only those values have been given which have been obtained with pure substances; in no case have values been included which were determined from crude substances ('Rohfraktion'). If several reliable estimates were available, then the one giving the higher value received preference.

[1] A. Wichmann, *Zeitschr. f. physiol. Chem.* **27**. 575 (1899).
[2] F. N. Schulz and R. Zsigmondy, *Hofmeister's Beitr.* **3**. 137 (1902).
[3] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **37**. 484 (1903).

	1	2	3	4	5	6	7
	Globin of the Oxyhaemoglobin of Horse's Blood.	Serum-albumin of Horse's Blood.	Serum-globulin.	Egg-white.	Egg-albumin crystallised.	Albumin of Yolk.	Caseinogen (see p. 397 for other products).
Glycocoll . . .	0 [9]	0 [10]	3·52 [95]	...	...	...	0 [45 95]
Alanin	4·19 [9]	2·68 [10]	2·22 [95]	...	...	...	0·9 [95]
Leucin	29·04 [9]	20·48 [10]	18·7 [95]	22·6 [40]	...	...	10·5 [95]
Phenylalanin . . .	4·24 [9]	3·08 [10]	3·84 [95]	+ [3]	...	...	3·2 [95]
α-Pyrrolidin-carboxylic acid	2·34 [9]	1·04 [10]	2·76 [95]	+ [3]	...	...	3·2 [1 9]
Glutaminic acid . .	1·73 [9]	1·52 [10]	2·20 [95]	+ [40]	+ [48]	...	10·7 [95]
Aspartic acid . . .	4·43 [9]	3·12 [10]	2·54 [95]	+ [37]	+ [48]	...	1·2 [95]
Cystin	0·31 [9]	2·53 [43]	1·51 [43]	0·4 [43]	0·29 [43]	...	0·065
Serin	0·56 [9]	0·6 [10]	...	...	...	...	0·43 [1]
Oxy-α-Pyrrolidin-carboxylic acid . .	1·04 [9]	...	...	...	...	...	0·25 [1]
Tyrosin	1·33 [9]	2·1 [10]	...	0·58 [66]	1·5 [31]	...	4·5 [36]
Lysin	4·28 [9]	...	...	+ [16 75]	...	...	5·8 [26]
Histidin . . .	10·96 [9]	...	...	+ [18]	+ [18]	+ [18]	2·6 [26]
Arginin	5·42 [9]	...	...	+ [17]	+ [16 17]	+ [17]	4·84 [2]
Tryptophane . . .	+ [9]	+ [10]	...	+ [80]	...	...	1·5 [54]
Ammonia . . .	0·93 [50]	1·2 [49]	1·75 [49]	...	1·5 [49]	...	1·8 [26]
Cystein	...	+ [44]	...	+ [44]	...	...	0 [44]
Amino-valerianic acid .	...	...	...	...	...	...	1· [95]
Glucosamin . . .	...	...	...	...	10–11 [34]	...	0 [67]
Diamino-trioxydodecanoic acid	...	...	...	...	...	...	0·75 [9]

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	9	10	11	12	13	14	15	16	17
						Wheat Gluten.			
	Fibrin.	Gelatine.	Serum-albumin—Human.	Edestin of Hemp-seed.	Zein of Maize.	Gliadin.	Mucedin.	Gluten-fibrin.	Gluten-casein.
.	+[45]	16·5[4]	+[13]	3·8[11]	0[12]	0·68[94]	...	...	...
.	...	0·8[4]	+[13]	3·6[11]	0·5[12]	2·66[94]	...	...	...
[51]	much	2·1[4]	+[13]	20·9[11]	11·25[12]	6·[94]	+[37]	+[37]	+[37]
.	+	0·4[4]	+[13 46]	2·4[11]	6·96[12]	2·6[94]	...	...	...
.	+[1]	5·2[4]	+	1·7[11]	1·49[12]	2·4[94]	...	...	...
.	0·66[25 69]	14[70]	+	6·3[11]	11·78[12]	27·6[94]	25[37]	13·07[21]	9·0[21]
.	1·1[25 69]	0·56[4]	+	4·5[11]	1·4[37]	1·24[94]	1·1		0·33[37]
.	1·17[43]	...	1·2[43]	0·25[11]	...	...	...	...	...
.	...	+[15]	...	0·33[11]	...	0·12[94]	...	...	...
.	...	3·0[15]	...	2·0[11]	...	...	...	...	...
[7 66]	3·82[66]	0	2·7[43]	2·13[11]	10·06[21]	2·37[94]	2·35[21]	4·43[21]	2·75[21]
[6 26]	4[25 69]	5–6[20]	...	1·65[27]	0[20 21]	0[20 94]	0[20]	0[20]	2·15[20]
[6 26]	+[69]	0·4[26]	...	2·19[27]	0·81[20]	1·2[20]	0·43[20]	1·53[20]	1·16[20]
[6 26]	3[25 69]	9·3[20]	...	14·17[27]	1·82[20]	2·75[20]	3·13[20]	3·05[20]	4·4[20]
.	+[81]	0	...	+[11]	0[85]	1·0[94]	...	...	...
[3 26]	+[39]	0·43[26]	...	1·74[27]	2·56[20]	4·1[21]	4·23[20]	3·89[20]	3·8[20]
.	...	...	...	+[44]	...	...	...	...	...
.	...	...	...	+[11]	+[12]	...	...	...	...
.	...	0	...	0[83 84]	...	...	...	...	...
.	...	...	...	...	...	...	...	...	...

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	18	19	20	21	22	23	24
			Proteids from the Seeds of				
	Conglutin from Seeds of Lupinus.	Legumin of Peas.	Abies.	Pinus silvestris.	Pinus Pinaster.	Picea.	Cucumber.
Glycocoll	...	...	...	...	...	...	...
Alanin	...	...	...	...	...	...	...
Leucin	much [35]	17·9 [40]	...	...	...	+[76]	...
Phenylalanin	+[72]	...	...	...	...	...	+[7]
α-Pyrrolidin-carboxylic acid	...	...	...	...	...	...	...
Glutaminic acid	3–5 [37]	1·5 [37]	...	...	...	...	...
Aspartic acid	4·1 [35]	3·5 [37]	...	...	...	...	...
Cystin	...	...	...	...	...	...	...
Serin	...	...	...	...	...	...	...
Oxy-α-Pyrrolidin-carboxylic acid	...	...	...	...	...	...	...
Tyrosin	3·2 [35]	2 [37]	...	...	...	+[76]	...
Lysin	2·1 [32]	5·05 [32]	0·5 [32]	0·25 [32]	0·79 [32]	1·2 [31]	1·6 [32]
Histidin	0·65 [32]	1·1 [32]	0·7 [32]	0·62 [32]	0·78 [32]	2·0 [31]	0·77
Arginin	6·6 [32]	4·6 [32]	12·5 [76]	10·9 [32]	11·3 [32]	14·3 [31]	7·6 [32]
Tryptophane	...	...	...	...	...	...	...
Ammonia	...	...	...	...	...	...	...
Cystein	...	...	...	...	...	...	...
Amino-valerianic acid	+[74]	...	...	...	...	...	...
Glucosamin	...	...	...	...	...	...	...

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25	26	27	28	29	30	31	32	33
Keratin from								
Horn Filings.[1]	Human Hair.	Egg-shells.	Gorgonin, Iodine-keratin from Corals.	Fibroin of Silk.	Silk-Gelatine.	Conchiolin of Mussel-shells.	Elastin.	Spongin.
0·34 [8]	...	...	...	36 [2]	0·1–0·2 [5]	4 [55]	25·75 [93]	+ [60]
1·2 [8]	...	...	...	21 [2]	5·0 [5]	...	6·58 [93]	...
3·3 [8]	14 [70]	+ [65]	+ [58]	1·5 [2]	+ [59]	+ [55]	21·38 [93]	+ [60]
3·0 [8]	...	...	...	1·5 [2]	...	+ [55]	3·89 [93]	...
3·6 [8]	...	...	...	0·3 [14]	...	...	1·74 [93]	...
1 [70]	12 [70]	...	...	...	...	...	0·76 [93]	12 [20]
2·5 [8]	+ [70]	...	...	...	...	...	0 [63]	..
3·8 [43]	13·92 [43]	7·62 [43]	...	...	...	...	1 [93]	...
5·70 [8]	...	...	...	1·6 [5]	6·6 [5]	...	...	...
...	...	...	...	...	...	...	...	...
1·58 [36]	3 [70]	+ [65]	2·5 [57]	10 [2]	5 [59]	5 [55]	0·34 [64]	...
...	...	...	1·5 [58]	+ [5]	+ [5]	...	+ [20]	3–4 [20]
...	...	...	+ [57]	+ [5]	...	...	...	...
2·25 [17]	...	...	2·2 [58]	1·0 [5]	4 [5]	...	0·3 [56]	5–6 [20]
+ [79]	...	...	+ [57]	+	...	...	...	...
uch [70]	much [70]	...	...	...	1·87 [55]	0·7 [55]	...	...
...	...	...	...	...	...	...	...	...
5·7 [8]	...	...	...	+ [2]	...	...	+ ? [63]	...
0 [73]	...	...	...	...	...	...	...	...

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	34	35	36	37	38	39	40
	Hetero-albumose from Syntonin.	Hetero-albumose from Witte-Pepton.	Proto-albumose from Syntonin.	Histone from Thymus.	Histone from Sperma of *Gadus morrhua*.	Histone from Sperma of *Lota vulgaris*.	Protamin from Sperma of Salmon : Salmin.
Glycocoll	+[45]	+[45]	0[45]	...	...	...	...
Alanin	...	...	...	...	...	...	...
Leucin	...	+[71]	+[71]	...	...	...	...
Phenylalanin	...	+[71]	0[71]	...	...	...	...
α-Pyrrolidin-carboxylic acid	...	...	...	...	...	...	+[89]
Glutaminic acid	...	...	...	3·66[21]	...	...	...
Aspartic acid	...	...	...	...	...	...	...
Cystin	...	...	...	...	...	...	0[20]
Serin	...	...	...	...	...	...	+[89]
Oxy-α-Pyrrolidin-carboxylic acid	...	...	...	...	...	...	...
Tyrosin	...	0[71]	+[71]	6·31[21]	...	...	0[20]
Lysin	7·03[26]	3·5[22]	3·08[26]	7·7[20]	8·3[20]	3·17[24]	0[20]
Histidin	1·12[26]	2·2[22]	3·35[26]	1·21[20]	2·34[20]	2·85[24]	0[20]
Arginin	8·52[26]	4·9[22]	4·55[26]	14·36[20]	15·52[20]	12·0[24]	84·3[20]
Tryptophane	...	0[71]	+[71]	...	...	...	...
Ammonia	0·97[26]	0·8[22]	0·76[26]	1·66[20]	0·74[20]	0·66[24]	0[20]
Cystein	...	...	...	...	...	...	0
Amino-valerianic acid	...	...	...	...	...	...	+[89]
Glucosamin	...	0[71]	0[71]	...	...	+[24]	...

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41	42	43	44	45	46	47	48	49	50	51
Protamin of the Sturgeon: Sturin.	Protamin of the Herring: Clupein.	Protamin of the Seahare: Cyclopterin.	Protamin of the Mackerel: Scombrin.	Protamin of the Carp: α-Cyprinin.	Protamin of the Carp: β-Cyprinin.	Proteid from Yeast.	Digestible Proteid of Pancreas.	Digestible Proteid from Gland of Mid-gut of Octopus.	Bence-Jones's Proteid.	Amyloid.
...	...	...	...	...	...	...	...	...	0^{78}	...
...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	$+^{23}$	+	$+^{68}$	$+^{78}$	$+^{77}$
...	...	...	...	...	...	$+^{88}$	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	$0{\cdot}66^{25}$	...	$+^{78}$	$+^{77}$
...	...	...	...	...	...	...	$1{\cdot}1^{25}$	...	...	$+^{77}$
0^{20}	0^{20}	0^{20}	0^{20}	0^{87}	0^{87}	$+^{88}$	...	...	...	...
...	$+^{87}$	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...
0^{20}	0^{20}	$8{\cdot}3^{20}$	...	0^{87}	$+^{87}$	$+^{23}$	+	$+^{68}$	$+^{78}$	$3{\cdot}9^{77}$
12^{20}	0^{20}	0	0^{19}	$+^{87}$	$+^{87}$	$11{\cdot}34^{88}$	$3{\cdot}82^{25}$	$+^{68}$	...	...
$12{\cdot}9^{20}$	0^{20}	0	0^{19}	0^{87}	0^{87}	$1{\cdot}98^{88}$	$0{\cdot}41^{25}$	$+^{68}$	...	...
$58{\cdot}2^{20}$	$82{\cdot}2^{20}$	$62{\cdot}5^{20}$ $+^{86}$	$+^{19}$	$+^{87}$	$+^{87}$	$3{\cdot}32^{88}$	$3{\cdot}02^{25}$ +	$+^{68}$	...	...
...	...	...	...	...	...	...	...	...	...	...
0^{20}	0^{20}	...	...	...	...	$+^{23}$	$+^{25}$	$+^{68}$	$1{\cdot}6^{78}$	...
0	0	0	0	0	0	...	...	...	...	...
...	$+^{20}$	...	...	...	...	...	...	...	...	...
..	...	...	...	...	...	...	...	...	...	...

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The Nitrogen Radicals of the Albumin-molecule

To facilitate the difficult quantitative estimation of dissociation-products, attempts have repeatedly been made to determine at least some of the groups quantitatively. The most important method in this respect is that which was worked out by E. Schulze,[1] Hausmann,[2] and others for the determination of the different forms in which nitrogen is present.

Hausmann's method of studying the distribution of nitrogen in the proteid-molecule consists of the following operations :—

1. One gram of the substance under investigation is dissociated with boiling hydrochloric acid.
2. The nitrogen which has been split off as ammonia, and which is present as ammonium chloride, is distilled off with magnesia. This N. is the so-called 'amid-nitrogen,' 'ammonia-nitrogen,' or 'readily displaceable nitrogen.' (It is absent in the protamins.)
3. The fluid, freed from ammonia, is precipitated with phosphotungstic acid, and the nitrogen present in the precipitate is determined by Kjeldahl's method. This nitrogen is the 'diamino-nitrogen' or 'basic nitrogen' of arginin, lysin, and histidin.
4. The nitrogen which is not driven off by magnesia, and which is not precipitated by phosphotungstic acid, is then determined by Kjeldahl's method as the 'mono-amino-nitrogen.'

Against this method Friedmann,[3] and particularly Kutscher,[4] have raised the objection, that the phosphotungstates of the bases are not insoluble, and that the bases are readily soluble in an excess of phosphotungstic acid. As the latter is used for washing out the bases, too low values are obtained for the bases, and these values differ also according to the way in which the washing-out process is performed. E. Fisher and Abderhalden[5] have recently overcome this difficulty in not washing out the phosphotungstic acid precipitate at all, but by pressing it out under a hydraulic press. But whether this method is practicable has still to be determined. Kutscher[6] has investigated casein by Hausmann's method, and has arrived at the conclusion that

[1] E. Schulze and E. Winterstein, *Zeitschr. f. physiol. Chem.* **33**. 574 (1901), **35**. 210 (1902).

[2] W. Hausmann, *ibid.* **27**. 95 (1899), **29**. 136 (1900).

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[6] Compare with Hausmann and Kutscher, *l.c.* F. Müller and J. Seemann, *Deutsch. med. Wochensch.* 1899, p. 209.

this method does not give reliable results. This view is, however, not shared by Osborne and Harris,[1] who believe Hausmann's method to be a good one, and the same conclusion Gümbel has arrived at. The method has also been used by Schulze[2] and Pick.[3]

The most recent and a very thorough investigation is that of Gümbel,[4] which is based on the work of Osborne and Harris.[1] Gümbel discusses the value of this method for determining the three groups of nitrogen-radicals supposed to exist in the albumin-molecule.

1. Amid-nitrogen-Determination

Gümbel tabulates the results which various authors have obtained and shows how closely they agree :—

Table I.

Author.	Substance.	Acid used.	Duration of boiling in Hours.	Average Percentage of Nitrogen.
Hausmann and Gümbel	Edestin of hemp-seed	Saturated HCl ,, HCl	5 5	1·87
Henderson .	,,	,, HCl 5–40 per cent H_2SO_4	7–40	1·88
Osborne and Harris	,,	20 per cent HCl	7–10	1·88
Henderson .	Casein	Saturated HCl	7–20	1·63
Gümbel .	,,	,, HCl	5	1·60
Kutscher .	,,	HCl of 1, 19 sp. gr.	6	1·60
Osborne and Harris	,,	20 per cent HCl	7–10	1·61
Henderson .	,,	5–40 per cent H_2SO_4	5–20	1·60

The amid-nitrogen can therefore be determined accurately by Hausmann's method, as long as we do not forget that albuminous substances, which are not readily attacked by acids, especially weak ones, require boiling for a longer time than do those albumins which part easily with their amid-nitrogen. That, on the other hand, excessive action of concentrated acids, *e.g.* H_2SO_4, for very long periods will induce secondary changes is also evident. The exact time at which all the amid-nitrogen has been split off may be determined either by

[1] T. B. Osborne and J. F. Harris, *Journ. Amer. Chem. Soc.* **25**. 323 (1903).
[2] E. Schulze, *Zeitschr. f. physiol. Chem.* **25**. 360 (1898).
[3] E. P. Pick, *ibid.* **28**. 219 (1899).
[4] Theodor Gümbel, *Hofmeister's Beiträge*, **5**. 297 (1904).

following Hausmann's directions, namely, to continue the boiling till no appreciable alteration in the amid-nitrogen-value occurs; or by showing that the biuret-reaction is no longer obtainable, a plan adopted by Osborne and Harris.

Gümbel adds one more precautionary measure: Embden has found that cystin gives off considerable amounts of ammonia if it is boiled for a long time with magnesia, while no ammonia is given off during the distillation with magnesia if the distillation is done in vacuo at a temperature not exceeding 40-42°. Distillation at 40° is the safest, because Schwarzschild[1] has shown that at this temperature even readily decomposable acid-amides, such as asparagin, are not affected. The distillation at low temperatures is especially indicated when substances rich in cystin (hair, horn, etc.) have to be investigated. A possible source of error is further the formation of melanin as shown below.

2. Di-amino-nitrogen-Determinations

Hausmann's method is not so perfect for the determination of di-amino-acids as for mono-amino-acids, because

1. The phosphotungstates of the di-amino-acids are not absolutely insoluble, and therefore the values obtained by Hausmann's method must be too low.
2. Mono-amino-acids in concentrated solutions are also apt to be precipitated by phosphotungstic acid.
3. The nitrogen of the melanins (humin-substances) is estimated along with the di-amino-nitrogen, and therefore increases its value.

Sub. 1. The solubility of arginin-phosphotungstate, according to Gulewitsch,[2] is as follows: On precipitating a solution of arginin-sulphate with phosphotungstic acid, there remains in solution, if sufficient phosphotungstic acid be taken, about 0·07 grm. in 1 litre of fluid (1 : 14,000); while if the phosphotungstic acid is in insufficient amount, the loss amounts to 0·2 grm. per litre (1 : 5000).

Gümbel determined experimentally the solubility of arginin, as it is set free during Hausmann's process. He made a 1 : 700 solution of arginin chloride, corresponding to 0·118 per cent arginin, and added varying amounts of phosphotungstic acid. He found the precipitate to be most readily soluble in water, less in a mixture of dilute HCl and phosphotungstic acid, and still less in the latter mixture, if the

[1] Schwarzschild, *Hofmeister's Beiträge*, **4**. 155 (1904).

[2] Gulewitsch, *Zeitschr. f. physiol. Chem.* **27**. 195 (1899).

precipitate was allowed to stand for twenty-four hours before it was mixed with the diluting fluid, because during this time the originally flocculent precipitate becomes crystalline. The phosphotungstate precipitate of arginin, of 0·00118 grm. (= 0·00038 grm. nitrogen), passes into solution only after 26-30 ccm. of phosphotungstic acid have been added, *i.e.* after a dilution of 1 : 22,000 to 1 : 25,000. In Hausmann's process, in estimating the nitrogen of 1 grm. of albumin, the volume of fluid dealt with amounts to 80 ccm., and in this quantity there will remain in solution 0·0035 arginin (= 0·001 grm. nitrogen), which, if we take the arginin-content of the albumin-molecule to equal 10-20 per cent, amounts to a loss of 1·8 to 3·5 per cent. If the precipitate be washed for a long time, the loss may amount to 10 per cent.

Lysin behaves quite analogously to arginin.

Histidin reacts, however, quite differently. It is readily precipitable by phosphotungstic acid, but redissolves if there be a trace of excess of phosphotungstic acid, provided the solution is concentrated. If, however, the solution of phosphotungstic acid is very dilute, then an excess of the acid does not dissolve the histidin-phosphotungstate precipitate. Therefore have 1 part of the diamino-nitrogen to 1000-1500 ccm. of solution. If the histidin has not been completely precipitated, then a precipitate will be found on diluting the filtrate or on adding some more dilute phosphotungstic acid.

If the rules laid down above are followed out, the error in the estimation of the total di-amino-nitrogen will amount to 5-10 per cent, *i.e.* instead of 4 per cent di-amino-nitrogen only 3·6-3·8 per cent will be obtained.

Sub. 2. Kutscher's objection to Hausmann's method, on the ground that mono-amino-acids are also precipitated by phosphotungstic acid if they are in very concentrated solutions and in the presence of very strong acid (Stolte [1]), is well known ; but mono-amino-acids in 0·5 per cent strengths are not precipitated, and inasmuch as this percentage is not exceeded if Hausmann's directions as to dilution to 70-80 ccm. are followed out, Kutscher's objections to the method are groundless.

Phenylalanin and cystin must also be taken into consideration, especially since Schulze and Winterstein [2] have recommended phosphotungstic acid for the separation of these amino-acids. Gümbel points out, however, that according to Winterstein 0·1 grm. of phenylalanin in 50 ccm. of water is no longer precipitated by phosphotungstic acid,

[1] Stolte, *Hofmeister's Beiträge*, **5**. 19 (1904).

[2] Schulze and Winterstein, *Zeitschr. f. physiol. Chem.* **29**. 155 (1901), and **33**. 374 (1902).

and as more than 0·2 grm. of this mono-amino-acid is not present in any one albumin, its presence does not vitiate the results of Hausmann's method. Cystin, according to Mörner,[1] is not precipitated in a hydrochloric acid solution by phosphotungstic acid, while according to Winterstein it is precipitated from a dilute sulphuric acid solution. Judging by experiments carried out in Hofmeister's laboratory, 0·05 per cent cystin prepared from egg-white is not precipitated by phosphotungstic acid, if a large excess be avoided, and therefore the cystin-factor may also be neglected as cystin occurs in most albumins in very minute quantities, and even horn-filings, so rich in cystin, do not seem to have given Gümbel any difficulty.

Sub. 3. Melanins are related to the indol-forming radicals of the albumin-molecule (Samuely[2]), and therefore to the tryptophane-grouping, and hence Hausmann holds that the melanin which is precipitated by phosphotungstic acid, and which contains 0·16 per cent N. (*i.e.* a little more than 1 per cent of the total nitrogen) increases by this amount the reading in the di-amino-fraction. It has been shown, however, by Udránszky[3] and Samuely, that nitrogen-containing humin-substances are formed when carbohydrates are boiled in the presence of amides or ammonium salts, with acids, and therefore, at present, we are not in a position to tell whence the nitrogen in the melanin-fraction is derived, and therefore it is best to follow Osborne and Harris and Gümbel, and to make separate determinations of the melanin-nitrogen.

3. Mono-amino-nitrogen-Determinations

The values obtained depend on the care with which the di-amino-fraction has been determined. As was pointed out above, some di-amino-nitrogen always remains in solution, and this amount is sufficient to raise the value of the total mono-amino-fraction 1 to 2 per cent, *i.e.* instead of 60 per cent of mono-amino-nitrogen one may get as high as 61 or 62 per cent.

This mono-amino-fraction is not always readily determined, because Kjeldahl's method is difficult to apply owing to the high percentage of salt present, but it should always be determined, to see whether the total-nitrogen equals the sum obtained by adding up the amid-N + di-amino-N + mono-amino-N + melanin-N.

Gümbel gives for crystalline serum-albumin and edestin of hemp-seeds, casein, keratin, and cartilage the following values, including those obtained by Hausmann and Osborne and Harris :—

[1] Mörner, *ibid.* **28**. 603 (1899).

[2] Samuely, *Hofmeister's Beiträge*, **2**. 355 (1902-3).

[3] Udránszky, *Zeitschr. f. physiol. Chem.* **12**. 389 (1888).

TABLE II.

Substance.	Observer.	Amid-N.	Melanin-N.	Di-amino-N.	Mono-amino-N.
Serum-albumin with 14·60 per cent Nitrogen as the mean measurement	Gümbel	0·95	0·15	4·86	8·81
Edestin from hemp-seed	Hausmann	1·90	7·07		10·19
,, ,,	Osborne and Harris	1·88	0·12	5·91	10·78
,, ,,	Gümbel	1·79	0·29	6·50	10·51
Casein, mean measurements	Kutscher	1·60	0·23	3·87	10·14
,, ,,	Osborne and Harris	1·65	——	3·46	——
,, ,,	Gümbel	1·64	0·31	4·25	9·82
,, ,,	Hausmann	2·10	1·84		11·93
Keratin, mean measurements	Gümbel	1·17	0·42	2·95	11·81
Cartilage . .	Gümbel	1·35	0·36	1·35	7·95
Chondro-sulphuric-acid	Gümbel	0·85	0·23	0·79	0·52

The inter-relation of cartilage and its component parts, namely, gelatine and chondro-sulphuric-acid, is well seen in the following table, in which, in each case, the total nitrogen = 100 per cent :—

	Amid-N.	Melanin-N.	Di-amino-N.	Mono-amino-N.
Cartilage . . .	12·27	3·27	12·27	72·27
Gelatine (Hausmann) .	1·61	35·83		62·56
Chondro-sulphate of potassium	35·27	9·54	32·78	21·57

Hausmann's method, notwithstanding the inaccuracies in the di-amino-fraction, gives results, in the hands of Osborne and Harris [1] and of Pick,[2] which show that differences in the construction of the albumin-molecule can be revealed, which by ordinary analysis would remain hidden.

Sörensen and Andersen [3] drew attention to the fact that if lower

[1] Osborne and Harris, *Journal of the Amer. Chem. Soc.* **25**.

[2] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899).

[3] S. P. L. Sörensen and A. C. Andersen, *ibid.* **44**. 429 (1905).

values in nitrogen-determinations are obtained by Kjeldahl's than by Gunning-Arnold's method when working with albuminous substances, that these contain either ring-like nitrogenous compounds which are not decomposed, such as pyridin or piperidin-compounds, or substances which by undergoing ring-formation give rise to pyridin or piperidin-complexes.

Effront[1] has pointed out that 'amides, imides, nitril bases, acid-amides, and amino-acids react at ordinary temperature on alkaline hypochlorite, and that thereby a loss of chlorine occurs which is proportional to the weight of the added substance.' 'Ammonium bases do not react with the hypochlorite solution.' The chlorine is estimated with an arsenious acid, As_2O_3, solution. The total figures obtained for egg-albumin, casein, Witte's peptone, etc., differ considerably from those given above.

Halogen-substituted Albumins

Oswald[2] has drawn attention to still another characteristic of albumins. It is possible to substitute halogens for the hydrogen-atoms of such aromatic nuclei as phenylalanin, tyrosin, and perhaps tryptophane (see Chapter VII.), and therefore the amount of halogen which is taken up by an albumin is a measure of the amount of aromatic nuclei present in that albumin. Casein contains 7 per cent tyrosin and phenylalanin, and at least 1·5 per cent tryptophane, while gelatine contains no tyrosin or tryptophane, and only 0·4 per cent phenylalanin. Correspondingly we find the casein-iodine compound to contain 11·43 to 13·45 per cent of iodine, while the gelatine-iodine compound contains only 1·34 to 2·0 per cent iodine. As long, however, as we do not know how many atoms of iodine are actually taken up by each of the aromatic nuclei, we can, of course, only make approximate estimations. See further p. 230.

B. Other Dissociation-Products not yet classified

Up till now only those dissociation-products have been enumerated of which we know definitely that they are primary ones, and the constitution of which is also sufficiently known. In the following pages are enumerated some substances which have not been definitely identified, or of which it is doubtful whether they are formed directly from albumin or only secondarily out of those primary substances described above.

[1] J. Effront, *Ber. d. deutsch. chem. Ges.* **37**. 4290 (1904).

[2] A. Oswald, *Hofmeister's Beiträge*, III. 514 (1903).

1. Amino-butyric Acid, $C_4H_9NO_2 = CH_3.CH_2.CH(NH_2).COOH$, or butalanin. Schützenberger[1] first described it as one of the dissociation-products to be obtained by heating albumins under pressure with barium hydrate. E. Fischer[2] discovered in fibroin and in gelatine, by means of the ester-method, a body which probably was amino-butyric acid, but which could not be identified with certainty; and Levene[3] found it amongst the dissociation-products of auto-digested testes.

2. α-Thiolactic Acid, $C_3H_6SO_2 = CH_3 . CH(OH) . CSOHCS(OH)$.
(Lactic acid is: $C_3H_6O_3 = CH_3 . CH(OH) . COOH$).

α-Thiolactic acid was prepared by Friedmann[4] from horn along with cystin and cystein. It cannot be regarded as a derivative of horn-cystein, as in the latter the SH group is in the β position (compare p. 56). Lovén[5] prepared synthetically α-thiolactic acid from pyro-uvic acid (propanonic acid) $CH_3CO . COOH$ (Brenztraubensäure) and β-thiolactic acid from β-iodo-propionic acid. Mörner[6] gives characteristic reactions for both acids and also new methods for preparing them in a pure state, and he further states[7] that not only in serum-albumin, serum-globulin, horn, and hair, but also in fibrinogen, egg-albumin, casein, and in the shell-membrane of hens' eggs, the whole of the sulphur is in some form of cystin (see p. 56) and none present as a thiolactic acid.

3. Pyro-uvic Acid, $CH_3 . CO . COOH$, or Brenztraubensäure of the Germans, is, according to Mörner,[8] a very constant, secondary dissociation-product of proteids.

4. Diamino-acetic Acid, $C_2H_6N_2O_2 = NH_2 . CH(NH_2) . COOH$. Drechsel[9] found it along with other bases in casein, but its occurrence is denied by Willstätter[10] and Sörensen,[11] as diamino-acetic acid prepared synthetically has quite different properties.

5. Glucosamin, $C_6H_{13}NO_5$ (for constitutional formula see p. 158).

[1] M. P. Schützenberger, *Bull. de la Soc. chim.* **23**. 161, 193, 216, 242, 385, 433, **24**. 2, 145 (1875).

[2] E. Fischer and A. Skita, *Zeitschr. f. physiol. Chem.* **33**. 177 (1901); E. Fischer, P. A. Levene, and R. H. Aders, *ibid.* **35**. 70 (1902).

[3] P. A. Levene, *Amer. Journ. of Physiology*, **11**. 437 (1904).

[4] E. Friedmann, *Hofmeister's Beitr.* III. 184 (1902).

[5] J. M. Lovén, *Journ. f. prakt. Chem.*, N.F., **29**. 366 (1884).

[6] K. A. H. Mörner, *Zeitschr. f. physiol. Chem.* **42**. 349 (1904).

[7] K. A. H. Mörner, *ibid.* **42**. 365 (1904).

[8] K. A. H. Mörner, *ibid.* **42**. 121 (1904).

[9] E. Drechsel, *Ber. d. Sächs. Ges. d. Wissenschaften zu Leipzig, math.-physik. Kl.* 1892. p. 115.

[10] R. Willstätter, *Ber. d. deutsch. chem. Ges.* **35**. II. 1378 (1902).

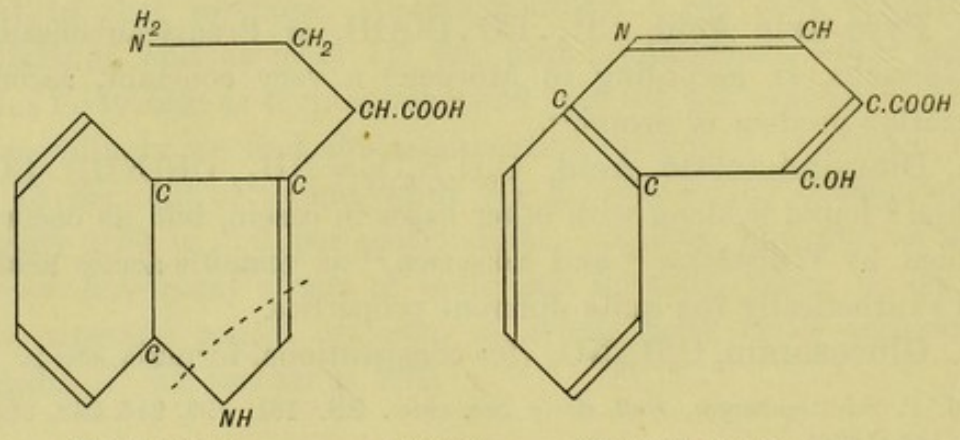
[11] S. P. L. Sörensen, *Compt. rend. des travaux du Laboratoire de Carlsberg*, **6**. 1 (1903).

Its occurrence in egg-white, and the so-called glyco-albumins were first observed by Fr. Müller.[1] Glucosamin is, however, according to Steudel[2] and Fränkel,[3] not a primary dissociation-product, but is formed secondarily out of a primary product.

6. Galactosamin takes the place of glucosamin in the mucus forming the covering of frogs' eggs (Schulz and Ditthorn).[4]

7. Kynurenic Acid, $C_{10}H_7NO_3$ = γ-oxy-β-quinolin-carboxylic acid.[5] Liebig discovered it in the urine of dogs, and Glässner and Langstein[6] have ascertained that, in the body, its mother-substance occurs amongst the alcohol-soluble, acetone-insoluble autodigestion products of the pancreas. After Camps[7] had shown that kynurenic acid was γ-oxy-β-quinolin-carboxylic acid, Ellinger[8] has proved experimentally that feeding dogs with tryptophane, or injecting it in dilute soda solution subcutaneously, leads to an excretion of kynurenic acid.

Hopkins and Cole have found that tryptophane may be converted into an oxy-quinoline derivative, having the formula C_9H_7NO, an observation which Ellinger believes to strengthen his view that kynurenic acid is a derivative of tryptophane, and that tryptophane possesses an arrangement by means of which it is readily converted into a quinoline. Compare with p. 51.



Tryptophane becomes Kynurenic acid.

On being heated kynurenic acid is converted into kynurin.

[1] Fr. Müller, *Zeitschr. f. Biol.* **42**. 468 (1901).
[2] H. Steudel, *Zeitschr. f. physiol. Chem.* **34**. 353 (1901).
[3] S. Fränkel, *Monatshefte f. Chem.* **19**. 747 (1898).
[4] F. N. Schulz and F. Ditthorn, *Zeitschr. f. physiol. Chem.* **32**. 428 (1901).
[5] For literature see: L. B. Mendel and H. C. Jackson, *Americ. Journ. of Physiol.* **2**. 1 (1898); A. Josephsohn, *Inaugural Dissertation*, Königsberg, 1898.
[6] K. Glässner and L. Langstein, *Hofmeister's Beiträge*, II. 34 (1902).
[7] R. Camps, *Zeitschr. f. physiol. Chem.* **33**. 390 (1901).
[8] Alexander Ellinger, *ibid.* **43**. 325 (1904).

8. Skatosin, $C_{10}H_{16}N_2O_2$, is a base which Baum[1] and Swain[2] isolated from the autodigested pancreas; on being melted with alkalies it yields skatol, and hence its name. It is not identical with tryptophane, as the latter does not possess strongly basic properties. Skatosin hydrochloride contains 3 molecules of hydrochloric acid, which fact is still unaccounted for. It is a yellowish-white substance which melts and simultaneously decomposes between 345° and 355°. Langstein[3] has isolated from serum-albumin, after prolonged peptic digestion, a body which in its properties resembles skatosin.

9. Lysatinin, $C_6H_{13}N_3O_2$. Along with lysin, it was isolated by Drechsel,[4] from casein as one of the first basic substances, but since Hedin[5] prepared arginin from it, it is generally supposed not to be a chemical individual but a mixture of lysin and arginin (see above, pp. 37 and 38), or a double salt of these bases crystallising in definite proportions. Siegfried[6] states, however, that he has tried unsuccessfully to prepare lysin or arginin from lysatinin, and therefore believes lysatinin to be a definite chemical compound. As it is only formed on dissociating casein with hydrochloric acid and zinc chloride, but not if sulphuric acid is used, Siegfried believes lysatinin to be perhaps another base which has become secondarily transformed.

10. Leucin-imide, $C_{12}H_{22}N_2O_2$

$$\begin{array}{l} C_4H_9—CH—NH—CO \\ \qquad\qquad\quad | \qquad\quad | \\ \qquad\quad CO—NH—CH—C_4H_9, \end{array}$$

is 3·6-di-isobutyl-2·5-di-acipiperazin (butyl alcohol = $CH_3(CH_2)_3OH$). Ritthausen,[7] Cohn,[8] and Abderhalden[9] showed its presence after dissociating proteids with acids, and Salaskin[10] after peptic digestion; but there can be but little doubt that it is formed secondarily.[9] Curtius[11] and E. Fischer[12] have pointed out that the derivatives of the amino-acids have a tendency to ring-formation, and that they

[1] F. Baum, *Hofmeister's Beiträge*, **3**. 439 (1903).
[2] R. E. Swain, *ibid.* **3**. 442 (1903).
[3] L. Langstein, *ibid.* **1**. 518 (1901).
[4] E. Drechsel, *Arch. f.* (*Anat. u.*) *Physiol.* 1891, p. 248.
[5] S. G. Hedin, *Zeitschr. f. physiol. Chem.* **21**. 297 (1895).
[6] M. Siegfried, *ibid.* **35**. 192 (1902).
[7] H. Ritthausen, *Eiweisskörper der Getreidearten*, Bonn, 1872; *Ber. d. deutsch. chem. Ges.* **29**. II. 2109 (1896).
[8] R. Cohn, *Zeitschr. f. physiol. Chem.* **29**. 283 (1900).
[9] E. Abderhalden, *ibid.* **37**. 484 (1903).
[10] S. Salaskin, *ibid.* **32**. 592 (1901).
[11] T. Curtius and F. Göbel, *Journ. f. prakt. Chem.* (2), **37**. 150 (1888).
[12] E. Fischer, *Ber. d. deutsch. chem. Ges.* **34**. I. 383 (1903).

give rise to compounds analogous to glycocoll-anhydride; the formation of such compounds is readily explained, for Abderhalden boils amino-acids for several hours with mineral acids and then esterifies them. This explanation does not hold good in the case of Salaskin, but peptic digestion is apt to give rise to bodies after the type of leucyl-leucin (see below, p. 127), and from the latter leucinimide may readily be formed.[1] Therefore the leucinimide, found after dissociating albumins with acids, represents leucin which has been changed, and must be calculated as leucin and be added to the latter. Its melting-point is 271°.

Compounds analogous to leucinimide are formed probably also from the other mono-amino-acids, for the same reasons as leucinimide is formed from leucin, and they seem to have only escaped observation because of the small amounts in which they are formed.

11. Prussic Acid, HCN. When employing Neumann's method [2] for converting albumins into ash, Aders Plimmer [3] noticed the presence of silver cyanide in the precipitate of silver chloride. He found that freshly precipitated silver cyanide is quantitatively converted into prussic acid, HCN, on being boiled with dilute nitric acid. After having destroyed the amino-groups of the albumin-molecule with nitrous acid, the same amount of HCN was obtained as previously, and he therefore concluded 'that the prussic acid originates from a decomposition-product which does not contain nitrogen in the form of amino-groups.' The same amount of prussic acid is obtained as with Neumann's method, if oxidation is brought about with potassium bichromate and sulphuric acid in suitable proportions, while 'oxidation with manganese dioxide and sulphuric acid, potassium permanganate and sulphuric acid, and concentrated sulphuric acid does not give rise to prussic acid, or in very small amount only.' In a second paper [4] Aders Plimmer describes how the amount of prussic acid obtained by oxidation with chromic acid is in general greater than by oxidation with Neumann's nitric and sulphuric acid mixture.

The mean-percentages of HCN after oxidising with chromic acid are as follows:—

Gelatine	2·75	Fibrin (Merck) . .	1·11
Casein (hydrolysed) . .	1·25	Witte's peptone . .	0·94
Hæmoglobin (Merck) .	1·13	Egg-albumin . . .	0·88

[1] E. Fischer and E. Fourneau, *Ber. d. deutsch. chem. Ges.* **34**. II. 2868 (1901).

[2] A. Neumann, *Zeitschr. f. physiol. Chem.* **37**. 115 (1902), and **43**. 32 (1904).

[3] R. H. Aders Plimmer, *Journ. of Physiol.* **31**. 65 (1904). Here too is given the older literature.

[4] R. H. Aders Plimmer, *ibid.* **32**. 51 (1904).

Casein (Merck) . .	0·82	Pyrrolidine-carboxylic acid	0·37
Glycocoll . . .	11·10	Arginin carbonate . .	0·12
Aspartic acid . . .	7·70	Lysin hydrochloride . .	0·10
Leucin	0·68	Glucosamin hydrochloride	0·08

Negative results were obtained with alanin, glutaminic acid, tyrosin, and tryptophane.

The formation of prussic acid from glycocoll probably takes place over nitroso-acetic acid which, according to Cramer,[1] breaks down when heated to 120° into prussic acid, carbonic acid, and water, thus:

$$NH_2 . CH_2—COOH + 2O = NOH . CH—COOH + H_2O$$
$$NOH . CH—COOH + \text{heat} = HCN + CO_2 + H_2O.$$

Plimmer believes the formation of oxaminic acid as observed by Kutscher and Schenk, when they oxidised gelatine with calcium permanganate, to be also explainable on the supposition that nitroso-acetic acid is the first oxidation-product of glycocoll, if, as v. Peckmann[2] suggests, the nitroso-acetic acid undergoes the Beckmann rearrangement into the isomeric acid amide, namely, oxaminic acid.

$$NOH . CH—COOH \text{ becomes } NH_2 . CO . COOH.$$

The formation of prussic acid from aspartic acid is as yet unexplainable.

Humin Substances or Melanoidins

The term 'humin' was introduced in 1838 by Berzelius,[3] as a substitute for the expressions 'ulmin' and 'géine,' which he had used previously in describing certain deeply-coloured constituents of 'humus' or mould.

Mulder then showed that albumins on being boiled with strong hydrochloric or sulphuric acids separate off flocculi of a brown or black colour, which resemble the deeply-coloured bodies seen in putrefying matter.

These substances have been investigated later, especially by v. Udránszky,[4] Hoppe-Seyler,[5] Schmiedeberg,[6] and Samuely.[7] Humin-substances are not only formed from albumins, but also from many

[1] C. Cramer, *Ber. d. deutsch. chem. Ges.* **25**. 715 (1892).

[2] H. v. Peckmann and K. Wehsarg, *ibid.* **21**. 2991 (1888).

[3] Berzelius, *Poggendorff's Ann.* **44**. 375 (1838).

[4] L. v. Udránszky, *Zeitschr. f. physiol. Chem.* **11**. 537, where the older literature is given; **12**. 33 (1887).

[5] F. Hoppe-Seyler, *ibid.* **13**. 66 (1889).

[6] O. Schmiedeberg, *Arch. f. experiment. Path. u. Pharm.* **39**. 1 (1897).

[7] F. Samuely, *Hofmeister's Beiträge*, **2**. 355 (1902).

other organic compounds, and in particular from carbohydrates. Hoppe-Seyler states that 25 per cent of cane-sugar may be converted into humin-substances in a few hours. The humin-substances prepared from sugar are, in their dry state, black or brown powders having a peculiar glitter. In water and acids they are insoluble, while in alkalies a certain percentage becomes peculiarly slippery, while the greater percentage—Mulder's humic acid—is readily soluble. From such alkaline solutions they are precipitated by acids. Hoppe-Seyler gives for humin prepared from cane-sugar this percentage composition :

$$C = 63{\cdot}88,\ H = 4{\cdot}64,\ O = 31{\cdot}48.$$

The high percentage of carbon and the low percentage of hydrogen is characteristic of all humin substances, whatever their source may be. They contain protocatechuic acid, in addition to which Udránsky isolated, by fusion with alkalies, formic acid, oxalic acid, and pyrocatechin 1 : 2 $C_6H_4(OH)_2$. Samuely further found pyridin, C_5H_5N, and pyrrol, C_4H_4NH.

If, in addition to carbohydrates, ammonia or other nitrogenous substances are in solution, then the humins combine with the ammonia and become thereby nitrogenous. Analogously they may also take up sulphur and iron. For a preparation which was made by boiling serum albumin with 25 per cent hydrochloric acid, Schmiedeberg calculated the following percentage :—

$$C = 66{\cdot}27,\ H = 5{\cdot}49,\ N = 5{\cdot}57,\ \text{and a little sulphur.}$$

For another preparation, made from Witte's peptone,

$$C = 60{\cdot}34,\ H = 4{\cdot}86,\ N = 8{\cdot}09,\ S = 0{\cdot}96.$$

Schmiedeberg lays special stress on the fact that the composition in the two preparations is not the same. Humin substances owe their existence undoubtedly to a secondary reaction. Schmiedeberg has shown that the greater part of an albumin-molecule absorbs water and then gives rise to amino-acids ; but this change is accompanied by an accessory reaction, as is usually the case amongst organic bodies, in consequence of which 1 to 2 per cent of the albumin is transformed into a compound rich in carbon, but poor in hydrogen and nitrogen. Perhaps it is more probable that the dissociation-products, which are formed at first, undergo subsequently a secondary change, as held by Langstein [1] and Samuely. Amongst the dissociation-products may be mentioned :

1. Glucosamin and other carbohydrates.

[1] L. Langstein, *Zeitschr. f. physiol. Chem.* **31**. 49 (1900).

2. Tyrosin, which, according to v. Fürth and Schneider [1] and Ducceschi,[2] is converted by ferments and other oxidising media into dark-coloured substances (see index under Tyrosinase).
3. Lysin, according to Hart.[3]
4. Tryptophane, according to Nencki,[4] Hopkins, and Cole.[5]

To put it shortly, Samuely holds that normal pigments are formed by the indol-, pyrrol-, pyridin-, and tyrosin-radicals of the albumin molecule. In this connection Ellinger's formula of tryptophane (p. 53) would explain the tendency to a closure of the pyridin ring in tryptophane, and the assumption of a pyridin-nucleus in the albumin-molecule [6] become therefore unnecessary, as this assumption is based on the fact that melanoidins on being reduced give pyridin (Samuely).[7]

Samuely also observed a more or less abundant formation of humin on subjecting carbohydrates along with amino-acids or other nitrogenous bodies to the action of boiling hydrochloric acid. Glucose and tyrosin together give an especially large amount of humin. Schmiedeberg [8] noticed further that nucleic acid, which contains a carbohydrate along with xanthin-bases, gives rise to melanoidin. Similarly, egg-white is very apt to form humin because it contains a large amount of carbohydrate. Antipeptone, analogously, does not form melanin,[9] because it is deficient in glucosamin, tryptophane, and tyrosin.

The formation of melanoidin depends on oxidation, for Samuely states that access of oxygen is as necessary as in the case of 'tyrosinase,' [10] and this explains why v. Fürth obtained xanthomelanin. (See p. 94, under 'Disintegration with Nitric Acid.')

Hart [3] has pointed out that the formation of humin introduces a considerable uncertainty, when lysin and ammonia have to be estimated, as humin is formed to a much greater extent when sulphuric acid alone is used than when sodium chloride is added as well: in the latter case much more lysin and ammonia are obtained. Differences between the action of hydrochloric and of sulphuric acid were also observed by Hoppe-Seyler. Langstein explains the absence of glucosamin amongst the dissociation-products of egg-white resulting from the action of strong hydrochloric acid, by assuming that the glucosamin unites with

[1] O. v. Fürth and H. Schneider, *Hofmeister's Beiträge*, **1**. 229 (1901).
[2] V. Ducceschi, cited from Samuely, *ibid.* **2**. 355 (1902).
[3] E. Hart, *Zeitschr. f. physiol. Chem.* **33**. 347 (1901).
[4] M. Nencki, *Ber. d. deutsch. chem. Ges.* **28**. I. 560 (1895).
[5] F. G. Hopkins and S. W. Cole, *Journ. of Physiology*, **27**. 418 (1901).
[6] F. Hofmeister, *Ergebnisse der Physiol.* **1**. 768 (1902).
[7] Samuely, *Hofmeister's Beiträge*, **2**. 355 (1902).
[8] O. Schmiedeberg, *Arch. f. experiment. Path. u. Pharmakol.* **43**. 57 (1899).
[9] F. Müller, *Zeitschr. f. physiol. Chem.* **38**. 279 (1903).
[10] M. Gonnermann, *Pflüger's Arch.* **82**. 289 (1900).

the ammonia, which is formed simultaneously, and thereby gives rise to humin.

Schmiedeberg has drawn attention to the fact that humins, as far as appearance, properties, and composition are concerned, show a great resemblance to the melanins or the normally occurring dark pigments of the hair, skin, etc. He therefore calls them melanoidins, and those with acid characters melanoidinic acids. According to the recent account of Spiegler,[1] however, melanins may show quite different chemical reactions. The darkening of urine which occurs on boiling it with acids depends, according to v. Udránsky, on the formation of humin out of the reducing substances in the urine; he calls the dark pigment seen in urine after poisoning with carbolic acid also humin. Hlasiwetz[2] has pointed out that some relation exists between humin substances and the deeply coloured bodies in the bark, etc., of plants, and has called these dark bodies phlobaphenes.

The melanotic pigments found during pathological conditions have been investigated by Zdarek and Zeynek,[3] Brandl and Pfeiffer,[4] and Wolff.[5]

C. Secondary Dissociation-Products derived from the Amino-Acids

Before discussing how the primary dissociation-products are linked together, and how thereby the configuration of the albumin-molecule is determined, it is necessary to study the secondary dissociation-products, by which we mean those substances which result from a disintegration of the primary products, namely, the amino-acids. These secondary products were at one time of the highest importance for the study of the chemistry of albumins, while now they are of especial interest in connection with physiological research.

(*a*) *The Disintegration of Albumins by means of Boiling Alkalies*

Schützenberger[6] was the first to disintegrate albuminous substances with barium hydrate under pressure; he was followed by Schulze and Bosshard,[7] while later Maly[8] and Bernert[9] used the same method in

[1] E. Spiegler, *Hofmeister's Beitr.* **4**. 40 (1903).
[2] H. Hlasiwetz, *Liebig's Ann.* **143**. 290 (1867).
[3] Zdarek and Zeynek, *Zeitschr. f. physiol. Chem.* **36**. 493 (1902).
[4] Brandl and Pfeiffer, *Zeitschr. f. Biol.* **26**. 348 (1890).
[5] Hans Wolff, *Hofmeister's Beitr.* **5**. 476 (1904).
[6] P. Schützenberger, *Bull. de la Soc. chimique*, **23** and **24**. (1875).
[7] E. Schulze and E. Bosshard, *Zeitschr. f. physiol. Chem.* **9**. 63 (1884).
[8] R. Maly, *Monatshefte f. Chem.* **6**. 107 (1885), **9**. 258 (1888).
[9] R. Bernert, *Zeitschr. f. physiol. Chem.* **26**. 272 (1898).

dealing with oxyprot-sulphonic and peroxyprot-sulphonic acids, two oxidation-products of albumin. E. Fischer [1] and Steudel [2] have decomposed casein under ordinary pressure by boiling with caustic soda or barium hydrate. In doing so, albumins pass through the stage of alkali-albuminates and subsequently form albumoses and peptones, therefore at first the same substances are obtained as after treating albumins with acids. Schützenberger found leucin, amino-valerianic acid, amino-butyric acid, alanin, tyrosin, phenylalanin, aspartic and glutaminic acids, besides other amino-acids which were not isolated; Schulze and Bosshard found leucin, tyrosin, phenylalanin, aspartic and glutaminic acids; Bernert: lysin and histidin; Steudel: lysin and tyrosin; E. Fischer: pyrrolidin-carboxylic acid.

As has already been mentioned, treatment with fixed alkalies renders all amino-acids optically inactive, but, in addition, it splits off ammonia, so that instead of and along with the amino-acids we obtain the corresponding simple acids, namely acetic, propionic, butyric, and valerianic acids, and of course large amounts of ammonia; Habermann and Ehrenfeld [3] found 3·58 per cent in casein.

Accompanying the above changes are still other processes which lead to the formation of formic and carbonic acids. Schützenberger stated oxalic acid to be also formed, but Habermann and Ehrenfeld were unable to confirm this observation. Because of all these secondary changes, the usual dissociation-products can no longer be found; thus Steudel failed to obtain arginin and histidin.

By fusing albumins directly with solid potash the disintegration becomes even more marked, as has been shown by Bopp [4] and Hinterberger,[5] and later by Kühne,[6] Nencki,[7] Sieber and Schoubenko,[8] and Rubner.[9] At first, again, amino-acids, leucin, and tyrosin[10] are formed, but then the amino-acids give rise to the corresponding fatty acids; thus indol-amino-propionic acid is changed into skatol and indol, substances first observed by Kühne and Nencki, while cystin yields sulphuretted hydrogen [11] and mercaptane.[12]

[1] E. Fischer, *Zeitschr. f. physiol. Chem.* **35**. 227 (1902).

[2] H. Steudel, *ibid.* **35**. 540 (1902).

[3] J. Habermann and R. Ehrenfeld, *ibid.* **30**. 453 (1900).

[4] N. Bopp, *Liebig's Ann.* **69**. 29 (1847). [5] F. Hinterberger, *ibid.* **71**. 70 (1849).

[6] W. Kühne, *Ber. d. deutsch. chem. Ges.* **8**. I. 206 (1875).

[7] M. Nencki, *ibid.* **8**. I. 336 (1875); *Journ. f. prakt. Chem.* (2), **17**. 97 (1878).

[8] N. Sieber and G. Schoubenko, *Arch. d. Sciences biol. de St. Pétersbourg*, **1**. 314 (1892). [9] M. Rubner, *Arch. f. Hygiene*, **19**. 136 (1893).

[10] N. Bopp, *Liebig's Ann.* **69**. 29 (1847); F. Hinterberger, *ibid.* **71**. 70 (1849).

[11] N. Sieber and G. Schoubenko, *Arch. d. Sciences biol. de St. Pétersbourg*, **1**. 314 (1892).

[12] N. Sieber and G. Schoubenko, *ibid.*; M. Rubner, *Arch. f. Hygiene*, **19**. 136 (1893).

By mixing chemically pure tryptophane or indol-amino-propionic acid with eight to ten times its weight of caustic potash, adding some water and heating for only a short time after water has ceased to come off, allowing to cool, adding some more water, and heating again and repeating this addition of water three or four times, Hopkins and Cole[1] obtained in the distillate 65 per cent of what would be the theoretical yield of skatol. The distillate also contained an abundance of ammonia, and gave a slight nitroso-indol reaction. In the non-volatile residue from the potash-fusion abundant oxalic acid could always be detected, and at times it was possible to show the presence of glyoxylic acid. These two products, along with ammonia, are derived from the side-chain of the indol-amino-propionic acid under the combined hydrolytic and oxidative influence of potash.

Skatol, on being fused with potash, yields β-indol-carboxylic acid, according to Ciamician and Magnanini,[2] and Ciamician and Zatti.[3]

Dry distillation of the most diverse albumins also gives rise, according to Rubner, to sulphuretted hydrogen, H_2S; ethyl-mercaptane, $C_2H_5 . SH$; and methyl-mercaptanes, $CH_3 . SH$.

(*b*) *The Disintegration of Albumins by Superheated Steam*

At first, again, amino-acids are liberated, of which Lubavin[4] found leucin and tyrosin, while Steudel[5] demonstrated aspartic acid. Later on, according to Steudel,[5] all hexone bases disappear completely. (See also p. 202.)

(*c*) *The Disintegration by means of Oxidising Media*

The primary changes produced by oxidising media in albumins are fully discussed on pp. 237 to 249.

1. *Potassium Permanganate in Combination with Sulphuric or Chromic Acid.*—This method was employed under the direction of Liebig by Guckelberger,[6] who investigated egg-white, fibrin, casein, and gelatine, but only the volatile products were isolated. He obtained formic, acetic, propionic, butyric, valerianic, and caproic acids; benzoic acid, an aldehyde; ammonia, oil of bitter almonds, different nitrites; finally, a 'heavy oil smelling of cinnamon.' According to E. Fischer,[7] phenyl-

[1] Hopkins and Cole, *Journ. of Physiol.* **29**. 463 (1903).
[2] Ciamician and Magnanini, *Ber. d. deutsch. chem. Gesellsch.* **21**. 673 (1888).
[3] Ciamician and Zatti, *ibid.* **21**. 1933 (1888).
[4] N. Lubavin, *Hoppe-Seyler's mediz.-chem. Untersuch.* p. 463 (1871).
[5] H. Steudel, *Zeitschr. f. physiol. Chem.* **35**. 540 (1902).
[6] Guckelberger, *Liebig's Ann.* **64**. 39 (1848).
[7] E. Fischer, *Zeitschr. f. physiol. Chem.* **33**. 151 (1901).

alanin gives rise to phenyl-acetaldehyde on being treated with sulphuric acid and bichromate.

2. *Potassium Permanganate in Alkaline Solutions.*—The products formed at first, still retain their albuminous character, and are discussed in Chapter V. Subsequently, according to Bernert,[1] are formed acetic, propionic, butyric, and valerianic acids; lysin, histidin, pyrrol, and ammonia. Kutscher[2] has shown arginin to give rise at first to guanidin-butyric acid and then to guanidin and succinic acid, when it is treated with barium permanganate. Lossen[3] has obtained guanidin also directly from albumin.

3. *Calcium Permanganate in Boiling Solutions.*—Zickgraf[4] has employed boiling solutions of calcium permanganate for oxidising gelatine, because the calcium oxide which is set free is at once rendered inert through the carbonic acid and the oxalic acid resulting from the oxidation of the gelatine, and the oxidation taking place in a boiling solution results in a rapid and complete dissociation of the gelatine-molecule and prevents the formation of partly oxidised substances such as oxy-protsulphonic acid.

Kutscher and Schenk,[5] by oxidising gelatine with calcium permanganate, obtained large amounts of oxaminic acid, which is a derivative of glycocoll. The oxaluria which results from feeding with gelatine (Lommel)[6] is readily explained by assuming that the glycocoll of the gelatine is converted in the body into oxamin, and that the latter then splits up into ammonia and oxalic acid.

4. *Hydrogen Peroxide in Acid Solution.*—Neuberg and Blumenthal[7] prepared from gelatine, and Orgler[8] from crystallised egg-albumin, the two substances acetone and isovaleric aldehyde. The latter is derived from leucin, while the acetone may be derived either from leucin or from a hitherto not yet isolated amino-isobutyric acid.

The action of nitric acid is discussed below.

To Neumann's excellent method for converting albumin into ash by heating albuminous matter with a mixture of equal volumes of concentrated nitric and sulphuric acids special attention is drawn.[9]

[1] R. Bernert, *Zeitschr. f. physiol. Chem.* **26**. 272 (1898).

[2] E. Benech and F. Kutscher, *ibid.* **32**. 278 (1901); F. Kutscher, *ibid.* **32**. 413 (1901).

[3] W. Lossen, *Liebig's Ann.* **201**. 369 (1880).

[4] G. Zickgraf, *Zeitschr. f. physiol. Chem.* **41**. 259 (1904).

[5] Fr. Kutscher and Martin Schenk, *ibid.* **43**. 337 (1904).

[6] Lommel, *Deut. Arch. f. klin. Med.* 1899.

[7] C. Neuberg and F. Blumenthal, *Hofmeister's Beitr.* **2**. 238 (1902); *Deutsche mediz. Wochenschrift*, 1901, p. 6.

[8] A. Orgler, *Hofmeister's Beitr.* **1**. 583 (1902).

[9] A. Neumann, *Zeitschr. f. physiol. Chem.* **37**. 115 (1902), and **43**. 32 (1904).

Here may also be mentioned Friedenthal's[4] views on the oxidation of albuminous substances in living organisms. As completely dry carbon will not burn in completely dry oxygen, he has arrived at the conception that oxidation depends on an accumulation of OH-ions; on subjecting various foodstuffs to the oxidising action of alkalies, *i.e.* of OH^1-compounds, he found albumins, colloidal carbo-hydrates, fats and soaps not to become oxidised, while the lower dissociation products of albumins and carbohydrates readily became oxidised. As the higher albuminous substances are broken up in the body, it is assumed that they are hydrolysed in the first instance before being oxidised.

The oxidation of uric acid in alkaline solutions is discussed by Behrend.[2]

(*d*) *Dissociation by Means of Acid, and Treatment of the Resulting Amino-Acids with Nitrous Acid and Reduction with Metallic Sodium*

By this means Ducceschi[3] obtained from egg-white, serum-albumin and horn; and Spiro[4] from casein and gelatin, cinnamic acid, which is derived from phenyl-alanin.

$$\begin{matrix} C_6H_5 \\ CH_2 \\ CHNH_2 \\ COOH \end{matrix} - NH_3 = \begin{matrix} C_6H_5 \\ CH \\ \| \\ CH \\ COOH. \end{matrix}$$

Phenyl-alanin — ammonia = cinnamic acid.

By an analogous process aspartic acid gives rise to fumaric acid.

$$\begin{matrix} COOH \\ CH_2 \\ CHNH_2 \\ COOH \end{matrix} - NH_3 = \begin{matrix} COOH \\ CH \\ \| \\ CH \\ COOH. \end{matrix}$$

Aspartic acid — ammonia = fumaric acid.

(*e*) *Disintegration with Nitric Acid*

This method produces entirely different results. Mühlhäuser,[5] v. Fürth,[6] and Habermann and Ehrenfeld[7] have boiled casein with

[1] H. Friedenthal, *Arch. f.* (*Anat. und*) *Physiol.* 1904, p. 371.
[2] Behrend, *Liebig's Annalen*, **333**. 141 (1904).
[3] V. Ducceschi, *Hofmeister's Beiträge*, **1**. 339 (1901).
[4] K. Spiro, *ibid.* **1**. 347 (1901).
[5] Mühlhäuser, *Liebig's Ann.* **90**. 171 (1854), **101**. 176 (1857).
[6] O. v. Fürth, Strassburger Habilitationsschrift, Strassburg, 1899.
[7] J. Habermann and R. Ehrenfeld, *Zeitschr. f. physiol. Chem.* **35**. 231 (1902).

nitric acid and thereby obtained very large quantities—up to 30 per cent—of oxalic acid, which is not obtainable by the ordinary methods (see below, under *f*). Habermann and Ehrenfeld found in addition oxyglutaric acid, evidently derived from glutaminic acid and leucic acid, while v. Fürth obtained xanthomelanin. This is a friable, blackish-brown powder having a bitter taste; it is very slightly soluble in water, ether, or chloroform, but readily soluble in ethyl-, methyl-, amyl-alcohol, and in acetone; it is most soluble in glacial acetic acid, and is precipitated from such a solution by the addition of water. In soda solution and in ammonia xanthomelanin dissolves with a reddish-brown colour, and is evidently that complex which gives rise to the xantho-proteic reaction (see p. 6). Xanthomelanin prepared from casein has, according to v. Fürth, the following percentage composition :—

C	H	N	S	NO_2
50·03	4·93	10·7	0·59	16·86.

Fürth could arrive at no definite conclusion as to the sulphur radical. A preparation obtained from horn filings had a somewhat different composition.

On reducing the nitro-group with stannous chloride an acid was obtained; on being melted with a fixed alkali a distinct smell of indol or skatol could be detected, which seems to indicate a certain resemblance to tryptophane. This may also explain the remarkable fact that on disintegrating casein with nitric acid no tyrosin is found amongst the disintegration-products.

(*f*) *Disintegration by means of Nitrous Acid*

The reaction which is supposed to take place when albuminous substances are brought into contact with either nitrous acid or an alkali-nitrite + acetic acid may be represented, according to Levites,[1] thus :

$$R - NH_2 + NO \cdot OH = 2N + H_2O + R - OH.$$

Schiff is of the opinion, as is Nasse and Hausmann [2] and Hofmeister,[3] that in the albumin-molecule are contained at least two $CONH_2$-groups, as is explained on p. 141, where the biuret-reaction is discussed. Schiff has stated that desamination leads to a disappearance of the $CONH_2$-groups, and that this disappearance also explains the absence of the biuret-reaction with desamino-peptones.

[1] S. Levites, *Zeitschr. f. physiol. Chem.* **43**. 202 (1904).
[2] Hausmann, *ibid.* **27**. 95 (1899), and **29**. 136 (1900).
[3] F. Hofmeister, *Ergebnisse der Physiol.* I., i., p. 159 (1902).

If the reaction between nitrous acid and albuminous matter takes place according to the equation given above, then the amid-nitrogen ought to be completely removed from the albumin-molecule. Levites has examined desamino-albumin prepared from egg-white, freed from globulins and ovomucoid; desamino-casein (Hammarsten-Grübler) and desamino-glutin from purified gelatine.

Desamino-albumin and desamino-casein gave both the biuret-reaction and the reaction of Millon, while desamino-glutin gave a distinct biuret-reaction.

In the following table the author has compiled "mean readings" from the figures given by Levites:—

Substance.	Total-Nitrogen in Percentage of Substance.	Amid- or Ammonia-Nitrogen in	
		Percentage of Substance.	Percentage of Total-Nitrogen.
Egg-albumin	14·81	1·33	8·98
Desamino-albumin (average)	14·166	1·48	10·50
Casein	15·00	1·58	10·53
Desamino-casein	14·03	1·67	11·93
Gelatine	17·78	0·33	1·85
Desamino-glutin	16·60	0·31	1·86

This table shows that the amid-nitrogen remains nearly unaltered after treatment with nitrous acid, if it is not even increased in amount, while the total-nitrogen is considerably diminished. According to Levites the occurrence of $CONH_2$-radicals in the albumin-molecule is therefore not yet proved, nor can the biuret-reaction be said to depend on these $CONH_2$-groups, especially as the pepsin-glutin-peptones of Scheermesser contain no trace of amid-nitrogen.

(*g*) *Disintegration of Albumins with Bromine*

Hlasiwetz and Habermann[1] have heated eggwhite under pressure with an excess of bromine in watery solution, and they obtained from 100 grammes eggwhite (see also Chapter VII., p. 230):

29·9 grammes Bromoform.
22 „ Brom-acetic acid.
12 „ Oxalic acid.

[1] H. Hlasiwetz and J. Habermann, *Liebig's Ann.* **159**. 304 (1871).

23·8 grammes Aspartic acid (and perhaps glutaminic acid).
22·6 ,, Leucin.
1·5 ,, Bromanil.

Further, carbonic acid, the amount of which was not determined. Other albumins yielded the same products, but in different proportions.

(*h*) *Disintegration by means of Sulphur*

On mixing egg-white with flowers of sulphur or with precipitated sulphur, there is soon liberated sulphuretted hydrogen, which blackens lead-acetate-paper. After various explanations had been offered which were purely speculative, Nasse and Rösing[1] recognised that the process was one of oxidation. They assumed that in egg-albumin a labile, *i.e.* loosely attached hydrogen-atom, was replaced by a hydroxyl-radical of water, and that the H derived from the albumin, along with the remaining H of the water-molecule, united with sulphur to form H_2S. This view is supported by the fact that benzaldehyde, acetaldehyde or œnanthol by autoxidation give rise to H_2S in the presence of sulphur and water.

According to Engler,[2] however, autoxidation takes place in a different manner. An oxygen-molecule takes the place of the labile hydrogen-atom in the autoxidator, giving rise thereby to a peroxide. The hydrogen-atoms which are set free are then supposed to unite with sulphur to form H_2S.

Against the view of Nasse may be urged, that aldehyde-groups have not yet been shown to occur in albumins; and against Engler, that egg-white is unable to oxidise an acceptor, *i.e.* a substance which under otherwise equal conditions is not directly oxidisable, such substances being, *e.g.*, arsenious acid and indigo-sulphuric acid.

Nasse and Rösing were right in assuming that an oxidation-process is set up by the action of sulphur on egg-white, as traces of other oxidising media will prevent the sulphur from being changed into H_2S, according to Heffter,[3] who believes that the sulphur simply takes away the H from the albumin-molecule, as happens also when diphenyl-methane and sulphur are heated together, there being formed tetraphenyl-ethylene and H_2S (Ziegler [4]).

[1] Rösing, *Untersuchungen über die Oxydation von Eiweiss in Gegenwart von Schwefel.* Dissertation (under O. Nasse), Rostock, 1891.

[2] Engler, *Ber. d. deutsch. chem. Ges.* **33**. 1097 (1900).

[3] A. Heffter and Max Hausmann, *Hofmeister's Beiträge*, **5**. 213 (1904). Here complete literature.

[4] Ziegler, *Ber. d. deutsch. chem. Ges.* **21**. 779 (1888).

$$2(C_6H_5)_2CH_2 + 2S = (C_6H_5)_2C : C(C_6H_5)_2 + 2H_2S.$$

Phenyl-hydrazin reacts similarly (E. Fischer [1]). Certain thio-compounds are especially apt to split off hydrogen. For example, the following substances become oxidised by the oxygen of the air during evaporation and then give rise to disulphides: Thiophenol (Hübner and Alsberg [2]), thio-benzoic acid (Engelhardt [3]), and benzyl-mercaptane (Märcker [4]).

$$2C_6H_5SH + O = C_6H_5S—S \,.\, C_6H_5 + H_2O.$$
Thiophenol = Diphenol-disulphide.

This thiophenol in presence of water and sulphur readily forms H_2S. If thiophenol be first oxidised with ferricyanide of potash, it does not split off H_2S.

Ethyl-mercaptane also readily splits off H_2S, and as Mörner has shown that egg-white contains a sulphur radical which is not cystin and which is volatile, Heffter assumes egg-white to contain a mercaptane which brings about the formation of H_2S, when egg-white and flowers of sulphur are mixed. Serum-albumin and serum-globulin contain only cystin, and they do not form H_2S. The presence of an unstable hydrogen-atom in albumins explains the reduction of iodates into iodides, ferri-compounds into ferro-compounds, etc., and according to Mann [5] also heat-coagulation.

(*i*) *Disintegration by means of Enzymes*

Amino-acids are very resisting to boiling with acids and alkalies, and also to the action of pepsin and trypsin (see below), for which reason the amino-group cannot be detached by these means. It can, however, be removed by enzymes, which seem to be common both in plants and in animals. Butkewitsch [6] has studied the action of lower plant-life on albumins and on amino-acids, and Bertel [7] has attempted to show that tyrosin when oxidised gives rise to homogentisinic acid, ammonia, and carbon-dioxide. Czapek [8] believes this transformation to be a good proof of the des-amination of amino-acids by means of enzymes, and attributes to the tyrosin-ferment both

[1] E. Fischer, *Ber. d. deutsch. chem. Ges.* **10**. 1334 (1877).
[2] Hübner and Alsberg, *Ann. d. Chem.* **156**. 330 (1865).
[3] Engelhardt, Latschinoff, and Malyscheff, *Zeitschr. f. Chem.* 1868, p. 353.
[4] Märcker, *Ann. d. Chem.* **136**. 75 (1865).
[5] Gustav Mann, *Physiological Histology*, 1902, p. 66.
[6] K. Butkewitsch, *Pringsheim's Jahrb. f. wissensch. Botanik*, **38**. 147.
[7] R. Bertel, *Ber. d. deutsch. bot. Gesellsh.* **20**. 460.
[8] F. Czapek, *Hofmeister's Beiträge*, **2**. 588 (1902).

oxidative and des-aminative properties. Saitos,[1] however, has shown that the des-amination of tyrosin cannot be the function of the ferment tyrosinase, because the ammonia- and tyrosinase-reactions do not run parallel with one another. This view is shared by Shibata.[2] The only other enzyme known in plants is the urease, or urea-splitting enzyme.

In animals special enzymes seem to be present. Loewi[3] has described a urea-forming ferment in the liver, and has shown that glycocoll and leucin give rise to an alcohol-ether soluble body with an easily dissociable amid-radical. Jakoby[4] found that liver-juice preserved under toluol showed a marked increase of the ammonia-nitrogen (amid-nitrogen), at the expense of the amino-acid-nitrogen (see p. 77). He failed, however, in getting the liver-juice to act on glycocoll.

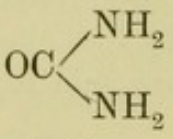
That tryptic digestion splits off ammonia from the albumin-molecule has been shown by Hirschler,[5] Kutscher,[6] and others. Pepsin acts similarly on albumin according to Zunz.[7] But that neither trypsin nor pepsin have any action on amino-acids has been shown by Gulewitsch.[8] His observations are confirmed by Schwarzschild,[9] who found that trypsin was unable to split off ammonia from asparagin, acetamide, urea, biuret, oxamide, benzamide, glycinamide, etc. But there is one exception to this rule, namely, the glycin-base of Curtius, or hexaglycyl-glycin-ethylester, as it does give off ammonia when acted upon by trypsin, while with pepsin it does not react at all.

Schmiedeberg[10] has shown that liver-pulp can convert hippuric acid into glycocoll and benzoic acid, and Gonnermann finds that acetamide, oxamide, benzamide, etc., mixed with liver-pulp, containing 1 per cent of sodium-fluoride as an antiseptic, are split up in some cases.

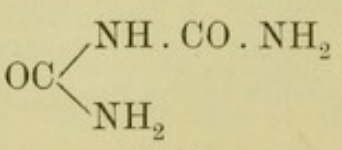
Shibata[2] has shown that in addition to the uro-bacteria, urea is also broken up by *Aspergillus niger*; and further, that this mould has some action on biuret, a very slight action on urethan, and

[1] K. Saitos, *Botanical Magazine* (Tokio), No. 201, Nov. 1903.
[2] K. Shibata, *Hofmeister's Beiträge*, **5**. 384 (1904).
[3] O. Loewi, *Zeitschr. f. physiol. Chem.* **25**. 511 (1898).
[4] M. Jakoby, *ibid.* **30**. 149 (1900).
[5] A. Hirschler, *ibid.* **10**. 302 (1886).
[6] E. Kutscher, *Endprodukte der Trypsinverdauung*, 1899 (Dissertation).
[7] E. Zunz, *Zeitschr. f. physiol. Chem.* **28**. 132 (1899).
[8] Wl. Gulewitsch, *ibid.* **27**. 540 (1899).
[9] M. Schwarzschild, *Hofmeister's Beiträge*, **4**. 155 (1904).
[10] O. Schmiedeberg, *Arch. f. experim. Pathol. u. Pharm.* **14**. 288 (1881).

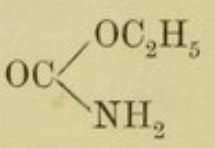
no action on guanidin (an amidine), and on the ureides: allantoin and sodium urate.



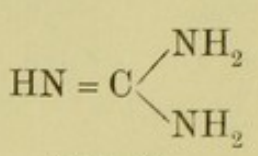
Urea.



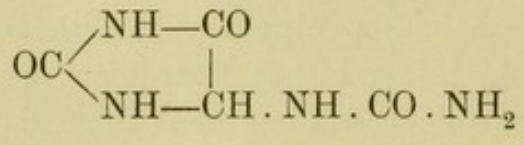
Biuret.



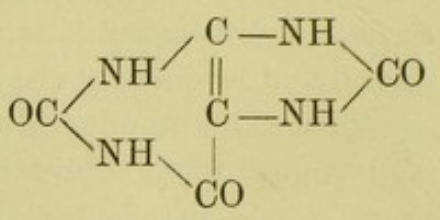
Urethan.



Guanidin.

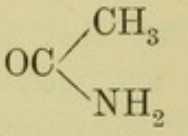


Allantoin.

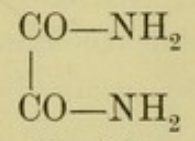


Uric acid.

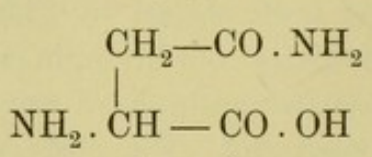
Aspergillus further liberates ammonia from acetamide and oxamide, traces from asparagin but none from benzamide (C_6H_5—$CO.NH_2$).



Acetamide.



Oxamide.



Asparagin.

It splits hippuric acid, $C_6H_5CO.NH—CH_2—COOH$, up into glycocoll, $CH_2.NH_2—COOH$, and benzoic acid.

1. *Disintegration of Albumins by Putrefactive Organisms.* — The disintegration of albumins by means of the ubiquitous putrefactive bacteria and the bacteria of the alimentary canal has been extensively studied by E. and H. Salkowski,[1] Nencki,[2] Baumann,[3] and Brieger,[4] further by Hoppe-Seyler,[5] Schultzen and Ries,[6] Blumenthal[7] and Rubner.[8] As has already been mentioned, these researches shed

[1] E. and H. Salkowski, *Zeitschr. f. physiol. Chem.* **8**. 417 (1884), **9**. 8 (1884), **9**. 491 (1885) (summary of previous papers); E. Salkowski, *ibid.* **27**. 302 (1899); H. Salkowski, *Ber. d. deutsch. chem. Ges.* **31**. II. 776 (1898).

[2] M. Nencki, *ibid.* **7**. II. 1593 (1874), **8**. I. 336 (1875), **10**. I. 1032 (1877); *Journ. f. prakt. Chem.* [2], **26**. 47 (1882); *Zeitschr. f. physiol. Chem.* **4**. 371 (1880); *Zentralbl. f. d. med. Wissensch.* 1878, Nr. 47.

[3] E. Baumann, *Ber. d. deutsch. chem. Ges.* **12**. II. 1450 (1879); *Zeitschr. f. physiol. Chem.* **4**. 304 (1880), **6**. 183 (1882), **7**. 282, 553 (1883), **20**. 583 (1895); Baumann and L. Brieger, *ibid.* **3**. 149 and 284 (1879).

[4] L. Brieger, *Journ. f. prakt. Chem.* [2] **17**. 124 (1877); *Ber. d. deutsch. chem. Ges.* **10**. I. 1027 (1877), **12**. II. 1986 (1879); *Zeitschr. f. physiol. Chem.* **2**. 241 (1878), **3**. 134 (1879), **4**. 414 (1880), **5**. 366 (1881); *Die Ptomaine*, Berlin, 1886.

[5] F. Hoppe-Seyler, *Pflüger's Arch. f. d. ges. Physiol.* **12**. 1 (1876); *Zeitschr. f. physiol. Chem.* **2**. 1 (1878).

[6] Schultzen and Ries, *Acute Yellow Atrophy of Liver*, Berlin, 1869.

[7] F. Blumenthal, *Virchow's Arch.* **137**. 539 (1894) (here also the older literature).

[8] M. Rubner, *Arch. f. Hygiene* **19**. 136 (1893).

much light on the existence of aromatic nuclei in albumins, long before these aromatic bodies themselves had been isolated.

Instead of studying the effect of impure cultures of so-called 'putrefactive bacteria,' pure cultures of definite bacteria were used later on. Thus Nencki [1] and his pupils [2] examined the bacillus of quarter-evil (Rauschbrand) under anaerobic conditions; Zoja [3] employed other anaerobes; Kühne [4] investigated the tubercle bacillus; Emmerling,[5] the Streptococcus longus; Taylor,[6] the Bacterium coli and Proteus vulgaris; Kutscher,[7] the yeast; Mörner,[8] the bacteria of the 'Gährströmling,' a Scandinavian food-stuff, which results from the action of apparently very definite micro-organisms on salt fish. These micro-organisms differ greatly from one another in some respects, and therefore the results obtained by the above researches have a high biological interest, but for the chemistry of the albumin-molecule the investigations of Hoppe-Seyler, Baumann, Ellinger,[9] and Spiro [10] are of greater importance, inasmuch as these investigators did not subject the albumin as a whole, but only its different primary dissociation-products, to the action of bacteria.

Bacteria behave, in the first instance, exactly as do trypsin or boiling acids, for they form at first albumoses and peptones, and subsequently the amino-acids. The action of bacteria does not stop, however, at this point, for Czapek [11] and Emmerling [12] have pointed out that the α-amino-acids are the very best nutritive media for bacteria.

These amino-acids are acted upon by bacteria in two distinct ways:—

(1) The amino-acids are converted into the corresponding simple acids in exactly the same way as if they were acted upon with fixed alkalies or with oxidising media. Owing to the elimination of ammonia, there are formed: acetic, propionic, butyric, valerianic,

[1] M. Nencki, *Monatshefte f. Chem.* **10**. 506 (1889).

[2] M. Nencki and N. Sieber, *ibid.* **10**. 526; L. Nencki, *ibid.* **10**. 862; R. Kerry, *ibid.* **10**. 864; L. Selitrenny, *ibid.* **10**. 908 (1889).

[3] S. Zoja, *Zeitschr. f. physiol. Chem.* **23**. 236 (1897).

[4] W. Kühne, *Zeitschr. f. Biologie*, **29**. 1 (1892), **30**. 221 (1894).

[5] O. Emmerling, *Ber. d. deutsch. chem. Ges.* **30**. II. 1863 (1897).

[6] Al. E. Taylor, *Zeitschr. f. physiol. Chem.* **36**. 487 (1902).

[7] F. Kutscher, *ibid.* **32**. 419 (1900).

[8] C. T. Mörner, *ibid.* **22**. 514 (1896).

[9] A. Ellinger, *Ber. d. deutsch. chem. Ges.* **31**. III. 3183 (1898); **32**. III. 3542 (1899); *Zeitschr. f. physiol. Chem.* **29**. 334 (1900); Ellinger and M. Gentzen, *Hofmeister's Beiträge*, **4**. 171 (1903).

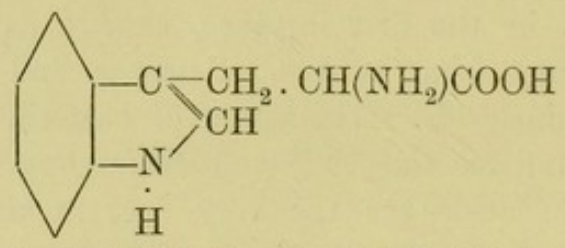
[10] K. Spiro, *ibid.* **1**. 347 (1901).

[11] F. Czapek, *ibid.* **1**. 538 (1902), **2**. 557 (1902), **3**. 47 (1902).

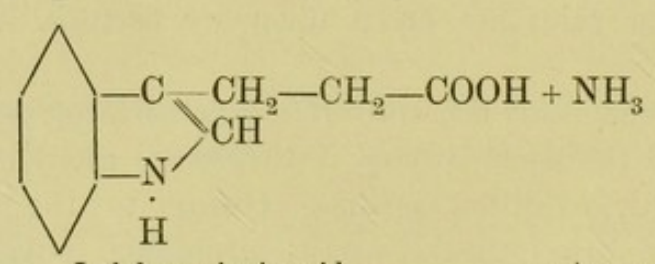
[12] Emmerling, *Ber. d. deutsch. chem. Ges.* **35**. II. 2289 (1902).

and caproic acids; further, δ-amino-valerianic acid, which may be derived either from ornithin, by a splitting off of the α-amino-group (H. Salkowski), or by an opening up of the pyrrol-ring of the α-pyrrolidin-carboxylic acid; succinic acid (Blumenthal); phenyl-propionic acid or cinnamic acid; *p*-oxyphenyl-propionic acid or hydro-para-cumaric acid; and indol-propionic acid.

The changes produced in chemically pure tryptophane or indol-amino-propionic acid by putrefaction have been carefully studied by Hopkins and Cole.[1] The first to observe that tryptophane is formed at an early period during the putrefaction of albumins was Claude Bernard, who observed the colour reactions with the halogens. Hopkins and Cole obtained indol and all the related substances—skatol, skatol-carboxylic (indol-acetic) acid, and skatol-acetic (indol-propionic) acid—by bacterial action, and therefore tryptophane is the precursor of these substances in putrefaction. Under the influence of anaerobic bacteria, tryptophane yields large amounts of indol-propionic acid, owing to the removal of amino-groups from the amino-acids:



Trytophane or indol-amino-propionic acid + Hydrogen =



Indol-propionic acid + Ammonia.

Baumann[2] found analogous changes in tyrosin, when it was digested with putrefying pancreas, and Nencki,[3] when tyrosin was subjected to the anaerobic growth of the Rauschbrand bacillus.

$$HO.C_6H_4.CH_2.CH(NH_2)COOH + H_2$$

Tyrosin or oxyphenylamino propionic acid + Hydrogen =

$$HO.C_6H_4.CH_2.CH_2.COOH + NH_3$$

Oxyphenyl-propionic acid + Ammonia.

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[1] Hopkins and Cole, *Journal of Physiology*, **29**. 451 (1903).

[2] Baumann, *Ber. d. deutsch. chem. Ges.* 1451 (1879).

[3] Nencki, *ibid.* **7**. 1593 (1874).

E. Schulze found that phenylamino-propionic acid is changed by aerobic bacteria in this manner. (1) By des-amination phenyl-propionic acid is first produced, and the latter then oxidised in the presence of air into phenyl-acetic acid. In Nencki's anaerobic experiments the change stopped short at the first stage—the simple removal of the amino-group by reduction—and only phenyl-propionic acid was obtained.

(2) Carbonic acid is split off, leading, as Ellinger has shown, to the formation of Brieger's diamines. Thus lysin is converted into penta-methylene-diamin or cadaverin, $C_5H_{14}N_2$,

Lysin $CH_2NH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH$ $-CO_2$ = $CH_2NH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2NH_2$ Cadaverin

while the ornithin of the arginin gives rise to tetra-methylene-diamin or putrescin, $C_4H_{12}N_2$,

Ornithin $CH_2NH_2 \cdot CH_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH$ becomes $CH_2NH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2NH_2$ Putrescin

and phenylalanin forms phenylethylamin (Nencki, Spiro).

$C_6H_5 . CH_2 . CH(NH_2)COOH$ becomes $C_6H_5 . CH_2 . CH_2(NH_2)$

Phenylalanin. Phenylethylamin.

The mother-substance of methylamin, which Mörner and Emmerling have found in some bacteria, may be glycocoll

$$[NH_2CH_2COOH] \text{ minus } [CO_2] = NH_2CH_3.$$

(3) It is possible for both processes mentioned above under (1) and (2) to take place. Kerry has found glycocoll to give rise to methane, CH_4. As a rule the change does not stop here, for the terminal C-atom is oxidised, the CO_2 is given off, and then reoxidation takes place, as is clearly shown by the following example :—

p-oxyphenylamino-propionic acid .	$C_6H_4 . OH . CH_2CHNH_2 . COOH$
p-oxyphenyl-propionic acid . . .	$C_6H_4 . OH . CH_2CH_2 . COOH$
p-oxyphenyl-acetic acid	$C_6H_4 . OH . CH_2COOH$
p-oxymandelic acid	$C_6H_4 . OH . CH(OH)COOH$
p-cresol	$C_6H_4 . OH . CH_3$
phenol	$C_6H_5 . OH$

The other two aromatic groups behave similarly: phenylamino-propionic acid → phenyl-propionic acid → phenyl-acetic acid; and indolamino-propionic acid → indol-propionic acid → indol-acetic acid → skatol → indol.

Benzoic acid has not yet been found.

Phenol, indol, and skatol are looked upon as the characteristic products of putrefaction, as they are readily recognised by their smell, and because they are absorbed as the final products of intestinal putrefaction and then excreted in the urine as paired sulphonic acids.[1] But these substances are by no means formed by all bacteria. Phenyl-acetic acid or α-toluylic acid is eliminated as phenaceturic acid.[2]

Phenyl-acetic acid. Phenaceturic acid.

The fatty series is represented by formic acid and carbonic acid, both of which are constantly present. They may be formed by a method analogous to that described above for the aromatic series, but whether the same holds good for the other aliphatic acids cannot as yet be considered as settled, for they may be derived either from amino-acids or from higher homologues.

Cystin gives rise to sulphuretted hydrogen, which is another characteristic product of putrefaction. Salkowski, Baumann, Rubner, Mörner, Nencki, and Zoja observed also methylmercaptane, but great caution is necessary in explaining its derivation, because Rubner has shown that it may be formed synthetically.

2. *Disintegration during the Metabolism of Plants, exclusive of Bacteria.*— Most light has been thrown on this question through the work of E. Schulze[3] and his pupils. There are contained in the seeds of plants certain albuminous substances which serve as a storehouse for the growing embryo, and these are made available through the action of proteolytic enzymes, whenever germination commences. These enzymes have been examined by v. Gorup-Besanez,[4] Neumeister,[5] Weis,[6] Butkewitsch,[7] and others; they act analogously to trypsin, for

[1] E. Baumann and E. Herter, *Ber. d. deutsch. chem. Ges.* **1**. 244 (1877).

[2] E. and H. Salkowski, *ibid.* **9**. 491 (1885).

[3] E. Schulze, *ibid.* **24**. 18 (1897), **26**. 411 (1899), **30**. 241 (1900), (in these three papers is a summary of the previous ones); E. Schulze and E. Winterstein, *ibid.* **33**. 547 (1901), **35**. 299 (1902).

[4] v. Gorup-Besanez, *Ber. d. deutsch. chem. Ges.* **7**. II. 1478 (1874); **8**. II. 1510 (1875).

[5] R. Neumeister, *Zeitschr. f. Biol.* **30**. 447 (1894).

[6] F. Weis, *Zeitschr. f. physiol. Chem.* **31**. 79 (1900).

[7] Wl. Butkewitsch, *ibid.* **32**. 1 (1901).

they give rise to amino-acids. As plants do not possess a circulatory system comparable to that of animals, the amino-acids remain *in situ*, *i.e.* either in the seed or in the cotyledons. Schulze has pointed out especially that the amounts of the amino-acids which are formed during germination agree with the amounts he was able to obtain by acting on the stored reserve-material with acids. He found leucin, amino-valerianic acid, phenylalanin and tyrosin, aspartic and glutaminic acids, lysin, histidin, arginin, guanidin, and ammonia.

Aspartic and glutaminic acids occur in the largest amounts, and these seem to take the place of one another in different plants, so that in some, even closely related, species one is present in large excess over the other. There occurs in plants, further, a synthesis brought about in this way :—A part of the ammonia of the mono-amino-acids is split off and then used for the conversion of some other mono-amino-acids into di-amino-acids. Thus aspartic and glutaminic acids are converted into asparagin and glutamin.

Aspartic acid, $CO\,.\,OH\,.\,CH_2\,.\,CH(NH_2)COOH$,
becomes $CO(NH_2)\,.\,CH_2\,.\,CH(NH_2)COOH$, Asparagin.

Glutaminic acid, $CO\,.\,OH\,.\,CH_2\,.\,CH_2\,.\,CH(NH_2)\,.\,COOH$,
becomes $CO(NH_2)\,.\,CH_2\,.\,CH_2\,.\,CH(NH_2)\,.\,COOH$, Glutamin.

Both are found as reserve material in germinating seeds, and may also be transported during germination. Through further complex syntheses they are then built up into albumin, taking up during this process also non-nitrogenous radicals derived from the ever-present carbohydrates, and perhaps also from the desaminated remainders of the mono-amino-acids. Corresponding processes take place also in other parts of the plant. The great importance of asparagin for the albumin-synthesis in plants is also shown by the results which Nägeli and Kühne [1] obtained with bacteria, as these can grow in media which contain no other nitrogenous substance beside asparagin.

Only in some conifers does arginin take the place of importance over asparagin and glutamin.[2] Arginin may be obtained in large quantities by boiling coniferous seeds,[3] and is therefore also a primary dissociation-product.

The fact that individual amino- and di-amino-acids are met with in very varying amounts can only partly be attributed to differences existing in the chemical constitution of the albumins from which they

[1] W. Kühne, *Zeitschr. f. Biol.* **29**. 1 (1892), **30**. 221 (1894).
[2] E. Schulze, *Zeitschr. f. physiol. Chem.* **22**. 435 (1896).
[3] E. Schulze, *ibid.* **24**. 276 (1897).

were obtained. In what amounts any acid will be found depends primarily on the stage which germination has reached in a plant.

Of especial importance are finally the investigations into the nature of tyrosinase, which were made by Bertrand,[1] Gonnermann,[2] and others. This tyrosinase is a ferment which is liberated in dying vegetable cells; it converts tyrosin into homogentisinic acid, and causes thereby the blackening or darkening of beetroot juice, of cuts into plants, etc. Biedermann,[3] v. Fürth and Schneider,[4] and Przibram[4] have also demonstrated the presence of tyrosinase in animals, *e.g.* in the secretion of the midgut of the mealworm,[3] in the hæmolymph of butterflies, and in the ink-sac of sepia.[5] It converts, according to v. Fürth and Schneider, tyrosin, but also pyrocatechin, hydroquinone, suprarenin, and oxyphenylethylamin, into dark bodies, which in their behaviour closely resemble the melanins. These dark bodies, and also melanin, are produced as the result of oxidation. Ducceschi[4] succeeded also in changing tyrosin into darkly coloured bodies by means of careful oxidation with chlorates. See also p. 580, chapter XII.

3. *Disintegration during the Metabolism of Animals.*—The changes which nucleo-proteids undergo as the result of auto-digestion are dealt with later. See p. 431.

The albumin, taken as food, is converted in the alimentary canal of the higher animals by means of the four proteolytic ferments, pepsin, trypsin, erepsin, and arginase, into primary crystalline dissociation-products, the amino-acids, etc., which are then absorbed in this form.[5] Whether a part of the albumin taken as food can or cannot be absorbed in the form of albumoses, peptones, and peptids, is a question which has not yet been settled,[6] and which cannot be discussed here.

Aromatic amino-acids have been specially studied by the following investigators:—

E. and H. Salkowski[7] believed that non-aminated acids which are

[1] Bertrand, *Compt. rend.* **122**. 1215 (1896); vgl. H. Steudel, *Deutsche med. Wochenschr.* 1900, p. 372.

[2] M. Gonnermann, *Pflüger's Arch. f. d. ges. Physiol.* **82**. 289 (1900).

[3] W. Biedermann, *ibid.* **72**. 105 (1898).

[4] O. v. Fürth and H. Schneider, *Hofmeister's Beiträge*, **1**. 229 (1901).

[5] A. Schmidt-Mülheim, *Arch. f. (Anat. u.) Physiol.* 1879, p. 39; O. Cohnheim, *Zeitschr. f. physiol. Chem.* **35**. 396 (1902), **36**. 13 (1902); F. Kutscher and J. Seemann, *ibid.* **34**. 528 (1902); O. Löwi, *Arch. f. experiment. Pathol. u. Pharmakol.* **48**. 305 (1902).

[6] E. Fischer and E. Abderhalden, *Zeitschr. f. physiol. Chem.* **39**. 81 (1903); G. Embden and F. Knoop, *Hofmeister's Beitr.* **3**. 120 (1902).

[7] E. and H. Salkowski, *Zeitschr. f. physiol. Chem.* **7**. 169 (1883).

homologous with benzoic acid became converted into benzoic acid if the side-chain contained more than two carbon atoms, or if the stability of the side-chain was weakened by the replacement of one H atom by OH, or the replacement of 2H by O, as in benzoyl-carboxylic acid; but Schotten[1] showed that mandelic acid $C_6H_5 . CH(CH)COOH$ remained unaltered, and explained the peculiar position occupied by phenyl-alanin and tyrosin amongst all aromatic-substances, inasmuch as these two acids are oxidised almost completely in the body, as due to the side-chain of the benzene-nucleus possessing a 3-C chain, the central C of which contains the NH_2 group. Bunge has explained the non-oxidation of phenyl-acetic, $C_6H_5—CH_2 . COOH$, and mandelic acid, $C_6H_5 . CH(OH)COOH$, by assuming that the non-oxidisable radicals C_6H_5 and COOH protect the CH_2 and CH(OH) groups. This view cannot, however, be accepted, for Pohl[2] has shown malonic acid, $COOH—CH_2—COOH$, in which CH_2 stands between two carboxyl-groups, to disappear in the body to the extent of over 90 per cent, and diphenyl-methane $C_6H_5—CH_2—C_6H_5$ is oxidised into $C_6H_5—CH_2—C_6H_4—OH$.

Knoop[3] has also studied the changes which the aromatic fatty acids undergo in the body. Such radicals as CH_3, $CH_2—OH$, CHO, and $CH_2—NH_2$ attached to benzene, C_6H_6, are as a rule oxidised to $CO . OH$, and the benzoic acid, $C_6H_5—CO . OH$, formed in this way then links on to glycocoll, $CH_2(NH_2)—CO . OH$, to form hippuric acid, $C_6H_5 . CO—NH . CH_2COOH$.

Tyrosin, phenylalanin, and α-amino-cinnamic acid, $C_6H_5 . CH_2 . CH(NH_2) . COOH$, are completely oxidised, while phenyl-propionic acid and cinnamic acid, $C_6H_5 . CH : CH . COOH$ are changed into benzoic acid. The carbon-chain remains unaltered in phenylacetic acid, $C_6H_5—CH_2 . COOH$, and also in its substitution products: mandelic acid, $C_6H_5 . CH(OH)COOH$, and phenyl-amino-acetic acid, $C_6H_5 . CH(NH_2)COOH$, only that in the latter the amino-group, NH_2, is replaced by OH.

Phenyl-propionic acid on being converted into benzoic acid cannot pass through the stage of phenyl-acetic acid, as otherwise the latter compound ought to appear in the urine. It follows, therefore, according to Knoop, that the oxidation of phenyl-propionic acid can only occur in the β- and not in the α-position.

Administered in gelatine capsules to dogs, 2 grammes of—

Phenyl-propionic acid was converted into hippuric acid,

[1] Schotten, *Zeitschr. f. physiol. Chem.* **8.** 68 (1883-4).
[2] Pohl, *Arch. f. experim. Pathol.* **37.** 413 (1896).
[3] Franz Knoop, *Hofmeister's Beiträge*, **6.** 150 (1904).

Phenylacetic acid was converted into		phenaceturic acid (no hippuric acid),
Ethyl-benzene	—→	hippuric acid (no phenaceturic acid),
Mandelic acid	remained	unchanged.

There is thus a marked difference between the oxidation of an alcohol (ethyl-benzene and its corresponding acid (phenylacetic acid).

Phenyl-butyric, phenyl-α-lactic, phenyl-pyro-uvic (and other five acids examined by Knoop) containing more than 2C in the side-chain did not give rise to benzoic acid.

It has already been pointed out that phenyl-propionic acid can only become oxidised in the β-position, and this rule seems to hold good for all saturated, normal, terminally phenyl-substituted fatty acids; analogous changes seem to take place in diabetes when oxy-butyric and acet-acetic acid appear in the urine. In the case of phenyl-alanin and other α-substituted phenyl-propionic acids, and in phenyl-α-amino-cinnamic acid, the α-substitution seems to make β-oxidation impossible, and it must be assumed that the substances last mentioned undergo some other change before they become oxidised.

Nencki and Schultzens[1] were the first to show that leucin and glycocoll administered in the food give rise to urea. Salkowski[2] confirmed this observation, and obtained similar results with sarcosin and alanin, while Knieriem[3] and Salkowski[4] found aspartic acid also to increase the urea output.

The extent to which the administration of various amino-acids will allow animals to maintain their nitrogen equilibrium was first studied by Loewi,[5] who showed that such end products of digestion, which no longer give the biuret-reaction, are still able to replace the albumins destroyed during the metabolism.

That amino-acids, when given in moderate amounts [glycocoll up to 5 grms., *i*-alanin (3 grms.), leucin (8 grms.), phenylalanin (3 grms.)], are completely broken up, has been shown by Abderhalden and Bergell.[6] Given in excess (25 grms. to a dog), glycocoll appears partly unchanged in the urine (Salkowski), and tyrosin given to rabbits gives rise to tyrosin-hydantoin, according to Blendermanns.[7]

[1] Nencki and Schultzens, *Zeitschr. f. Biol.* **8**. 124 (1872).
[2] Salkowski, *Zeitschr. f. physiol. Chem.* **4**. 100 (1880).
[3] Knieriem, *Zeitschr. f. Biol.* **10**. 263 (1874).
[4] Salkowski, *Zeitschr. f. physiol. Chem.* **42**. 213 (1904).
[5] O. Loewi, *Arch. f. experim. Pathol. u. Pharm.* **48**. 303 (1902).
[6] Abderhalden and Bergell, *ibid.* **39**. 9 (1903).
[7] Blendermanns, *ibid.* **6**. 234 (1882).

Stolte [1] injected one rabbit weighing about 3000 grms. with pure preparations of glycocoll, alanin, aspartic and glutaminic acids, phenylalanin, tyrosin, and leucin, with this result:

1. The aromatic mono-amino-acids (tyrosin and phenylalanin) do not give rise to an increased urea output.
2. Certain mono-amino-acids (alanin, aspartic acid, and glutaminic acid) increase the urea output, but appear also partially in the urine.
3. Certain mono-amino-acids, if given even in large amounts (glycocoll and leucin), are broken up so completely in the body as practically never to appear in the urine. They, of course, give rise to a large increase in the urea output.

The change which amino-acids undergo in the body is probably over the stage of oxy-acids, as occurs, *e.g.*, in plants and in alcaptonuria when tyrosin and phenylalanin are changed into homogentisinic acid (see p. 114). It is quite an open question whether the carbon-chain, after the splitting off of the nitrogen which forms urea, breaks up still further, or whether it is made use of in the building up of other non-nitrogenous substances, such as carbohydrates and fat.

V. Henriques and C. Hansen [2] have fed rats on casein hydrolysed by H_2SO_4 or HCl and casein digested by trypsin + erepsin in toluol water, and found that casein hydrolysed by acids gives rise to products which cannot keep an animal in N-equilibrium; while the products of casein digestion obtained with trypsin + erepsin not only cover the N-loss, but even allow of N being stored. The nitrogen-equilibrium is also kept up by that fraction of tryptically-digested casein which is not precipitable by phospho-tungstic acid, *i.e.* by mono-amino acids. Those compounds of tryptic digestion which are soluble in 96 per cent alcohol heated to 50° also maintain N-equilibrium, while the alcohol-insoluble fraction does not.

Abderhalden and Rona [3] also find that rats and dogs fed on the products obtained by hydrolysing casein by means of acids only live a few days longer than animals not fed at all, while dogs fed on casein-products digested tryptically till the biuret-reaction is no longer obtainable can maintain their N-equilibrium.

Wohlgemuth [4] on feeding rabbits with the inactive or racemised mono-amino acids, tyrosin, leucin, aspartic acid, and glutaminic acid, found that the inactive acids became dissociated during metabolism in

[1] Karl Stolte, *Hofmeister's Beiträge*, **5**. 15 (1903).
[2] V. Henriques and C. Hansen, *Zeitschr. f. physiol. Chem.* **43**. 417 (1905).
[3] E. Abderhalden and P. Rona, *ibid.* **42**. 528 (1904), and **44**. 198 (1905).
[4] J. Wohlgemuth, *Ber. d. deutsch. chem. Gesch.* **38**. 2064 (1905).

such a way that the component occurring normally in the body was oxidised as far as it could be assimilated, while the anormal ('körperfremde') component was excreted partly or completely in the urin. If, for example, the tyrosin occurring normally in animals is the dextro-rotatory tyrosin, then it would be acted upon, while the laevo-rotatory tyrosin which accompanies it in the raceme-tyrosin would be eliminated.

The part played by kreatin and kreatinin in metabolism is discovered by Czernecki,[1] while the fate of indol and skatol, when introduced into the body, has been studied by Grosser.[2]

Umber[3] believes to have altered the composition of albumin in the body during starvation by the administration of benzoic acid. The latter, by linking on to glycocoll, gives rise to hippuric acid, which, being excreted by the kidney, leads to a diminution in the glycocoll-context of albumins. Abderhalden, Bergell, and Dörpinghaus[4] do not agree with this, but the possibility of altering the composition of albumins is beyond doubt, for, apart from the histological evidence which the author possesses and which shows that it is possible to excite or suppress nuclear activity at will, and apart also from the evidence that protamins are derivatives of ordinary albumins (see p. 420), there is the strong evidence adduced by Wakemann,[5] who has shown phosphorous-poisoning to attack that nucleus of the albumin-molecule which contains most nitrogen, as is proved by the fact that the amount of arginin, histidin, and lysin becomes diminished to a greater extent than does that of the mono-amino-acids. The albuminous substances which are left behind in phosphorous-poisoning are poorer in nitrogen and in the basic constituents of the cell.

In the following pages the action of intracellular enzymes on the albumin molecule will be discussed.

Kossel in 1898[6] first expressed the opinion that proteolytic ferments might act on imide-groups of the albumin-molecule. In a second paper[7] Kossel and Dakin divide ferments into two classes:

1. Oxy-lytic ferments, which loosen the O-link by which the radicals are kept together in the fats and carbohydrates.

[1] W. Czernecki, *Zeitschr. f. physiol. Chem.* **44**. 294 (1905).

[2] P. Grosser, *ibid.* **44**. 321 (1905).

[3] F. Umber, *Berl. klinische Wochenschrift*, No. 39 (1903).

[4] E. Abderhalden, P. Bergell, and Th. Dörpinghaus, *Zeitschr. f. physiol. Chem.* **41**. 153 (1904).

[5] A. J. Wakemann, *ibid.* **44**. 335 (1905).

[6] A. Kossel, *ibid.* **25**. 188 (1898).

[7] A. Kossel and H. D. Dakin, *ibid.* **41**. 321 (1904).

2. Imino-lytic ferments [including also the amino-lytic ferments acting on the amino-groups of urea].

The imino-lytic ferments are again subdivided into—

(1) Trypsin and erepsin, which separate the imide NH from the neighbouring carbonyl CO, according to the general formula :

$$\underset{(1)}{CO}—\underset{(2)}{NH}—C \text{ becomes } \underset{(1)}{COOH}, \underset{(2)}{NH_2}—C;$$

or as in the case of arginin :

$$\underset{(1)}{CO}—NH—CNH—\underset{(2)}{NH}—C \text{ becomes}$$
$$\underset{(1)}{COOH}, \underset{(2)}{NH_2}—CNH—NH—C;$$

(2) Arginase, which separates off urea or $CO\begin{matrix}NH_2\\NH_2\end{matrix}$ from arginin.

$$NH_2 . CNH . NH . C_3H_6 . CHNH_2 . COOH + H_2O =$$
$$NH_2 . CO . NH_2 + NH_2 . C_3H_6 . CHNH_2 . COOH.$$

The changes induced by arginase in arginin are : proton (β-clupeon) → arginin → ornithin → urea → amino-valerianic acid. The arginase may be extracted from liver-substance with water or dilute acetic acid ; it is also present in the mucous membrane of the dog's intestine, and its, probably, universal occurrence explains why arginin is absent amongst the products of autolysis.[1]

A second paper on arginase by Kossel and Dakin[2] shows that ornithin remains attached to the albumin-molecule, while several or all of the arginin-compounds are attacked in such a manner as to liberate the urea-radical, or at least to become changed in some as yet unknown manner.[3]

That arginase also occurs in the yeast has been shown by Shiga[4] working under Kossel. Shiga also found that arginase does not act on guanidin, which is the mother-substance of arginin.

There are, further, some enzymes which behave analogously to putrefactive bacteria in splitting off the terminal carboxyl-group (see p. 103), and which convert lysin and ornithin into cadaverin and putrescin, and tyrosin into oxyphenylethylamin. Werigo[5] and

[1] Kutscher and Seemann, *Zeitschr. f. physiol. Chem.* **34**. 114 (1901), and **35**. 440 (1902).

[2] H. D. Dakin, *Journ. of Physiol.* **30**. 84 (1903).

[3] A. Kossel and H. D. Dakin, *Zeitschr. f. physiol. Chem.* **42**. 181 (1904).

[4] K. Shiga, *ibid.* **42**. 502 (1904).

[5] B. Werigo, *Pflüger's Arch. f. d. gesamte Physiol.* **51**. 362 (1892).

Steyrer[1] found cadaverin during pancreatic digestion, Lawrow[2] and Langstein[3] during gastric digestion; Lawrow found putrescin; oxyphenylethylamin was found by Langstein during gastric digestion, and by Emerson[1] during pancreatic digestion. These reactions depend, of course, on special enzymes, and not on pepsin or trypsin. Jacoby[4] has observed that the nitrogen which can be split off by means of magnesia, is increased during the autodigestion of the liver, and that the fixed nitrogen becomes loosened somehow.

The question of des-amination of amino-acids has been specially gone into by Lang,[5] who studied the action of finely-divided tissues (liver, kidney, lymph-glands, supra-renals, testes, pancreas, intestinal mucous membrane, spleen, muscle) on mono-amino-acids (glycocoll, tyrosin, phenylalanin, leucin, cystin), on acid-amides (asparagin, glutamin, acetamide), and also on urea and glucosamin.

All the tissues just mentioned act vigorously on asparagin and glutamin; glucosamin is readily broken up by the kidney and supra-renals, to a certain extent by the intestine, testis, liver, and spleen, very slightly by muscle, and not at all by the pancreas; urea was acted upon by the pancreas and to a certain extent by the liver, which also acts on glycocoll, acetamide, leucin, and uric acid. Lymph-glands and the spleen have no action on glycocoll. It will be seen that the power of desamination varies not only greatly amongst different organs but also as regards the individual amino-acid radicals which are subjected to the influence of the tissue-compounds.

When glycocoll is injected intravenously in dogs, a small percentage is excreted unchanged by the kidneys, while the greater part is desaminated in the tissues, leading thereby to an increase of ammonia in the blood, according to Salaskin and Kowatevsky.[6]

Amide-splitting enzymes are discussed by Shibata (see p. 99).[7]

Trypsin and erepsin do not split off ammonia,[8] while arginase splits off urea as shown above. Albumins are altered beyond the stage of the amino-acids, according to Kutscher,[9] for this observer failed to obtain arginin, tyrosin, glutaminic and aspartic acids from autodigested thymus.

[1] R. L. Emerson, *Hofmeister's Beitr.* **1**. 501 (1902).
[2] D. Lawrow, *Zeitschr. f. physiol. Chem.* **33**. 312 (1901).
[3] L. Langstein, *Hofmeister's Beiträge*, **2**. 229 (1902).
[4] M. Jacoby, *Zeitschr. f. physiol. Chem.* **30**. 149 (1900), **33**. 126 (1901).
[5] S. Lang, *Hofmeister's Beiträge*, **5**. 321 (1904).
[6] Salaskin and Kowatevsky, *Zeitschr. f. physiol. Chem.* **52**. 410 (1904).
[7] K. Shibata, *Hofmeister's Beiträge*, **5**. 384 (1904).
[8] J. Mochizuki, *ibid.* **1**. 44 (1901); O. Cohnheim, *Zeitschr. f. physiol. Chem.* **35**. 134 (1902).
[9] F. Kutscher, *ibid.* **34**. 114 (1901).

We may, secondly, obtain as the result of some pathological state certain intermediate products of metabolism. Although we cannot with certainty exclude the possibility that 'the car was not running previously on wrong lines,' it is generally believed that we are dealing with substances which arose normally, but the oxidation of which has somehow been interfered with, and that for this reason the substances leave the kidney in an unoxidised condition. This view is upheld in the case of alcaptonuria by Mayer,[1] Mittelbach,[2] Garrod,[3] Abderhalden and Falta;[4] Garrod describes the condition as a 'chemical abnormality'—one might add, as an abnormality due to arrest of development (Cohnheim).

The occurrence of mono-amino-acids in the urine during normal and pathological conditions has been studied by Abderhalden,[5] Abderhalden and Bergell,[6] Ignatowski,[7] and Abderhalden and Barker,[8] and Erben.[9] For methods of studying the diamines of the urine see the paper by Loewy and Neuberg.[10]

Cystin has been observed in the urin[11] and also in the tissues in a case of cystinuria.[12] Other substances occurring in the urine are cadaverin and putrescin,[13] which frequently accompany cystin, and the peculiar substances found in alcaptonuria. The chief substance seen in cases of alcaptonuria is, according to Baumann and Wolkow[14] and Huppert,[15] homogentisinic acid or dioxyphenyl-acetic acid, in addition to which Kirk[16] observed also uroleucic acid or dioxyphenyl-lactic acid. The latter is derived from tyrosin, and, as Falta and Langstein[17] have found, also from phenylalanin. The

[1] E. Mayer, *Deutsch. Arch. f. klin. Med.* **70**. 443 (1901).

[2] F. Mittelbach, *ibid.* **71**. 50 (1901).

[3] A. E. Garrod, *The Lancet*, 1901, II. 1484; 1902, II. 1616.

[4] E. Abderhalden and W. Falta, *Zeitschr. f. physiol. Chem.* **39**. 143 (1903); W. Falta, *Baseler Naturf. Ges.* **15**. Heft 2 (1903).

[5] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **38**. 557 (1903).

[6] E. Abderhalden and Peter Bergell, *ibid.* **39**. 9 and 464 (1903).

[7] Alexander Ignatowski, *ibid.* **42**. 371 (1904).

[8] E. Abderhalden and Lewellys. F. Barker, *ibid.* **42**. 524 (1904).

[9] Franz Erben, *ibid.* **43**. 320 (1904).

[10] A. Loewy and C. Neuberg, *ibid.* **43**. 355 (1904).

[11] E. Baumann and L. v. Udránszky, *ibid.* **13**. 562 (1889), **15**. 77 (1890). Compare with p. 56 of this book.

[12] E. Abderhalden, *ibid.* **38**. 557 (1903).

[13] E. Baumann and v. Udranszky, *ibid.* **13**. 562 (1889), **15**. 77 (1891).

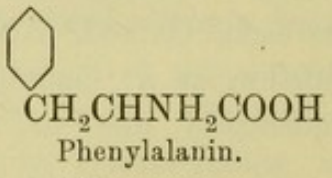
[14] M. Wolkow and E. Baumann, *ibid.* **15**. 228 (1891).

[15] Huppert, *Deutsch. Arch. f. klin. Med.* **64**. 129 (1899); *Zeitschr. f. physiol. Chem.* **23**. 412 (1897); Neubauer and Vogel's *Harnanalyse*, 10. Aufl., by H. Huppert, Wiesbaden, 1898, p. 243.

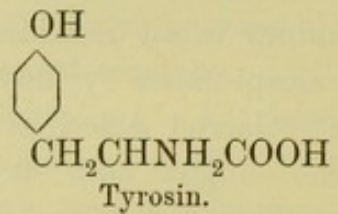
[16] R. Kirk, from Huppert, *Harnanalyse*.

[17] W. Falta and L. Langstein, *Zeitschr. f. physiol. Chem.* **37**. 513 (1903).

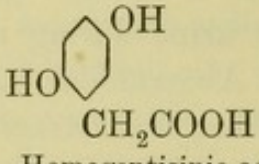
naturally-occurring, active phenylalanin is converted almost completely into homogentisinic acid, while the racemosed phenylalanin is only converted to the extent of 50 per cent. The four constitutional formulæ given below show that, because of the simultaneous oxidation and reduction, changes take place not only in the side-chain but also in the benzene ring.



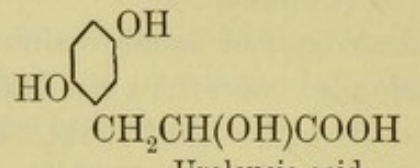
Phenylalanin.



Tyrosin.



Homogentisinic acid.



Uroleucic acid.

What changes aromatic acids undergo in the body in cases of alcaptonuria has been carefully studied by Neubauer and Falta,[1] who point out that α-phenyl-propionic acid behaves like phenyl-alanin and tyrosin in increasing the output of homogentisinic acid.

Baumann and v. Udránszky, Abderhalden, and Abderhalden and Falta have proved that alcaptonuria, cystinuria, and diaminuria are due to abnormalities in the metabolism and not due to intestinal putrefaction. The occurrence of amino-acids in the urine in cases of phosphorus poisoning and in acute yellow atrophy of the liver is also dependent on faulty metabolism.[2]

Drechsel[3] has found cystin also in the normal liver. The taurin of the bile is a derivative of cystin.[4] See p. 59.

By analogy we may further reason that all substances which on introduction into the body do not become oxidised, are not produced in metabolism (Cohnheim).

The carbohydrate-radical of albumins is discussed on p. 154.

Nothing is known as to relationship of albumins to oxyproteic acid,[5] uroproteic acid,[6] and uroferric acid,[7] substances which have been found in the urine.

[1] Otto Neubauer and W. Falta, *Zeitschr. f. physiol. Chem.* **42**. 81 (1904).

[2] E. Abderhalden and P. Bergell, *ibid.* **39**. 464 (1903).

[3] E. Drechsel, *Arch. f. (Anat. u.) Phys.* 1891, p. 243 ; *Zeitschr. f. Biol.* **33**. 86 (1896).

[4] G. v. Bergmann, *Hofmeister's Beitr.* **4**. 192 (1903) ; see also J. Wohlgemuth, *Zeitschr. f. physiol. Chem.* **40**. 81 (1903).

[5] Bondzynski and R. Gottlieb, *Centralbl. f. d. med. Wissensch.* 1897, p. 577 ; F. Pregl, *Pflüger's Arch. f. d. ges. Physiol.* **75**. 87 (1899) ; Bondzynski and K. Panek, *Ber. d. deutsch. chem. Ges.* **35**. II. 2959 (1902).

[6] M. Cloëtta, *Arch. f. exper. Path. u. Pharm.* **40**. 27 (1897).

[7] O. Thiele, *Zeitschr. f. physiol. Chem.* **37**. 251 (1903).

CHAPTER III

ON THE SYNTHESIS OF ALBUMINS

If the biuret-reaction is the most characteristic test for albumins, then we must regard Schaal[1] as having been the first to prepare—without knowing it, however—a synthetic compound resembling albumin, when he condensed aspartic-acid chloride in a stream of CO_2 at 200° into what Schiff[2] has subsequently called polyaspartic acids, (the octo-aspartic acid is described on p. 147). Grimaux then showed in 1882[3] that Schaal's compounds give the biuret-reaction as do also compounds he prepared himself by melting asparagin and urea together. The new substances formed by this last reaction were typically colloidal in nature.[4]

While working at the synthesis of hippuric acid, according to Desaignes' method,[5] Theodor Curtius found in 1881[6] that benzoyl-chloride acting on silver glycocollate produces hippuric acid, hippuryl-amino-acetic acid [or benzoyl-glycyl-amino-acetic acid or benzoyl glycylglycin],[7] and a γ-acid.

$$C_6H_5 . CO . Cl + NH_2 . CH_2 . CO . OAg =$$

benzoyl chloride + silver – glycocollate :

(1) hippuric acid.

$$C_6H_5CO—NH . CH_2 COOH$$

(2) benzoyl-glycyl-amino-acetic acid.

$$C_6H_5CO—NH . CH_2 . CO—NH . CH_2 . COOH$$

(3) benzoyl-pentaglycyl-amino acetic acid ; which is identical with the γ-acid.

$$C_6H_5CO . (NHCH_2CO)_5 . NHCH_2 . COOH$$

[1] Ed. Schaal, *Liebig's Annalen*, **157.** 24 (1871).

[2] H. Schiff, *Ann. d. Chem.* **303.** 183 (1898) and **307.** 231 (1899).

[3] Ed. Grimaux, *Bull. soc. chim.* **38.** 64 (1882).

[4] These references are quoted from Hofmeister's article in the *Ergebnisse d. Physiologie* I., 1, p. 790 (1902).

[5] Desaignes, *Jahresb. d. Chem.* 1857, p. 367.

[6] Theodor Curtius, *Journ. f. prakt. Chem.* [2] **24.** 239 (1881).

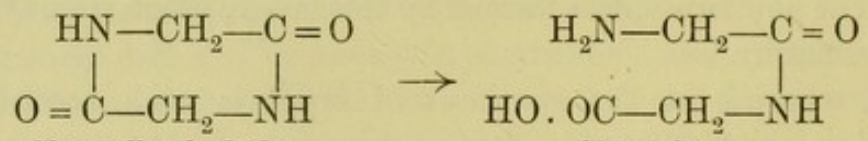
[7] Fischer and Fourneau have given the term glycyl to the radical (NH_2CH_2CO). Glycyl-glycin = 2 molecules of glycocoll or glycin minus one molecule of water. *Ber. d. deutsch. chem. Ges.* **34.** 2868 (1901).

The γ-acid of 1881 had its constitution, however, only explained in 1904 by Curtius and Benrath,[1] after Curtius and Wüstenfeld[2] had found, that in building up glycyl-chains by means of acid-azides all the higher chains beginning with the triglycyl compound gave the biuret-reaction.

The so-called 'biuret-base' of Curtius was first obtained in 1883[3] by the spontaneous decomposition of glycocollester, which can readily be obtained by suspending glycocoll in ethylalcohol; passing dry HCl into the alcohol; suspending the glycocollethylester chloride in ether and shaking it with dry silveroxide, then removing the silver-chloride by filtration, drying the ether with barium oxide and distilling. Glycocollethylester is a clear, basic fluid, boiling at 148° to 149°.[4]

The glycocoll-ester obtained in this way gave rise, in addition to the biuret-base, to glycin-anhydride $(NHCH_2CO)_2$.[5]

The nature of the 'biuret-base' could not be explained by Curtius[6] till E. Fisher[7] succeeded in converting the cyclic glycin-anhydride into the open-chain glycylglycin, which is the mother substance of the hippuryl-amino-acetic acid prepared by Curtius in 1881 (see above).



Glycocoll anhydride. Glycylglycin.

Schwarzschild[8] is of the opinion that the biuret-base of Curtius is built up of 7 glycocoll-molecules in an open chain, which would make the base into

$$NH_2 . CH_2 . CO\ (NHCH_2CO)_5\ NHCH_2 . CO . OC_2H_5$$

Aminoacetyl-pentaglycyl-amino-acetic acid-ethyl-ester.

Curtius, Gumlich, and Levy[9] state, however, that the biuret-base which results from the spontaneous transformation of glycocollester is a tetraglycyl-compound:

$$NH_2 . CH_2 . CO—(NHCH_2\ CO)_2—NHCH_2\ CO . OC_2H_5$$

Aminoacetyl-bisglycyl-amino-acetic-acid-ethyl-ester.

[1] Th. Curtius and Benrath, *Ber. d. deutsch. chem. Gesell.* **37.** 1279 (1904).

[2] Th. Curtius, *ibid.* **35.** 3226 (1902); R. Wüstenfeld, *Über d. Bildung von Glycylketten mittels Säureaziden*, Dissertation, Heidelberg, 1903.

[3] Th. Curtius, *Ber. d. deutsch. chem. Gesell.* **16.** 755 (1883).

[4] Curtius and Goebel, *Journ. f. prakt. Chem.* [2] **37.** 170 (1888).

[5] Th. Curtius, *ibid.* **23.** 3041 (1891).

[6] Th. Curtius and Goebel, *ibid.* [2] **37.** 170 (1888).

[7] Fischer and E. Fourneau, *Ber. d. deutsch. chem. Ges.* **34.** 2868 (1901).

[8] Schwarzschild, *Hofmeister's Beit.* **4.** 162 (1904).

[9] Th. Curtius, *Ber. d. deutsch. chem. Gesell.* **37.** 1284 (1904).

The substance which Schwarzschild examined must have been a mixture of the biuret-base and glycocoll-anhydride, for these two combine, according to Curtius, to form definite chemical compounds.

The drier the glycocoll-ester the greater is the yield of the biuret-base, and inversely the less the formation of glycin-anhydride. Levy working in Curtius's laboratory found that glycocoll-ester mixed with one third of absolute ether is converted, in the course of a few weeks, into the biuret-base to the extent of 99 per cent.

Gumlich has prepared an anhydride of the triglycylglycinester or biuret-base,[1] as has also E. Fischer.[2]

The biuret-base is readily soluble in warm water; gives a strongly alkaline reaction, and is slightly soluble in hot chloroform and acetic ester. When boiled with water it becomes converted into a gelatinous substance. It attracts the CO_2 of the air and in 10 per cent watery solutions gives the following reactions common to albuminous substances:

1. With caustic potash and copper sulphate the biuret reaction.
2. Ferric chloride gives a brownish red precipitate, soluble in an excess of the reagent and in much water.
3. Copper sulphate and acetate cause after a short time a turbidity and then a blue precipitate, very slightly soluble in excess of the reagents.
4. Mercuric chloride causes a precipitate which, at first, dissolves again, but then ultimately becomes permanent.
5. Lead acetate gives an immediate white precipitate insoluble in excess and on dilution.
6. Strong HCl and H_2SO_4 dissolve the base without altering it; strong HNO_3 decomposes the substance after a short time, gas being given off under a violent reaction.
7. Phosphomolybdic acid forms a yellow precipitate, soluble on heating and in much water.
8. Tannic acid gives at once a brown precipitate insoluble in excess and on dilution.
9. Picric acid throws down from saturated solutions of the base or its hydrochloride a precipitate which is fairly soluble in water and less soluble in alcohol.

Iodine-potassium-iodide produces in a short time a brown precipitate; mercury-potassium-iodide a white precipitate; bismuth-potassium-iodide a yellow precipitate soluble in much water. Ferro-

[1] Gumlich, *Ber. d. deutsch. chem. Ges.* **37.** 1300 (1904).

[2] E. Fisher, *ibid.* p. 2501.

cyanic acid and trichloracetic acid give no precipitate. From watery solutions the base crystallises in feebly anisotropous platelets, which begin to change at 218° and decompose at 270°, becoming black, without melting however.

Curtius[1] with the help of Wüstenfeld[2] has also prepared the following benzoyl-amino-acid compounds:

benzoyl-amino-acetic acid (hippuric acid). Melting point 187°.

$C_6H_5CO.NHCH_2.COOH.$

benzoyl-glycyl-amino-acetic acid. Melting point 206·5°.

$C_6H_5CO.NHCH_2CO. - NHCH_2.COOH.$

benzoyl-bis-glycyl-amino-acetic acid. Melting point 215°-216°.

$C_6H_5CO.(NHCH_2CO)_2 - NHCH_2.COOH.$

benzoyl-tri-glycyl-amino-acetic acid. Melting point 235°.

$C_6H_5CO.(NHCH_2CO)_3 - NHCH_2.COOH.$

benzoyl-tetra-glycyl-amino-acetic acid. Melting point 246°-252°.

$C_6H_5CO.(NHCH_2CO)_4 - NHCH_2.COOH.$

benzoyl-penta-glycyl-amino-acetic acid (γ-acid). Melting point about 268°.

$C_6H_5CO.(NHCH_2CO)_5 - NHCH_2.COOH.$

The benzoyl-bis- to the benzoyl-penta-compounds and all their derivatives give a violet colour with Fehling's solution. On boiling the above acids with concentrated HCl they dissociate into benzoic acid and the respective number of glycocoll-molecules. There is no tendency to ring-formation or to dissociation. The acids are slightly soluble in cold water, more so in hot water, giving a marked acid reaction; less soluble with alcohol; with alkaline metals they form readily soluble salts and are precipitated from such solutions by the addition of acids; their esters, hydrazides, and azides are readily prepared.[2]

With the view of introducing acyl-groups[3] into glycocoll, Curtius[4] has further employed acid-anhydrides instead of acid chlorides, thus:

$$(CH_3.CO)_2O + NH_2.CH_2.COOH = CH_3CO.NHCH_2.COOH$$

Acetic acid anhydride + glycocoll = aceturic acid.

[1] Th. Curtius, *Journ. f. prakt. Chem.* [2] **70.** 57 (1904).

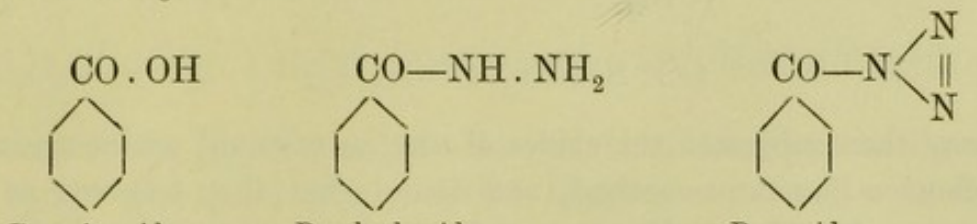
[2] Curtius and Wüstenfeld, *ibid.* N.F. **70.** 75 (1904).

[3] Acyl is a collective term which Liebermann introduced for the acid radical of fatty acids, thus in acetic acid, $CH_3.COOH$, acyl means the acetyl-remainder: $CH_3.CO$ while the analogous acid radicals of the homologous fatty acids are formyl: $H.CO$; propionyl: $CH_3.CH_2.CO$; butyryl: $CH_3.CH_2.CH_2.CO$.

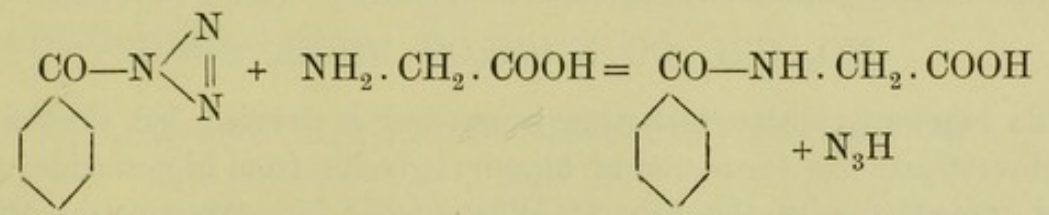
[4] Th. Curtius, *Ber. d. deutsch. chem. Gesell.* **17.** 1662 (1884).

In this paper, while describing the action of benzoyl chloride on silver glycocollate, Curtius states, 'in addition to hippuric acid, a series of acids is formed in which each successive member contains one "glycocoll minus water" or "$NH.CH_2.CO$-group" more than does the preceding one.' All the recent work of Curtius and his pupils[1] is based on Schotten-Baumann's method of benzoylation, and on the principle of substituting acid-azides for acid-chlorides.[2]

Hydrazides are derived from acids by substituting $-NH.NH_2$ for the hydroxyl group, OH; while azides are obtained by substituting the radical N_3:



Benzoic acid. Benzhydrazide. Benzaride.



Benzazide + glycocoll = hippuric acid + nitrohydric acid.

The synthesis of hippuric acid from benzazide and glycocoll is, according to Curtius,[3] a very satisfactory one, as shown by the results his pupils Hallaway and Darmstaedter obtained;[4] he therefore

[1] Th. Curtius, *Ber. d. deutsch. chem. Gesell.* **35.** 3226 (1902).
I. *Journ. f. prakt. Chem.* [2] **70.** 57 (introductory remarks).
II. Curtius and Wüstenfeld, *ibid.* p. 73 (glycylchains + hippurazide).
III. Curtius and L. Levy, *ibid.* p. 89.
IV. Curtius and E. Lambotte, *ibid.* p. 109 (α-alanin + hippurazide).
V. Curtius and C. F. van der Linden, *ibid.* p. 137 (linking up α-alanin and glycin by means of benzoyl-alanin-azide.
VI. Curtius and H. Curtius, *ibid.* p. 158 (linking up aspartic acid chains with hippurazide).
VII. Curtius and O. Gumlich, *ibid.* p. 195 (β-amino-α-oxypropionic acid and β-amino-butyric acid + hippurazide).
VIII. Curtius and E. Müller, *ibid.* p. 223 (linking up of hippuryl-γ-amino butyric acid and hippuryl-β-phenyl-α-alanin.
IX. Curtius and W. Lenhard, *ibid.* p. 230 (interaction between acid azides and urea; action of phenylcarbaminic acid-azide on glycocoll.

[2] The azide-reaction described above holds also good for β- and γ-amino-acids.

[3] Th. Curtius, *Journ. f. prakt. Chem.* [2] **50.** 285.

[4] R. R. Hallaway, *Über das Hydrazid und Azid der m-Nitrohippursäure*, Inaug. Diss. Heidelberg, 1901; E. Darmstaedter, *Über das Hydrazid der n-Tetramethylendikarbonsäure (Adipinsäure)*, Inaug. Dissert. Heidelberg, 1902. Printed by Karl Rössler.

extended the reaction to dibasic fatty acids, and prepared with Pringsheim [1] by uniting succinyl-azide and glycocoll, succinylglycocoll:

$$\begin{array}{l} CH_2CO\,.\,NHCH_2\,.\,COOH \\ | \\ CH_2CO\,.\,NHCH_2\,.\,COOH, \end{array}$$

and with Darmstaedter by combining adipinic-acid-azide with glycocoll, the adipinylglycin:

$$\begin{array}{l} CO\,.\,NHCH_2\,.\,COOH \\ | \\ (CH_2)_4 \\ | \\ CO\,.\,NHCH_2\,.\,COOH. \end{array}$$

Hallaway then subjected the azides of the substituted amino-acids to the Schotten-Baumann method, and found that they behaved as do amino-acid chlorides. He prepared from m-nitro-hippurazid the m-nitrohippuryl-amino-acetic acid:

$$NO_2\,.\,C_6H_4\,.\,CO\,.\,NHCH_2CO\,.\,NHCH_2\,.\,COOH.$$

The readiness with which this compound is formed led Curtius to reinvestigate the formation of hippuryl-glycins from hippurazide, and he succeeded with the help of Wüstenfeld [2] in converting hippuryl-amino-acetic acid:

$$\bigcirc\!\!\!\!\!\!\bigcirc CO\,.\,NHCH_2CO\,.\,NH\,.\,CH_2COOH$$

into the -ester, → hydrazide → azide and then linking the latter with glycyl-radials. In this way benzoyl-penta-glycylglycin was obtained:

$$\bigcirc\!\!\!\!\!\!\bigcirc CO\,.\,(NHCH_2CO)_5NH\,.\,CH_2\,.\,COOH.$$

This compound, as already pointed out, is the γ-acid which Curtius discovered in 1881.

Curtius and Levy by employing the glycylglycin and diglycylglycin discovered by Emil Fischer (see pp. 127, 128), have succeeded in building up the hexaglycylglycinester

$$NH_2\,.\,CH_2CO\,(NHCH_2CO)_5\,NH\,.\,CH_2\,CO\,.\,OC_2H_5,$$

and obtained also glycinhydrazide, $NH_2\,.\,CH_2CO\,.\,NH\,.\,NH_2$, the amino-hydrogen of which is as mobile as it is in the glycinester itself, for which reason glycinhydrazide is very suitable for building

[1] Hans Pringsheim, *Über das Hydrazid d. Pentamethylendikarbonsäure*, Inaug. Dissert. Heidelberg, 1901.

[2] Wüstenfeld, *Ber. d. deutsch. chem. Ges.* **35.** 3226 (1902).

up chains of amino-acids, as by the action of acid-chlorides or acid-azides, the acid radical enters the more strongly basic amino-group, while the hydrazin-remainder is preserved as such, and the azide of the resulting product is at once ready to unite with other amino-acids.

In addition to glycocoll Curtius has also coupled other amino-acids (such as alanin, aspartic acid, amino-butyric acid) by means of his azide-method. Thus, along with Lambotte, he first condensed hippur-azide with alanin, and then adding other alanyl-remainders obtained hippuryl-dialanyl-alanin :

$$C_6H_5CO—NH\,.\,CH_2CO\,.\,(NHCH(CH_3)CO)_2\,.\,NHCH(CH_3)COOH.$$

To remove the glycin remainder derived from the hippurazid, and thus to obtain glycyl-free alanyl-chains Curtius and Linden used Emil Fischer's benzoyl-alanin : $C_6H_5CO\,.\,NHCH(CH_3)COOH$; and they also added two glycin-remainders to benzoyl-alanin, and obtained thus benzoylalanyl-glycyl-glycin

$$C_6H_5CO—NHCH(CH_3)CO—NHCH_2CO—NHCH_2COOH.$$

Curtius senior with Hans Curtius have obtained from aspartic acid, by substituting for the Schotten-Baumann's reaction, the interaction of an azide and an amino-acid-ester dissolved in an indifferent medium, namely ether, the following branched compounds :—

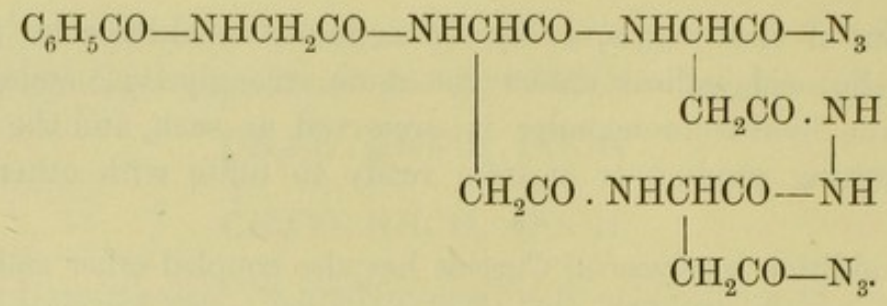
The slightly soluble, dibasic hippuryl-aspartic acid :

$$C_6H_5CO\,.\,NHCH_2CO\,.\,NHCHCOOH$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad CH_2COOH.$$

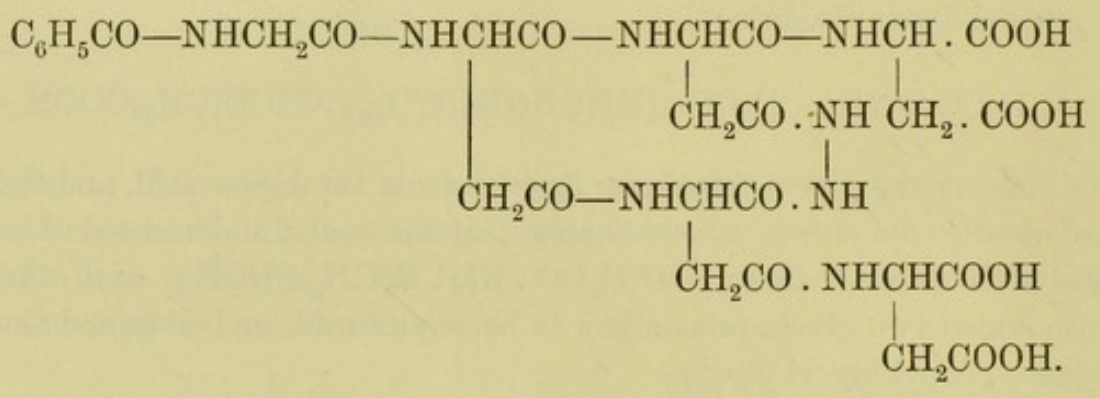
The very soluble 4-basic hippuryl-asparagyl-aspartic acid :

$$C_6H_5CO—NH\,.\,CH_2CO—NHCHCO—NHCH\,.\,COOH$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad CH_2\,.\,COOH$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad CH_2CO\,.\,NHCH\,.\,COOH$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad CH_2\,.\,COOH.$$

From the normal 4-acid hydrazide of the last compound on the addition of nitrous acid there resulted, owing to an internal splitting off of hydrazin, the formation of a dibasic hydrazi-azide :



On coupling this compound with two molecules of aspartic acid the 4-basic hippuryl-di-asparagyl-aspartic acid was obtained:



All the aspartic-acid-compounds give a violet colour with Fehling's solution.

Many of the compounds are characterised by separating out from their solutions in a very swollen state, and even dilute solutions may form so stiff a jelly that the vessel containing them may be inverted. Under the microscope they appear, with the exception of hippuryl-aspartic acid, as globules, without any crystalline character, *i.e.* as colloids.

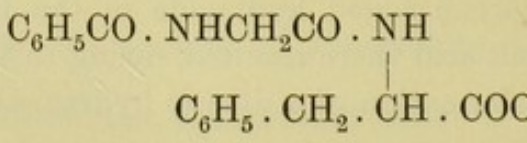
On endeavouring to introduce the hippuryl-group into the amino-group of β-amino-α-oxypropionic acid, Curtius and Gumlich only succeeded in getting hippuryl-α-oxy-β-amino-propionic acid

$$C_6H_5CO\,.\,NHCH_2CO\,.\,O\,.\,(CH_2NH_2)COOH,$$

while hippurazide and β-amino-butyric acid yielded as usual the corresponding hippuryl-β-aminobutyric acid:

$$C_6H_5CO\,.\,NHCH_2CO\,.\,NHCH(CH_3)CH_2\,.\,COOH.$$

By introducing the hippuric-acid-remainder into β-phenyl-α-alanin (or β-phenyl-α-amino-propionic acid) Curtius and E. Müller obtained the hippuryl-β-phenyl-α-alanin,



An attempt made by Curtius and Lenhard to build up long chains in which the carbaminic acid radical (NHCO), 'was to play the same part as the glycyl-radical ($NHCH_2CO$)' failed, because the carbaminic acid was decomposed into ammonia and carbon-dioxide, but phenyl-carbamindiglycylglycin was obtained:

$$C_6H_5NHCO \,.\, (NHCH_2CO)_2 \,.\, NHCH_2 \,.\, COOH.$$

Some dissociation-products of hippurazide-compounds.

If azides of mono-amino-acids are treated with ammonia or anilin or alcohol, according to circumstances, either normal saponification or transformation into urea-derivatives supervenes,[1] but on treating the azides of dibasic acids both phenomena may be observed in the same molecule, there being obtained compounds which are one half acid-amides and one half urea-derivatives. On hydrolysing these compounds the amino-acid chains are readily demonstrated.

Hippenyl-urethane, $C_6H_5CONHCH_2NH \,.\, CO \,.\, OC_2H_5$, formed from hippurazide and alcohol, on being hydrolysed yields benzoic acid, ammonia, formaldehyde, carbon-dioxide, and alkohol:

$$C_6H_5CO \,.\, NHCH_2 \,.\, NH \,.\, CO \,.\, OC_2H_5 + 3H_2O =$$
$$C_6H_2COOH + 2NH_3 + CHOH + CO_2 + C_2H_5OH.$$

The reaction leads thus from glycocoll to formaldehyde.

Analogously hippuryl-alanin-azide + aniline form the urea derivative: $C_6H_5CO \,.\, NHCH_2CO—NHCH(CH_3)NH \,.\, CO \,.\, NHC_6H_5$ and this on hydrolysis takes up three molecules of water, and is converted into

$$\underset{\text{Hippuric acid}}{C_6H_5CO \,.\, NHCH_2COOH} \quad + 2NH_3 \quad + CH_3CHO \quad + CO_2 \quad + C_6H_5NH_2$$
$$\text{+ ammonia + acetaldehyde + carbonic + anilin.}$$

By the action of anilin on hippuryl-aspartic-acid-azide is formed a compound which is half anilid and half carbanilid:

$$C_6H_5CO \,.\, NHCH_2CO \,.\, NHCHCO \,.\, NHC_6H_5$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad CH_2 \,.\, NHCONH \,.\, C_6H_5.$$

On hydrolising this compound besides hippuric acid, aniline and CO_2, also α-β-diamino-propionic acid is formed:

$$C_6H_5CO \,.\, NHCH_2CO \,.\, NHCHCO \,.\, NHC_6H_5$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad CH_2NH \,.\, CO \,.\, NC_6H_5H \quad + 3H_2O =$$

$$C_6H_5CO \,.\, NHCH_2COOH + NH_2CHCOOH$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad CH_2NH_2 \quad + 2C_6H_5NH_2 + CO_2\,;$$

[1] Th. Curtius, *Journ. f. prakt. Chem.* [2] **50.** 292 (1894).

this reaction illustrates therefore the transition of a dibasic mono-amino-acid into a mono-basic diamino-acid.

Hippuryl-aspartic-acid-azide + alcohol forms the normal urethane:

$$\begin{array}{l} C_6H_5CO\,.\,NHCH_2CO\,.\,NHCHNH\,.\,CO\,.\,OC_2H_5 \\ \qquad\qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\qquad\quad CH_2NH\,.\,CO\,.\,OC_2H_5. \end{array}$$

This compound by taking up four molecules of water gives rise, in addition to hippuric acid, ammonia, CO_2, and alcohol, to amino-acetaldehyde:

$$\begin{array}{l} C_6H_5CO\,.\,NHCH_2CO\,.\,NHCHNH\,.\,CO\,.\,OC_2H_5 \\ \qquad\qquad\qquad\qquad\quad | \qquad\qquad\qquad\qquad + 4H_2O = \\ \qquad\qquad\qquad\qquad\quad CH_2NH\,.\,CO\,.\,OC_2H_5 \\ C_6H_5CO\,.\,NHCH_2COOH + 2NH_3 + NH_2CH_2CHO + 2CO_2 + 2C_2H_5OH. \end{array}$$

This reaction is complementary to the last one, for by it a dibasic amino-acid is converted into the aldehyde of the monobasic glycocoll.

Gumlich has in addition shown that the urethane, formed by boiling hippuryl-β-amino-butyric-acid-azide with alcohol, on being hydrolysed, yields in addition to benzoic acid, glycocoll, CO_2, and alcohol, also propylenediamin. Out of a molecule of a substituted β-amino-butyric acid there is formed in this way a dibasic acid of the ethylene-diamin series.

Curtius points out the great interest of the conversion of glycocoll and other amino-acids into complex urea-like bodies which, on hydrolysis, yield partly formaldehyde and its homologues, and partly amino-aldehydes. "The transformation of dibasic mono-amino-acids to monobasic diamino-acids and the formation of a diamin of the putrescin-type from a monobasic mono-amino acid, opens up new, far-reaching vistas, for do we not see a number of substances, such as formaldehyde, which are of importance for the synthetic processes in organisms, and also others such as diamino-acids which are formed during the hydrolysis of albumins, stand in relatively simple genetic relationship to the various mono-amino acids, and do we not meet with bodies identical with those which are formed during the putrefaction of albuminous compounds."

Schützenberger[1] combined leucin and leuceine with urea by heating with phosphoric acid anhydride, and so obtained colloidal compounds.

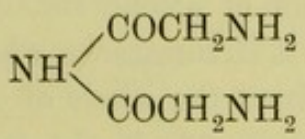
Balbiano and Frasciatti[2] converted glycocoll by heating with

[1] P. Schützenberger, "Récherches sur la synthèse des matières albuminoides et protéiques," *Compt. Rend.* **106.** 1407 (1888) and **112.** 198. (1891).

[2] Balbiano and Frasciatti, *Ber. d. deutsch. chem. Ges.* **33.** 2323 (1900); and Balbiano, *ibid.* **34.** 1501 (1900).

glycerine into an anhydride which differed from that which Curtius obtained. The constitution of the new anhydride could not, however, be determined.

Lilienfeld [1] states that with the help of Wolkowicz he succeeded in obtaining the following results: Glycocoll-ethyl-ester prepared by Curtius's method, see p. 116, is readily converted into the biuret-base by boiling with potassium bisulphate on the water-bath. Simultaneously di-methylamin, carbon dioxide, and ethylether are given off. The formula Lilienfeld attributed to the biuret-base is:



or 'di-mono-amidacetimid.'

On boiling the biuret-base or its carbonate a flocculent precipitate separated out, which was insoluble in water, alcohol, and dilute acids, readily soluble in pepsin + HCl at 37°.

By condensing the ethylesters of leucin, tyrosin, and glycocoll, he obtained a substance which was precipitable by ammoniacal basic lead-acetate, sublimate; tannic, phosphotungstic, phosphomolybdic, and picric acids, and by mercury-potassium iodide, but not precipitable by nitric and acetic acids or potassium-ferrocyanide. It further gave the reaction of Millon, the xanthoproteic test, the biuret-reaction, and the reactions of Adamkiewicz and Liebermann.

The author fails to see how Lilienfeld obtained the tryptophane reaction.

Lilienfeld also states to have prepared a typical albumin by treating the base of Curtius with amino-acid-esters in the presence of small amounts of formaldehyde and a 'condensing medium,' the nature of which, 'for obvious reasons,' he has not divulged.

A great step forward in synthetising substances resembling albumins was taken when Emil Fischer [2] commenced his important researches

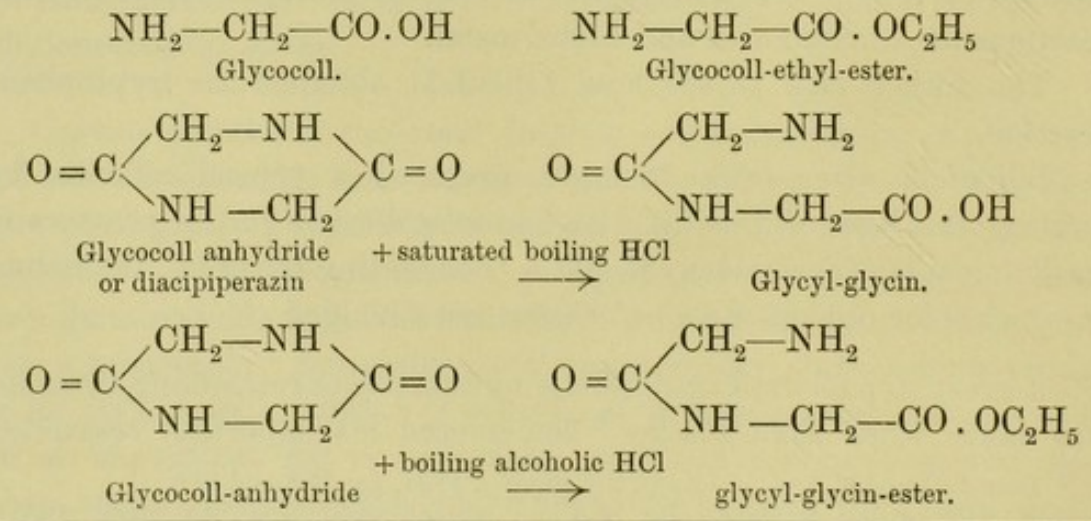
[1] Leon Lilienfeld, *Arch. f. (Anat. u.) Physiol.* 1894, pp. 383 and 555.

[2] E. Fischer and E. Fourneau, *Ber. d. deutsch. chem. Ges.* **34**. 2868 (1901); E. Fischer, *ibid.* **35**. 1095 (1902) [**I.**]; E. Fischer (and P. Bergell), 'Hydrolysis of Proteids,' *Vortrag auf der Karlsbader Naturforscherversammlung*, 1902. *Autoreferat Chemikerzeitung*, 1902, II. p. 939; E. Fischer, 'Derivatives of Polypeptids,' *Ber. d. deutsch. chem. Ges.* **36**. 2094 (1903); E. Fischer and E. Otto, 'Derivatives of Dipeptids,' *ibid.* **36**. 2106 (1903); E. Fischer and P. Bergell, 'Behaviour of Dipeptids towards Pancreatic Ferments,' *ibid.* **36**. 2592 (1903); E. Abderhalden and P. Bergell, 'Dissociation of Peptids in the Body,' *Zeitschr. f. physiol. Chem.* **39**. 9 (1903); E. Fischer, *Ber. d. deutsch. chem. Ges.* **36**. 2938 (1903); E. Fischer, 'Synthesis of Polypeptids' [**II.**], *Ber. d. deutsch. chem. Ges.* **37**. 2486 (1904); E. Fischer, and Umetaro Suzuki [**III.**], 'Derivatives of α-Pyrrolindin-carboxylic Acid,' *ibid.* **37**. 2842 (1904). E. Fischer, 'Derivatives of Phenylalanin' [**IV.**], *ibid.* **37**. 3062 (1904); E. Fischer and E. Abderhalden [**V.**], 'Derivatives of Prolin' (α-pyrrolidin-

detailed below. He had previously synthetised all the simple amino-acids, had split racemic amino-acids into their optically active components, see p. 20, had substituted, in preparing the esters of amino-acids, alkalies for the expensive silver-oxide-method of Curtius,[1] and had taught us to separate from one another the different amino-acids, in the form of their esters, by means of fractional distillation. Using these new methods he succeeded in demonstrating many amino-acids, which had hitherto not been found amongst the dissociation-products of albuminous substances, as already pointed out on p. 21.

That amino-acids by the introduction of alcohol-radicals lose their stability, as first noticed by Curtius, is a circumstance which has been taken advantage of by Fischer throughout the whole of his research. His aim has always been to unite amino-acids in the form of their esters, and to prevent the NH_2-radical from becoming split off.

Fischer and Fourneau[2] having prepared glycocoll-ethyl-ester according to the plan of Curtius and Goebel, see p. 116, converted it then into the di-glycocoll-anhydride, or, as it is now usually called, di-acipiperazin. This ring-compound was finally converted into the open-chain glycyl-glycin[3] by being boiled for a short time with concentrated hydrochloric acid. Treating glycocoll-anhydride with alcoholic hydrochloric acid on the other hand gave rise to glycyl-glycin-ester.



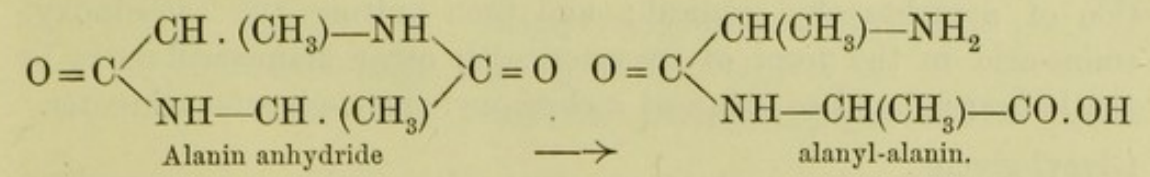
carboxylic acid), **37.** 3071 (1904); Hermann Leuchs and Umetaro Suzuki [**VI.**], *ibid.* **37.** 3306 (1904); E. Fischer and Umetaro Suzuki [**VII.**], 'Derivatives of Cystin,' *ibid.* **37.** 4575 (1904); E. Fischer and Ernest Koenigs [**VIII.**], 'Polypeptids and Amides of Aspartic Acid,' *ibid.* **37.** 4585 (1904); E. Fischer and Peter Bergell, 'Dissociation of some Dipeptids by means of Pancreatic Ferment,' *ibid.* **37.** 3103 (1904). E. Fischer [**IX.**] 'Chlorides of amino-acids and their acyl-derivatives,' *ibid.* **38.** 605 (1905). For references of papers X.-XIII., see p. 138.

[1] E. Fischer, *Sitzber. d. Berl. Akad.* (1900) 1062, and *Ber. d. deutsch. chem. Ges.* **34.** 433 (1901).

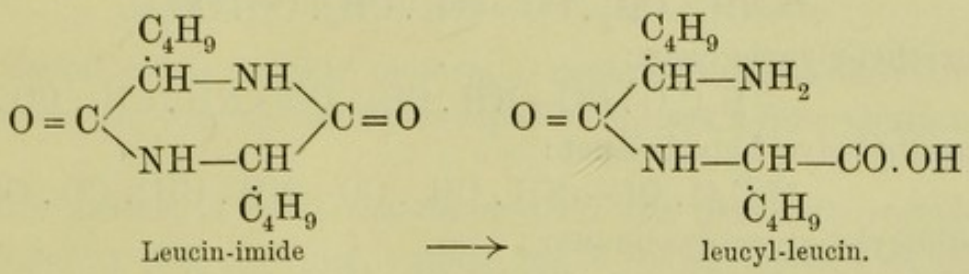
[2] E. Fischer and E. Fournean, *Ber. d. deutsch. chem. Ges.* **34.** 2868 (1901).

[3] Fisher and Fournean have given the term 'glycyl' to the radical [NH_2CH_2CO—] glycyl-glycin = 2 molecules of glycocoll, or glycin, minus one molecule of water.

From alanin anhydride was obtained alanyl-alanin:



By acting in an analogous manner on leucinimide[1] with fuming hydrobromic acid, leucyl-leycin was prepared:



The three substances just mentioned, namely:

Glycyl-glycin $NH_2 . CH_2 . CO—NH—CH_2—CO.OH$
Alanyl-alanin $NH_2 . CH(CH_3) . CO—NH—CH(CH_3)—CO.OH$
Leucyl-leucin $NH_2 . CH(C_4H_9) . CO—NH—CH(C_4H_9)CO.OH$,

Fischer has called 'dipeptids.' All three were obtained, as is explained above, by the opening-up of a diaci-piperazin-ring.

It is also pointed out in this paper that the instability of esters is cured by the introduction of radicals into the amino-group, for phenyl-cyanate-glycyl-glycin

$$C_6H_5 . NH . CO . NHCH_2CO . NHCH_2CO . OH$$ and

carbethoxyl-glycyl-glycin-ester

$$C_2H_5O . OC—NHCH_2CO . NHCH_2CO . OC_2H_5,$$

are very stable bodies.[2]

The desire of building up simple anhydrides of amino-acids was awakened in Fischer by his discovery amongst the products of partially dissociated silk fibroin,[3] of a body which was composed of glycocoll + alanin, and he therefore set about finding means for protecting the easily displaceable NH_2-radical of amino acids.

[1] First prepared by Bopp, *Ann. d. Chem.* **69**. 28 (1849).

[2] To make carbethoxyl-glycyl-glycin-ester dissolve rapidly, by shaking, 5 grammes of gycyl-glycin-ester in 20 grammes of water; cool in ice-water; add 4·25 grammes (=1¼ mol.) of chlorocarboxylic-ethyl-ester, Cl—CO . OC_2H_5, then add in three portions, shaking vigorously, 2·3 grammes of sodium carbonate dissolved in 20 ccm. of warm water, and then cooled. The amount of sodium carbonate is just sufficient to bind all the halogen of the chlorocarboxylic-ethyl-ester. The carbethoxy-glycyl-glycin-ester is precipitated as a thick crystalline deposit; wash it with a little water; dissolve in 11 parts of hot ester and precipitate with petroleum ether.

[3] Emil Fischer, *Chemiker Zeitung*, No. 80 (1902).

He fell on the plan of protecting the NH_2 group by the introduction of a carboxethyl-radical; and then uniting the carbethoxyl-amino-acid in the form of its ester with other amino-acid-esters by gentle heating, and so obtained carbęthoxyl-glycyl-glycyl-leucin-ester.

Glycyl-glycin:

$$NH_2 . CH_2 . CO—NH . CH_2 . CO . OH$$

Glycyl-glycin-ester:

$$NH_2 . CH_2 . CO—NH . CH_2 . CO . OC_2H_5$$

Carboxethyl-glycyl-glycin:

$$H_5C_2O . OC—NH . CH_2 . CO—NH . CH_2 . CO . OH$$

Carboxethyl-glycyl-glycin-ester:

$$H_5C_2O . OC—NH . CH_2 . CO—NH . CH_2 . CO . OC_2H_5$$

Carbethoxyl-diglycyl-leucinester:

$$C_2H_5O . OC—NHCH_2CO . NHCH_2CO—NH . CH(C_4H_9)CO . OC_2H_5.$$

As complex esters are less given to form condensation-products along the line just laid down, Fischer converted amino-acids into their acid chlorides by adopting Hans Mayer's plan of preparing chlorides of pyridin-carboxylic acid by means of thionyl-chloride.[1] The amino-acids are first converted into carbethoxy-compounds, and the latter by gentle warming with thionyl chloride into carbethoxy-amino-acid chlorides, for thionyl chloride, $SOCl_2$, acts on organic acids analogous to PCl_5 or SO_2Cl_2, giving acid chlorides; for example, butyric acid + thionyl chloride react thus:

$$C_3H_7CO . OH + SOCl_2 = C_3H_7COCl + SO_2 + HCl.$$

As the chlorides of carboxethyl-amino-acids readily unite with the esters of the same or other amino-acids, according to the equation,

$$H_5C_2O . OC—NH . CH_2CO—Cl + NH_2CH_2CO . OC_2H_5 =$$

Carboxethyl-glycin-chloride + glycin-ester =

$$H_5C_2O . OC—NHCH_2CO—NHCH_2CO . OC_2H_5$$

carboxethyl-glycyl-glycin-ester.

Fischer found it easy to prepare the esters of carbethoxyl-glycyl-glycin and of di-glycyl-glycin. The latter has the formula:

$$(C_2H_5OOC) . NH . CH_2CO—NHCH_2CO—NHCH_2CO . OC_2H_5.$$

By repeating the process with the products of synthesis, such as the dipeptid of glycocoll, it is possible to build up compounds containing 4 glycocoll-radicals in the form of anhydrides or carbethoxy-triglycyl-glycin-ester: $C_2H_5O . OC—[NHCH_2CO]_4—OC_2H_5$.

By saponifying these compounds the corresponding acids are obtained, while treatment with ammonia converts one of the ester-groups into an amide:

[1] Hans Mayer, *Monatshefte f. Chem.* **22**. 11 (1901).

$HO.OC[NHCH_2CO]_3—NHCH_2CO.OH$ =
triglycylglycin-dicarboxylic acid ;
$C_2H_5O.OC—[NHCH_2CO]_3NHCH_2CO.NH_2$ =
carbethoxy-triglycyl-glycin-amide.

Analogously by uniting HCN with glycyl-glycin-ester there is formed a urea-compound, namely, carbamino-glycyl-glycin-ester :

$$NH_2CO.NHCH_2CO.NHCH_2CO.OC_2H_5,$$

and similarly it is possible to form β-naphthalinsulpho-derivatives of dipeptids, and thereby readily to separate dipeptids from mixtures.

This method of converting amino-acids into their carboxethyls and subsequently into chlorides, enables one to couple mono- with di- and with oxy-amino acids, but it has the disadvantage that the carbethoxyl-group, $CO.OC_2H_5$, or in the free acids, the carboxyl-radical, $CO.OH$ cannot be got rid of.[1]

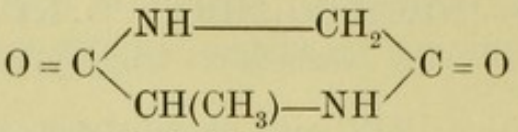
To overcome this difficulty Fischer devised an entirely new method. Instead of linking amino-acids together by their carboxyl-COOH-end, he joined them by their other or NH_2-end. We saw above that after converting an amino-acid into its carbethoxyl-derivative it was possible to introduce into the carboxyl-radical a chlorine atome, which during synthesis became replaced by the NH-radical of an amino-acid-ester. Now, Fischer introduced a halogen-containing acid-chloride into the NH_2-radical of an amino-acid, and then substituted in the synthetised product a molecule of ammonia for the chlorine-atom.

Such acid radicals, as just referred to, are, for example, chloracetyl-chloride, $CH_2Cl—COCl$, and the chlorides of brompropionyl, α-brom-iso-capronyl, α-δ-dibromvaleryl, and phenyl-α-brompropionyl. By means of these five compounds it was possible to introduce glycyl, alanyl, leucyl, prolyl, and phenyl-alanyl, on the following lines :

By the action of chloracetyl chloride $ClCH_2—COCl$, on alanin-ester, there is formed $ClCH_2CO—NH.CH(CH_3)CO.OC_2H_5$, or chloracetyl-alanin-ester, which, on being treated with alcoholic NH_3, has its chlorine replaced by NH_2 ; alcohol is split off simultaneously and ring-formation occurs, there being formed methyl-diacipiperazin,

[1] By the use of an excess of alkalies, glycyl-glycin-ester is converted into glycyl-glycin-carboxylic acid, $HO.OC.NHCH_2CO.NHCH_2.CO.OH$, which can again be converted into a neutral ester by means of alcoholic HCl, but the newly formed ester differs in its properties from the original one. This remarkable fact seems to hold good also for other amino-acids. Fischer calls the original ester, the α-ester, and the secondarily-formed ester, β-ester.

consisting of the anhydrides of the two different aliphatic amino-acids, namely, amino-acetic and amino-propionic acid:



Methyl-diacipiperazin.

Acting in the same way with chloracetyl-chloride on glycyl-glycin-ester, $ClCH_2 . CONHCH_2CO . NHCH_2CO . OC_2H_5$, or chloracetyl-glycyl-glycin-ester, was obtained. By carefully saponifying the latter there resulted $Cl . CH_2CO . NHCH_2CO . NHCH_2CO . OH$, or chloracetyl-glycyl-glycin, which when treated with concentrated watery ammonia did not give rise to a diacipiperazin, but to the simple tripeptid or di-glycyl-glycin, $NH_2CH_2CO—NHCH_2CO—NHCH_2 . COOH$.

Amino-acid chains containing no acyl-radicals [1] Fischer has called peptids; and the method just described of obtaining peptids, is of the highest importance, for it allows of the union of the most diverse amino-acids, and in the case of glycin even yields tetra-glycyl-glycin: $NH_2CH_2CO[NHCH_2CO]_3 . NHCH_2COOH$.

In his fourth paper Fischer further shows that, in addition to introducing an acid-chloride radical into the amino-group, it is also possible after having protected the amino-group by means of the acid-halogen chloride, to introduce another Cl-atom into the carboxyl-group of an amino-acid by means of phosphorus pentachloride, PCl_5,[2] and then to combine the chlorinated acid-chloride-amino-acid with other amino-esters. Thus, α-brom-isocapronylglycin-chloride + glycin-ester yields α-bromisocapronyl-glycyl-glycin-ester. By saponification and subsequent treatment with ammonia, this ester is converted into its corresponding tetra-peptid.

Other acyl-derivatives of amino-acids, for example, benzoyl-compounds, react similarly. Thus $C_6H_5CO . NH . CH_2 . COCl$, or hippuryl chloride, is obtained by shaking together finely divided hippuric acid with acetyl-chloride and phosphorus-penta-chloride.

Hippuryl-chloride reacts readily with the esters of the amino-acids or with alkaline solutions of the latter (see later) giving rise, for example, to benzoyl-glycyl-glycin. This latter after being converted into the chlorinated compound by means of PCl_5, will combine with glycin-ester to form the benzoyl-di-glycin-ester.

It is thus possible to obtain the same benzoyl-bodies which

[1] See footnote on p. 118.

[2] Phosphorus penta-chloride, PCl_5, is generally used for substituting Cl for OH. It also acts as a dehydrating agent, converting amides into nitrites, and it forms anhydrides out of dibasic acids.

Theodor Curtius and his pupils obtained by means of the azides; see p. 119.

Free amino-acids, with the exception of glycocoll, react with PCl_5, giving rise to chlorinated amino-acid chlorides, according to the general formula:

$$\begin{array}{l} R.CH.COCl \\ | \\ NH_3Cl. \end{array}$$

Leucyl-chloride hydrochlorate, $C_4H_9.CH(NH_3Cl).COCl$, is prepared by placing 5 grammes of pure, synthetic, inactive leucin, carefully powdered and dried into 100 ccm. of fresh acetyl chloride in a glass cylinder of 200 ccm. capacity with a well-fitting glass stopper. This mixture is cooled and then 8 grammes (= 1 mol.) of fresh PCl_5 are rapidly finely divided and added to the mixture, which is then shaken for two hours at the ordinary temperature. The PCl_5 disappears completely, and the leucin is converted into the chlorinated chloride. To ensure that no free leucin is present, add another 1·5 grammes of finely divided PCl_5 and shake for another hour. To isolate the compound, filter it off and wash it out with acetyl chloride and petroleum ether. A special apparatus is required for keeping out all moisture.

Leucyl-chloride is very important in manufacturing polypeptids. In combination with glycocoll-ester it gives rise to leucyl-glycin-ester, which splits off alcohol and then passes into leucyl-glycin-anhydride:

$$C_4H_9.HC\begin{array}{l}\diagup CO{-}NH \diagdown \\ \diagdown NH{-}CO \diagup\end{array}CH_2.$$

It is therefore possible to build up polypeptids from the amino-acids over the chlorides of the latter.

The last method requiring special mention is that of forming dipeptids by splitting up diacipiperazins,[1] by means of dilute cold alkalies, instead of using acids as Fischer had done at first.[2] Thus, shaking finely powdered glycin-anhydride with equi-molecular amounts of normal soda solution, produces a salt of glycyl-glycin. If this solution be then shaken with acid-chlorides, such as benzoyl-chloride or brom-iso-capronyl-chloride, the corresponding acyl-derivatives are formed, namely benzoyl-glycyl-glycin and brom-iso-capronyl-glycyl-glycin.

The ready dissociation of diacipiperazin-rings under the action

[1] See p. 126. [2] For some compounds, acids are still indispensable.

of dilute, cold alkalies does away with the objection hitherto raised against the occurrence of diacipiperazin compounds in the albumin molecule. It used to be argued, as no diacipiperazin compounds were obtainable after digesting albumins by means of ferments acting in dilute alkaline solutions, that such ring compounds could not be present, but Fischer points out that diacipiperazin-rings are readily formed during the synthesis of polypeptids, and says: "I hold it for probable that they are also present in some of the proteid compounds. They probably play a part during the denaturalisation of native albumins, and also during the formation of alkali-albuminates."

List of the Polypeptids and some of their Derivatives built up by E. Fischer's School

The figures I. to IX. refer to the consecutive papers published by Emil Fisher and his school. *See footnotes on pp.* 125-126.

Glycyl-glycin-Derivatives (I.)

Glycyl-glycin: $NH_2 . CH_2 . CO . NH . CH_2 . CO . OH.$

Glycyl-glycin-ester: $NH_2 . CH_2 . CO . NH . CH_2 . CO . OC_2H_5.$

Glycyl-glycin phenyl-cyanate:

$C_6H_5 . NH . CO . NH . CH_2 . CO . NH . CH_2 . COOH.$

Carbethoxyl-glycyl-glycin-ester:

$C_2H_5O . OC . NH . CH_2 . CO . NH . CH_2 . CO . OC_2H_5.$

Carbamino-glycyl-glycin-ester:

$NH_2 . CO . NH . CH_2 . CO . NH . CH_2CO . OC_2H_5$ (?).

Di-glycyl-glycin-Derivatives

Di-glycyl-glycin: $NH_2CH_2CO . NHCH_2CO . NHCH_2CO . OH.$

α-brom-propionyl-glycyl-glycin:

$CH_3 . CHBr . CO . NHCH_2CO . NHCH_2CO . OH.$

Alanyl-glycyl-glycin:

$CH_3 . CH(NH_2) . CO . NHCH_2CO . NHCH_2CO . OH.$

Carbethoxyl-alanyl-glycyl-glycin:

$C_2H_5CO_2 . NH . CH(CH_3) . CO . NHCH_2CO . NHCH_2CO . OH.$

α-bromiso-capronyl-glycyl-glycin: $C_{10}H_{17}O_4N_2Br.$

Leucyl-glycyl-glycin :

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\rangle CH \,.\, CH_2 \,.\, CH(NH_2) \,.\, CO \,.\, NHCH_2CO \,.\, NHCH_2CO \,.\, OH.$$

Phenyl-carbamino-leucyl-glycyl-glycin :

$$C_6H_5 \,.\, NH \,.\, CO \,.\, NH \,.\, CH(C_4H_9) \,.\, CO \,.\, NHCH_2CO \,.\, NHCH_2CO \,.\, OH.$$

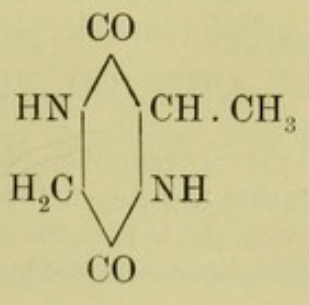
Leucyl-glycyl-glycin-ethyl-ester-hydrochlorate : $C_{12}H_{23}O_4N_2HCl$.

The polypeptids derived from mono-amino-acids have a structure which can be directly deduced from the synthesis, while their stereochemistry is somewhat more difficult. All amino-acids derived from albumins, with the exception of glycocoll, possess an asymmetrical C-atom. In polypeptids there are present as many asymmetric C-atoms as are present in amino-acids (except in glycocoll) linked together as anhydrides, and the number of independent optic isomers is expressed by van't Hoff's formula 2^n. Thus a dipeptid having the general formula, $NH_2 \,.\, \overset{*}{C}HR \,.\, CO \,.\, NH \,.\, \overset{*}{C}HR \,.\, COOH$, has two asymmetric C-atoms marked with a star, and therefore four active forms, *dd*, *ll*, *dl*, and *ld*, must exist, of which each two may form a racemic compound. If, on the other hand, active components are used for building up polypeptids, such as active amino-acids and active halogen-containing acid radicals, there are formed optically active polypeptids, and if one of the components is racemic, then one may expect also two isomers. Thus the natural, active tyrosin, in combination with inactive α-brom-iso-caproic-acid, may exist in the two steric combinations *dl* and *ll*.

Various Polypeptids (II.)

The following polypeptids of glycocoll, of inactive alanin, leucin, and of active *l*-tyrosin have been prepared by combination with chlorides of chlor-acetyl, brompropionyl and inactive α-brom-iso-capronyl.

1. Dipeptids.—Glycyl-glycin, alanyl-alanin, and leucyl-leucin, obtained by a splitting up of diazipiperazinc, contain two like amino-acids. The only known mixed diacipiperazine is glycin-alanin-anhydride :



Glycyl-alanin (inactive): $NH_2 . CH_2 . CO . NH . CH(CH_3) . COOH$.[1]

Leucyl-leucin (inactive):

Glycyl-*l*-tyrosin: $NH_2 . CH_2 . CO . NH . CH\begin{<}COOH\\CH_2 . C_6H_4 . OH\end{>}$.

Leucyl-*l*-tyrosin: $C_{15}H_{22}O_4N_2$ gives Millon's reaction.

Leucin-tyrosin-anhydride: $C_4H_9CH\begin{<}CO . NH\\NH . CO\end{>}CH . CH_2 . C_6H_4 . OH$.

2. Tripeptids.—The di-glycyl-glycin described above.

3. Tetrapeptids.—Tri-glycyl-glycin:

$$NH_2CH_2CO . NHCH_2CO . NHCH_2CO . NHCH_2 . COOH.$$

α-brom-iso-capronyl-leucyl-glycyl-glycin:

$$C_4H_9 . CHBr . CO . NH . CH(C_4H_9) . CO . NHCH_2 . CO . NHCH_2 . COOH.$$

Dileucyl-glycyl-glycin (inactive):

$$NH_2 . CH(C_4H_9) . CO . NH . CH(C_4H_9) . CO . NHCH_2CO . NHCH_2 . COOH.$$

4. Pentapeptid.—Tetra-glycyl-glycin:

$$NH_2CH_2CO . NHCH_2CO . NHCH_2CO . NHCH_2 . CO . NHCH_2 . COOH.$$

This peptid was prepared from chloracetyl-triglycyl-glycin:

$$ClCH_2 . CO[NH . CH_2 . CO]_3NH . CH_2 . COOH.$$

Derivatives of Pyrrolidin-carboxylic Acid or Prolin (III.)

The constitutional formula of pyrrolidin-carboxylic acid is given on p. 45.

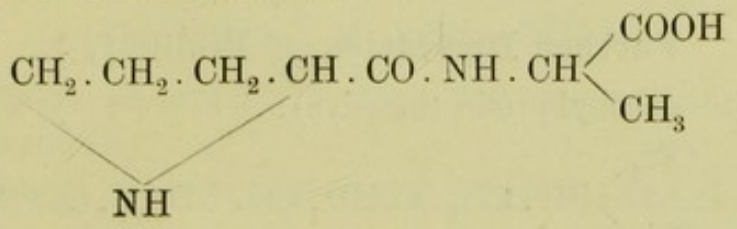
Polypeptids, which are derivatives of proline or α-pyrrolidin-carboxylic acid, have been prepared by E. Fisher and Suzuki. As Willstätter had noticed that α-δ-dibrom-valerianic acid is converted into α-pyrrolidin-carboxylic acid, Fischer and Suzuki attempted to join up pyrrolidin-carboxylic acid with other amino-acids by an analogous procedure, and they succeeded with alanin. If the α-δ-dibrom-valerianic acid is converted into the chlorinate by means of phosphorus-pentachloride and is then brought together with an alkaline solution of alanin, there is formed α-δ-dibrom-valeryl-alanin:

$$CH_2Br . CH_2 . CH_2 . CHBr . CO . NH . CH(COOH) . CH_3,$$

[1] On subjecting fibroin to the combined disintegrating action of hydrochloric acid, of trypsin, and of alkali, E. Fischer and Bergell ("Hydrolysis of Proteids," *Karlsbader Naturforscherversammlung* (1902), and in *Chemiker Zeitung*, 1902, II. p. 939), obtained a crystalline glycyl-alanin, which was, however, not identical with the synthetised product.

which, when acted upon by watery ammonia, is changed into pyrrolidin-carboxylic-acid-alanin, or shortly 'prolyl-alanin,' the term prolin standing for pyrrolidin-carboxylic acid.

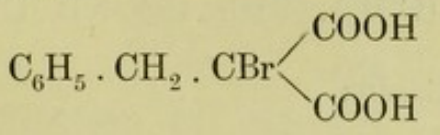
Prolyl-alanin has the formula:



It possesses a slightly acid reaction towards litmus, is nearly tasteless, very soluble in water, very slightly soluble in absolute-alcohol, and nearly insoluble in ether, benzol, chloroform, petrolether. The neutral as well as the acid solutions (H_2SO_4) are precipitated by phosphotungstic acid.

Derivatives of Phenyl-alanin (IV.)

To enable Fischer to couple the phenyl-alanin radical with other amino-acids he required the chloride of a phenyl-α-halogen propionic acid, and therefore prepared phenyl-α-brompropionic acid or α-bromhydrocinnamic acid, $C_6H_5 . CH_2 . CHBr - COOH$, from benzyl-malonic acid, which Conrad [1] had used for the preparation of hydrocinnamic acid, by introducing bromine and then decomposing by heat the benzyl-brom-malonic acid:



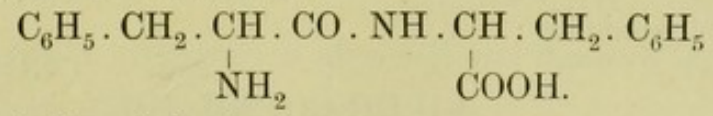
After the conversion of the phenyl-α-brompropionic acid into the chloride, this compound was coupled with glycyl-glycin and with phenyl-alanin. Cinnamoyl-plenyl-alanin and cinnamoyl-glycyl-glycin were also obtained.

The constitutional formula of phenyl-alanin is given on p. 47.

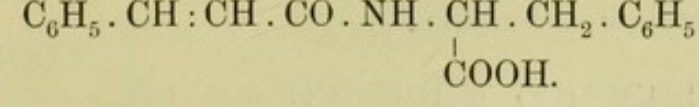
Phenyl-alanyl-glycyl-glycin:

$$C_6H_5 . CH_2 . CH(NH_2) . CO . NHCH_2CO . NHCH_2 . COOH.$$

Phenyl-alanyl-phenyl-alanin:

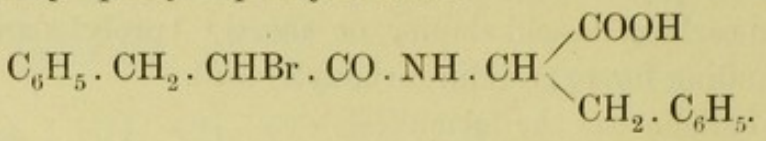


Cinnamoyl-phenyl-alanin:



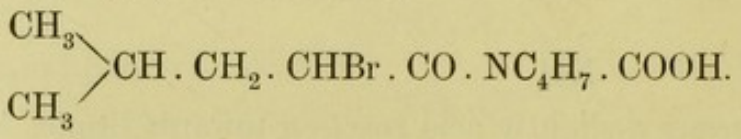
[1] M. Conrad, *Liebig's Ann. d. Chem.* **204**. 174 (1880).

Phenyl-brompropionyl-α-phenyl-alanin :

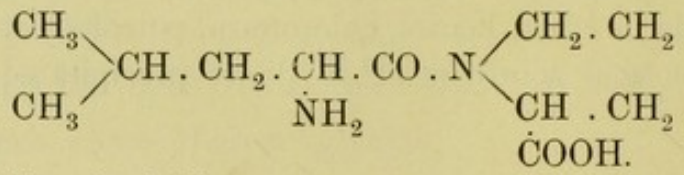


Further Derivative of Prolin (V.)

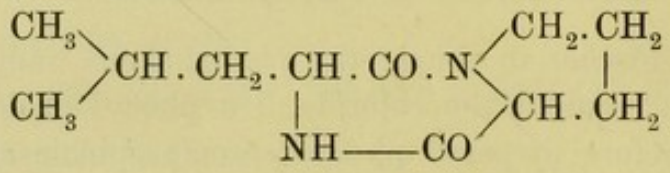
α-Brom-iso-capronyl-prolin (inactive) :



Leucyl-prolin :

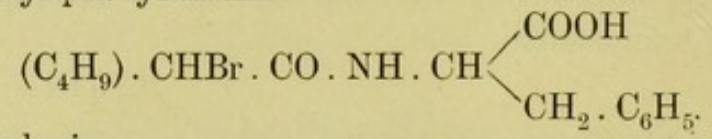


Leycyl-prolin-anhydride :

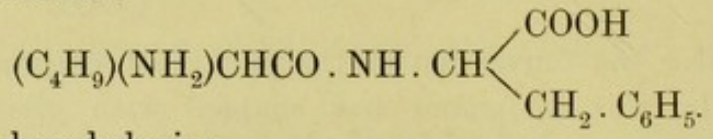


Further Derivatives of Phenyl-alanin (VI.)

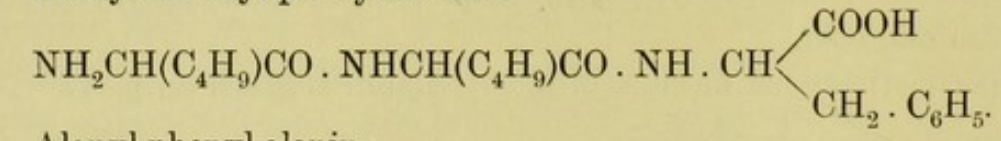
α-Brom-iso-capronyl-phenyl-alanin :



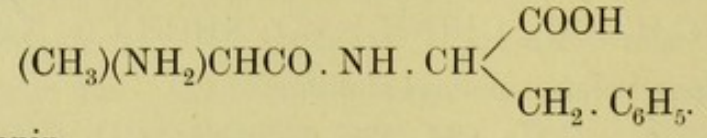
α-Leucyl-phenyl-alanin :



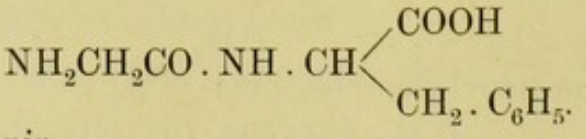
Leucyl-α-leucyl-phenyl-alanin :



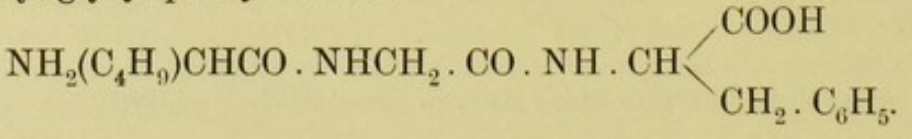
Alanyl-phenyl-alanin :



Glycyl-phenyl-alanin :



Leucyl-glycyl-phenyl-alanin :



Diglycyl-phenyl-alanin:

$$NH_2CH_2CO.NHCH_2CO.NH.CH\begin{cases}COOH\\CH_2.C_6H_5\end{cases}$$

Derivatives of Cystin (VII.)

The constitutional formula of cystin is given on p. 57.

Diglycyl-cystin:

$$\begin{array}{llllll} CH_2CO.NH & CHCH_2S.S.CH_2. & CHNH.COCH_2\\ \dot{N}H_2 & \dot{C}OOH & \dot{C}OOH & \dot{N}H_2. \end{array}$$

Di-alanyl-cystin:

$$\begin{array}{llllll} CH_3:CH.CO.NH. & CH.CH_2S.S.CH_2. & CH.NH.CO.CH.CH_3\\ \dot{N}H_2 & \dot{C}OOH & \dot{C}OOH & \dot{N}H_2. \end{array}$$

Dileucyl-cystin:

$$\begin{array}{llllll} C_4H_9.CH.CO.NH. & CH.CH_2.S.S.CH_2. & CH.NH.CO.CH.C_4H_9\\ \dot{N}H_2 & \dot{C}OOH & \dot{C}OOH & \dot{N}H_2. \end{array}$$

Derivatives of Aspartic Acid (VIII.)

The constitutional formula of aspartic acid is given on p. 34.

Glycyl-asparagin:

$$NH_2CH_2CO.NH.\dot{C}H\begin{matrix}CH_2.CONH_2\\ \dot{C}OOH\end{matrix}$$

Leucyl-asparagin:

$$(CH_3)_2CH.CH_2.CH(NH_2).CO.NH.\dot{C}H\begin{matrix}CH_2-CONH_2.\\ \dot{C}OOH\end{matrix}$$

Leucyl-aspartic acid:

$$(CH_3)_2.CH.CH_2.CH(NH_2).CO.NH.\dot{C}H\begin{matrix}CH_2\ CONH_2\\ \dot{C}OOH.\end{matrix}$$

Asparagyl-monoglycin (inactive):

$$\begin{array}{lll} CO.NH.CH_2.COOH & & COOH\\ \dot{C}H.NH_2 & or & \dot{C}H.NH_2\\ \dot{C}H_2.COOH & & \dot{C}H_2.CO.NH.CH_2.COOH. \end{array}$$

By hydrolysis it gives rise to glycocoll and inactive aspartic acid.

Fumaryl-di-aspartic-acid-ester:

$$\begin{array}{ll} CO.NH.CH.CH_2.COOC_2H_5\\ \dot{C}H \quad \dot{C}OOC_2H_5\\ \|\\ \dot{C}H \quad COOC_2H_5\\ \dot{C}O.NH.\dot{C}H.CH_2.COOC_2H_5. \end{array}$$

Chlor-succinyl-di-alanin:

$$HOOC . CH(CH_3) . NH . CO . CH_2 . CHCl . CO . NH . CH(CH_3) . COOH.$$

Asparagin-imide or di-acipiperacin-di-acetic-acid-di-amide:

$$H_2NOC . CH_2 . CH . CO . NH .$$
$$\dot{N}H . CO . \dot{C}H . CH_2 . CONH_2.$$

Chlorides of Amino-acid and their Derivatives (IX.)

Hippuryl-chloride: $C_6H_5 . CONH . CH_2 . CO . Cl.$

Hydrochloride of alanyl-chloride: $CH_3 . CH(NH_3Cl) - COCl$

Hydrochloride of α-amino-butyryl-chloride:

$$CH_3 . CH_2 . CH(NH_3Cl) . COCl.$$

Cystin-dimethyl-ester (Umetaro Suzuki):

$$[- S . CH_2 . CH(NH_3Cl) . CO . OCH_3]_2.$$

The following papers appeared too recently to be included:

Alanyl-glycin and leucyl-alanyl-glycin [1] (XI.).
Alanyl-alanin and derivatives [2] (XII.).
Chlorides of amino-acids and polypeptids [3] (XIII.).

[1] E. Fischer, *Liebig's Ann. d. Chem.* **340**. 123 (1905). E. Fischer and W. Axhausen, *ibid.* p. 128. (XI.)

[2] E. Fischer and K. Kantzsch, *Ber. d. deutsch chem. Ges.* **38**. 2375 (1905).

[3] E. Fischer, *ibid.* **38**. 2914 (1905).

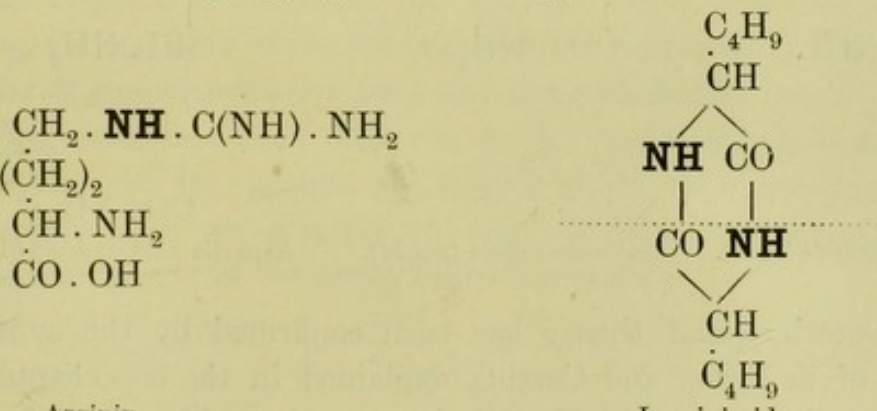
CHAPTER IV

THE CONSTITUTION OF ALBUMINS

(See also pp. 237-249)

The Linking of Amino-acids

SCHÜTZENBERGER[1] advanced, in 1875, the view that albumins ought to be considered as derivatives of urea, $NH_2—CO—NH_2$ and of oxamid, $NH_2—CO—CO—NH_2$, but this would only account for the guanidin-remainder, $—CNH.NH_2$, occurring normally in arginin.[2] The conception of Nasse,[3] that albumins are built up as esters, containing the grouping: $\equiv C—O—C\equiv$, will also only account for a small percentage of the total amount of albumin, for radicals containing the alcohol-group OH are but few, such as serin, tyrosin, oxyprolin, and the diamino-oxycarboxylic acids, mentioned on pp. 44, 45. For these reasons Hofmeister[4] advanced in 1892 the theory that albumins are linked up according to the general formula: $–CH_2—NH—CO—$ or $—NH—CH_2—CO—NH$. He based his view partly on the fact that this grouping occurs in arginin and in leucin-imide:



Arginin.

Leucinimide.

and partly on the fact that according to Löw[5] and Schiff,[6] relatively

[1] Schützenberger, 'Über d. Proteinkörper,' *Chem. Centralbl.*, 1875, 1876.

[2] That oxamide occurs normally is held by Kutscher and Schenk, see p. 244.

[3] O. Nasse, "Über d. Wirkung der Fermente," *Rostocker Zeitung*, 15th Dec. 1894.

[4] F. Hofmeister, *Ergebnisse d. Physiologie*, **1.** i., p. 159 (1902).

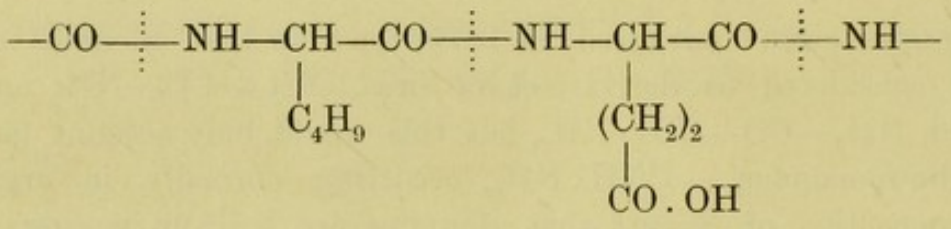
[5] O. Löw, *Journ. f. prakt. Chem.* **31.** 129.

[6] H. Schiff, *Ber. d. deutsch. chem. Ges.* **29.** 1354.

little NH_2 is present in albumins, judging by the amount of the nitrogen which is split off and the ease with which the biuret-reaction can be prevented on subjecting albumins to the action of nitrous acid.

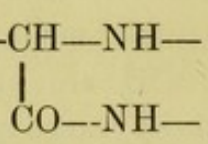
Although albumin treated with nitrous acid gives negative results with the biuret-reaction because of the disappearance of terminal $—CO—NH_2$ or $CS.NH_2$ or $=C(NH).NH_2—$ or $—CH_2.NH_2—$ radicals (Schiff),[1] it is easy to get once more positive results by hydrolising the albumin, which had been treated by nitrous acid, into its dissociation-products, as hereby NH groups become converted into NH_2 groups.

Hofmeister illustrated his theory by the following example in which leucin and glutaminic acid are linked together:

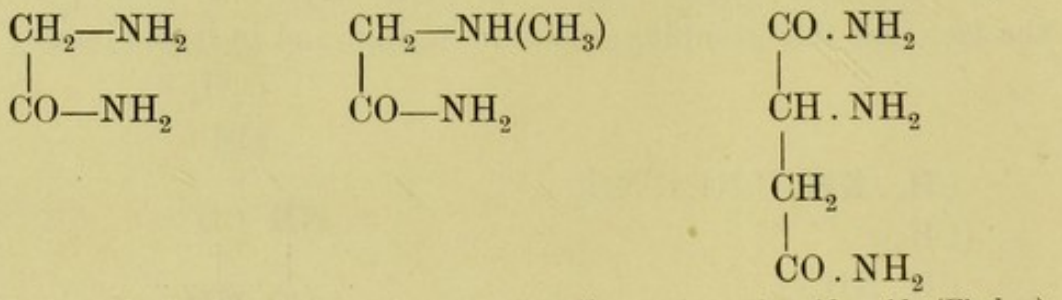


Leucin. Glutaminic acid.

In this compound the radical



occurs, which is also met with in the following compounds, which give the biuret reaction, namely,



Glycinamide (Schiff). Sarcosin-amide (Schiff). Aspartic-acid-amide (Fischer).

Hofmeister's sound theory has been confirmed by the synthetic researches of E. Fisher and Curtius, explained in the last chapter, for there cannot be any doubt that ordinary amino-acids are linked up to form neutral imino-compounds. Other methods of linking up exist also in albumins, but these will be discussed later, see p. 146, after the phenomena which are explainable on the imine-linking have been gone into.

[1] H. Schiff, *Ber. d. deutsch. chem. Ges.* **29**. 298, **30**. 2449; and Liebig's *Annalen*, **299**. 236, and **319**. 300 (see p. 142 of this book).

The grouping

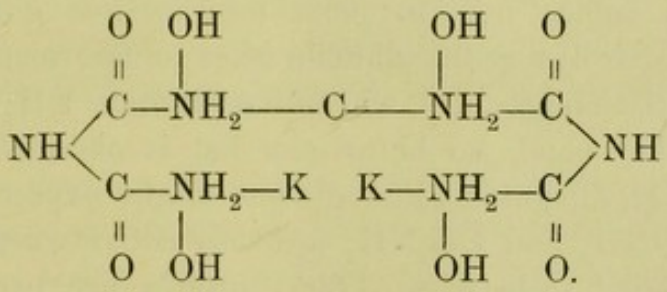
$$—CO\ |\ —NH—\underset{\times}{\overset{\cdot}{C}}H—CO—\ |\ NH—\underset{\times}{\overset{\cdot}{C}}H—CO—\ |\ NH—$$

allows us to explain the following properties exhibited by albumins :—

1. The fact that all albumins greatly resemble one another, although they are built up of such diverse dissociation-products.

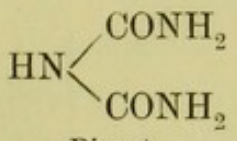
Now we understand why the albumins dissociate in so uniform a manner, even if the most divergent reagents are used, and why dissociation always takes place at the already existing 'locus minoris resistentiae' and why all albumins can be dissociated by trypsin, while each of the polysaccharids requires its own specific carbohydrate-ferment.

2. *The Biuret-Reaction.*—The red colour which biuret and similar substances exhibit, when they are treated with sodium hydrate and copper sulphate, depends, according to Schiff,[1] on the formation of a copper-potassium-biuret compound having this constitution :—

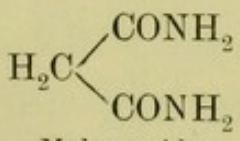


This substance Schiff isolated in the form of long, red, needle-like crystals. According to Goto,[2] protones give the biuret-reaction without the addition of an alkali, and Henze[3] states that the copper-containing hæmocyanin does not require the addition of a copper salt. Instead of copper salts one may also use nickel salts (see p. 6).

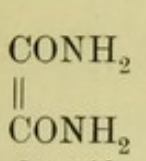
This reaction Schiff believes to be given by all compounds in which two $CONH_2$-groups are linked either to a carbon-atom or to a nitrogen-atom or directly to one another, and which therefore correspond to one of the three following types :—



Biuret.



Malonamide.



Oxamide.

One of the $CONH_2$-groups may also be replaced by a CH_2NH_2 group or a $CSNH_2$-group.

[1] H. Schiff, *Ber. d. deutsch. chem. Ges.* **29**. I. 298 (1896) ; *Liebig's Annalen*, **299**. 236 (1897) ; **319**. 300 (1901). [2] M. Goto, *Zeitschr. f. physiol. Chem.* **37**. 94 (1902).
[3] M. Henze, *ibid.* **33**. 370 (1901).

In proteids we meet with the combination

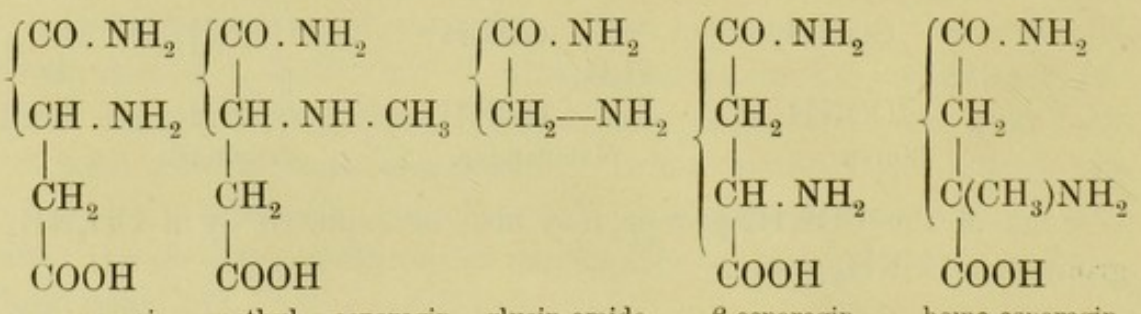
$$\begin{array}{l}\text{—CHNH—}\\ \quad\vert\\ \text{CONH—}\end{array}$$

According to Schiff, only one of the hydrogen-atoms attached to the nitrogen can be substituted, and the $CONH_2$-group must be free; but E. Fischer has observed the biuret-reaction with carb-ethoxyl-di-glycyl-glycin-ester and with triglycyl-glycin-carboxylic acid,—compounds in which a hydrogen-atom is substituted in both $CONH_2$-groups,—and Levites[1] has shown that treatment with nitrous acid, which completely destroys the $CO.NH_2$-group, does not alter egg-albumin, casein, or gelatine in such a way as to prevent the biuret-reaction, and Scheermesser's[2] pepsin-glutin-peptone gives the biuret-reaction, although it contains no amid-nitrogen, and therefore no $CO.NH_2$ groups. On p. 95, when discussing desamino-albumin, Levites' view as to the non-existence of $CO.NH_2$-groups in the albumin-molecule is dealt with. It will suffice now to point out, as was done above, the possibility of hydrolysing the albumin after its treatment with nitrous acid into dissociation-products, which must contain NH_2-groups.

On the other hand, no biuret-reaction is obtained with glycyl-glycin and other dipeptids, although one might expect it. It would appear that $CONH_2$ and CH_2NH_2 are only active when the $CONH_2$ group is terminal, otherwise several groups are necessary. The conditions determining the biuret-reaction are therefore not as yet cleared up.

The oldest synthetically prepared substances giving the biuret-reaction are fully discussed in Chapter III., p. 115.

Schiff found that α-asparagin (onion-red) and methyl-α-asparagin (red-violet), belonging to the oxamide type and glycocoll-amide (glycin-amide or amino-acetamide); further β-asparagin and homo-asparagin (blue-violet), belonging to the malon-amide type,—give the biuret-reaction.



α-asparagin. methyl α-asparagin. glycin-amide. β-asparagin. homo-asparagin.

[1] S. Levites, *Zeitschr. f. physiol. Chem.* **43**. 202 (1904).

[2] Scheermesser, *ibid.* **41**. 68 (1904).

E. Fisher,[1] in confirmation of Schiff's theory, obtained a biuret-reaction with the following three substances, which are comparable to glycin-amide :—

(1) carboxy-ethyl-glycyl-glycin-amide :

$$C_2H_5COO . NH . CH_2 . CO—NH . CH_2 . CO—NH_2.$$

(2) carboxy-ethyl-glycyl-glycin-leucin-ester :

$$C_2H_5COO . NHCH_2CO—NHCH_2CO—NH . CH(C_4H_9)CO . OC_2H_5.$$

(3) carbonyl-diglycyl-glycin-amide :

$$CO : [NH . CH_2 . CO . NHCH_2 . CO . NH_2]_2.$$

The free acid corresponding to carboxy-ethyl-glycyl-glycin-amide, namely, $COOH . NH . CH_2 . CO—NH . CH_2 . CO . NH_2$, does not give the biuret-reaction, probably owing to the presence of acid groups in the molecule. This principle cannot, however, be generalised, as α-asparagin (Schiff) does give a red reaction. The ordinary asparagin, $COOH . CH . (NH_2)CH_2 . CO . NH_2$, which, according to Schiff, gives a bluish-violet colour, Fischer says reacts so little as to hardly justify the expression 'biuret.' The diaminoaspartic acid, $NH_2 . CO . CH (CH_2) . CH_2 . CO . NH_2$, gives, however, according to Fischer a strong biuret-reaction.

Schiff[2] has further discovered that the polyaspartic acids and their derivatives also give the reaction. These bodies have the same configuration as the peptids of Fischer, for they too are imino-compounds, formed by the union of the amino-group of one molecule of aspartic acid with the carboxyl-group of another molecule.

The biuret-reaction is not a uniform one, for there are great differences in the colour produced, as just pointed out, and also in the ease with which it is obtained. According to Neumeister,[3] peptones can be demonstrated in dilutions of 1 : 100,000 ; they give a pure red colour. Albumoses, according to Kühne,[4] require to be more concentrated, and give a reddish-violet colour ; while natural albumins give a violet colour, and require to be fairly concentrated. The differences cannot be explained as due to differences in the size of the molecule or by similar factors, as Schiff and E. Fischer have seen similar differences in their synthetic products. What factors bring about the colour changes is not known, however. When dealing with the higher albumins a decision becomes

[1] E. Fischer and Fourneau ; E. Fischer and Otto. See footnotes on p. 125 and 126.

[2] H. Schiff, *Liebig's Annalen*, **303**. 183 (1898), **307**. 231 (1899), **310**. 37 and 301 (1899).

[3] R. Neumeister, *Zeitschr. f. Biol.* **26**. 324 (1890).

[4] W. Kühne, *ibid.* **29**. 308 (1892).

even more difficult, because albumins and albumoses are disintegrated by strong caustic soda. Siegfried[1] has also noticed that the biuret-reaction may be obscured by the presence of other bodies.

The biuret-reaction is generally supposed to be the most important of all colour tests given by albumins, because biuret, malonamide, oxamide, etc., do not occur in nature, and are not formed during the processes employed by physiological chemists. The reaction is further believed to be given only by true albuminous substances having the structural arrangement described above, and not to be given by the amino-acids derived from the albumins; and in this respect the biuret test differs from all the other colour tests. It used also to be held that albumins had been completely disintegrated, by either acids or by ferments, whenever the biuret-test gave negative results; but this is placing too high a value on the method, for bodies are known to exist which are not amino-acids and yet yield amino-acids on being dissociated—bodies which behave therefore as do the peptones, but which do not give the biuret-reaction. Such bodies have been obtained as the result of peptic digestion by Zunz,[2] Pick,[3] Pfaundler,[4] and Reach;[5] during tryptic digestion, by E. Fischer and Abderhalden;[6] by a combined treatment with hydrochloric acid, trypsin, and barium hydrate, by E. Fischer and Bergell.[7] Hofmeister[8] calls these bodies peptoids, while E. Fischer[9] calls them peptids, because he considers them to resemble his synthetised amino-acid compounds. As these substances are at least partially dissociated by proteolytic ferments, and as they yield the identical amino-acids as do peptones,[9] on being dissociated by acids, there is no reason to separate these bodies from peptones simply because they do not give the biuret-reaction. The gradual dissociation of albumins is also discussed in Chapter V.

3. *The Behaviour towards Trypsin.*—All albumins are dissociated by certain ferments—of which the pancreas ferment[10] is the most import-

[1] M. Siegfried, *Zeitschr. f. physiol. Chem.* **35**. 164 (1902).

[2] E. Zunz, *ibid.* **28**. 132 (1899); *Hofmeister's Beiträge*, **2**. 435 (1902), **3**. 339 (1902).

[3] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899).

[4] M. Pfaundler, *ibid.* **30**. 90 (1900).

[5] F. Reach, *Hofmeister's Beiträge*, **4**. 139 (1903).

[6] E. Fischer and E. Abderhalden, *Zeitschr. f. physiol. Chem.* **39**. 81 (1903).

[7] E. Fischer and P. Bergell, *Chemikerzeitung*, 1902, II. 939. (Vortrag von E. Fischer auf der Naturforscherversammlung zu Karlsbad, 1902.)

[8] F. Hofmeister, *Ergebnisse der Physiol. von Asher u. Spiro*, I. **1**. 759 (1902).

[9] E. Fischer and E. Abderhalden, *Zeitschr. f. physiol. Chem.* **39**. 81 (1903); E. Fischer and P. Bergell, *Chemikerzeitung*, 1902, II. 939. (Vortrag von E. Fischer auf der Naturforscherversammlung zu Karlsbad, 1902.)

[10] H. M. Vernon, *Journ. of Physiol.* **26**. 405 (1901). Vernon is of the opinion that

ant—into peptones, peptids, and finally into amino-acids. According to Gulewitsch [1] and Schwarzschild,[2] trypsin acts only on albumins, but not on acid-amides, esters, urea-derivatives, biuret, hippuric acid, etc. E. Fischer and Bergell have found, on the other hand, that some dipeptids, *e.g.* glycyl-*l*-tyrosin and glycyl-*l*-leucin, are readily and quickly dissociated by trypsin, while other dipeptids, such as glycyl-glycin and leucyl-tyrosin, are apparently not acted upon. Schwarzschild [2] observed, however, that the base of Curtius (see p. 116) is dissociated by trypsin. (See also footnotes on p. 125.)

4. *The Simultaneous Acid and Basic or 'Amphoteric' Character of Albumins.*—When dealing with the salts of the albumins in Chapter VI., it will be explained more fully how albumins, which in watery solutions are almost neutral, have the power of combining with acids and with bases to form salts. The amino-acids which build up the albumin-molecule behave in every respect as do the albumins themselves, because the amino-acids retain their acid and basic character owing to the fact that they become linked in such a manner that the amino-radical of one molecule unites with the carboxyl-radical of another molecule, as has already been explained on p. 116. No other method of linking amino-acids will preserve their double nature.

Glycyl-glycin,

$$H_2N . CH_2 . CO—NH . CH_2 . COOH,$$

and diglycyl-glycin,

$$H_2N . CH_2 . CO—NH . CH_2 . CO—NH . CH_2 . COOH,$$

are as much amino-acids as is glycocoll itself. If the union of amino-acids were brought about only through the amino-groups, the free carboxyl-groups would confer on the albumin-molecule an acid character, while, in the reverse case, the acid anhydrides or esters would possess such marked basic properties as are possessed, *e.g.*, by the esters of the amino-acids of Curtius and E. Fischer (Cohnheim).

The linking of radicals characteristic of albumins is therefore the same as in E. Fischer's peptids, namely, the joining of amino-acids to form imine-chains. It is, however, not permissible to consider albumins

trypsin is not a single chemical substance, because he found that trypsin is very rapidly destroyed by sodium carbonate, an active extract kept at 38° with 0·4 per cent Na_2CO_3 having about 65 per cent of its ferment destroyed in an hour; 1 per cent Na_2CO_3 destroys over 80 per cent, whilst pure water may destroy over 30 per cent an hour. This extreme sensitiveness is, however, only obtained with the least deteriorated glycerine, alcoholic, saline, and aqueous extracts of human, dog, pig, sheep, and ox pancreases, while with the most deteriorated (least active) trypsin only 7 per cent of the ferment is destroyed by 0·4 per cent Na_2CO_3.

[1] W. Gulewitsch, *Zeitschr. f. physiol. Chem.* **27**. 540 (1899).

[2] M. Schwarzschild, *Hofmeister's Beiträge*, **4**. 155 (1903).

simply as long chains built up of amino-acids linked to one another, for there exist still other connections within the albumin-molecule.

Other Modes of Union amongst Amino-Acids

In the substance arginin, the radical guanidin is linked to amino-valerianic acid as an imide and not as an amide, and for this reason it is impossible either to obtain the biuret-reaction or to act on arginin with trypsin, erepsin, or boiling acids. It has already been pointed out that arginin occurs in every albumin and even in the peptones (see pp. 150, 188), and therefore the imino-linking is as widely distributed as is the amino-grouping (Cohnheim).

With the exception of the protamins all albumins contain the so-called 'amino-nitrogen.' A certain percentage of the nitrogen in albumins is always given off as ammonia, whenever the albumin is dissociated, and this nitrogen is co-ordinated with the other dissociation-products, as none of the latter give off nitrogen on being boiled with acids. Schmiedeberg[1] observed that after a slight action of alkalies, a small percentage of nitrogen is given off, and that the remaining albumin is altered only to a slight extent; this remainder he calls 'desamido-albuminic acid.' According to Schiff,[2] nitrous acid exerts an action similar to that of an alkali, and he calls the compound which is left after the splitting off of some of the nitrogen 'desaminopeptone.'

This desaminopeptone does not give a proper biuret-reaction, a fact which is explained by Schiff, Hausmann,[3] and Hofmeister[4] as being due to the destruction of the terminal $CONH_2$-groups by the nitrous acid. Paal,[5] however, is of the opinion that amin-remainders are split off, basing this opinion on his investigation into the splitting off of nitrogen when glutin-peptone is treated with nitrous acid. The remaining product, the desaminonitrosopeptone possesses only one half the basicity of the original glutin-peptone. How the amounts of amino-nitrogen vary with the different albumins has already been discussed when dealing with the nitrogen radicals (p. 76), and can be read directly from the tables on p. 81.

That Levites does not believe in the existence of terminal $CO.NH_2$-groups has been pointed out on p. 142.

The existence of terminal amino-groups is, however, proved by the

[1] O. Schmiedeberg, *Arch. f. experimentelle Pathol. u. Pharmak.* **39**. 1 (1897).

[2] H. Schiff, *Ber. d. deutsch. chem. Ges.* **29**. II. 1354 (1896); *Liebig's Annalen*, **299**. 264 (1898).

[3] W. Hausmann, *Zeitschr. f. physiol. Chem.* **27**. 95 (1899).

[4] F. Hofmeister, *Ergebnisse der Physiologie von Asher u. Spiro*, I. **1**. 759 (1902).

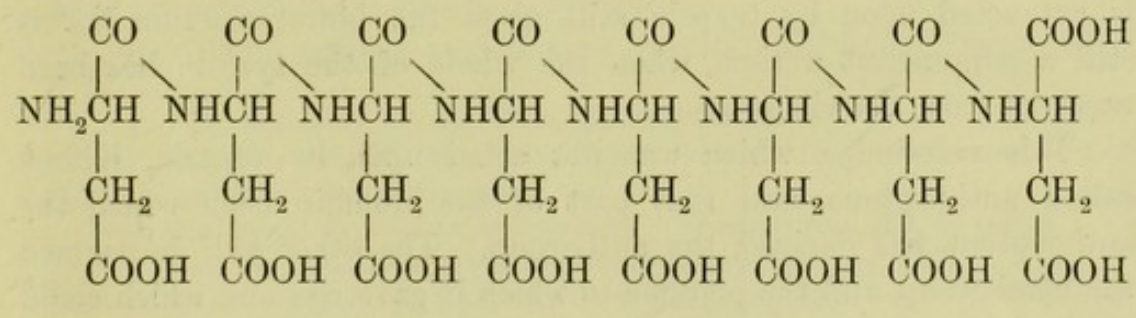
[5] C. Paal, *Ber. d. deutsch. chem. Ges.* **29**. 1084 (1896).

fact that albumins are pluri-acid bases. If an albumin resulted simply from a linking together of many amino-acids into one chain, we might expect it to be monobasic and monoacid, as are the simple amino-acids. This is, however, not the case. Determinations of the acid capacity by Erb[1] and others (Chapter V., p. 177) show that the equivalent weight of albumins is very low. Thus Erb estimates the equivalent weight of egg-albumin as not exceeding 152, while the molecular weight according to Hofmeister[2] is 5378 or a multiple of this number. Therefore egg-albumin must be at least a 35-acid base.

The terminal groups in the albumin-molecule are represented especially by the 'aminonitrogen,' but also by the NH_2-groups of lysin and arginin. The second and third N-atom of histidin are also joined in a ring-like formation (see p. 43). According to Kossel[3] the basicity of albumins increases in proportion to the increase in the amount of di-amino-acids.

It is questionable whether the NH_2-groups of ammonia, arginin, and lysin suffice to explain the high numbers of Erb. They do not suffice in the case of edestin and the hetero-albumose, where calculations can be made with a fair amount of accuracy. That the numerous basic groups of albumins are not of the same value will be shown later on, when discussing the salts of albumins (Cohnheim).

The power which albumins possess of combining with bases has been measured much less accurately than their capacity for acids; still here again the equivalent weights of albumins are very much lower than are the lowest possible molecular weights, and therefore albumins must also be pluri-basic acids. This has been proved by Pemsel and Spiro,[4] Laqueur and Sackur,[5] and others. Apart from the α-amino-groups, free carboxyl-groups are met with in glutaminic and in aspartic acids. Cohnheim draws attention to the interesting analogy, seen in octaspartic acid, which, according to Schiff,[6] has this constitution:



[1] W. Erb, *Zeitschr. f. Biol.* **41**. 309 (1901).
[2] F. Hofmeister, *Zeitschr. f. physiol. Chem.* **24**. 158 (1897).
[3] A. Kossel, *Ber. d. deutsch. chem. Ges.* **34**. III. 3214 (1901).
[4] W. Pemsel and K. Spiro, *Zeitschr. f. physiol. Chem.* **26**. 233 (1898).
[5] E. Laqueur and O. Sackur, *Hofmeister's Beiträge* **3**. 192 (1903).
[6] H. Schiff, *Liebig's Annalen*, **303**. 183 (1898), **307**. 231 (1899), **310**. 301 (1899).

In this compound eight molecules of aspartic acid are linked together in such a way that in seven of them one of the carboxyl groups remains free, while the other one is used to establish the link with the next molecule. This octaspartic acid, containing nine carboxyl-groups, is, however, only octovalent. The manner in which its carboxyl-groups differ from one another and their linking together throws also some light on certain phenomena observed in albumins. For Siegfried[1] has shown that the simple peptones, without exception, are strongly acid, and that they all contain glutaminic acid. Amongst the acid radicals of albumins differences also exist, while neutral albumins must either possess an equal number of free basic and free acid complements, the preponderance of one over the other determining whether an albumin is acid or basic in its character, or be in the pseudo-acid–pseudo-basic state. (See Index.)

Against the theory of a uniform linking together of amino-acids must also be mentioned the existence of the hemi- and the anti-groups, which differ from one another to a marked extent.

The Hemi- and the Anti-Groups (by Cohnheim)

Schützenberger and Kühne first pointed out that the albumin-molecule shows different degrees of resistance to the action of acids or ferments. Subsequently it was shown that the portion which is readily dissociated contains tyrosin and tryptophane, while the portion which is not easily acted upon is characterised by the presence of phenylalanin, glycocoll, and pyrrolidin-carboxylic acid. These differences in the building material determine how firmly the various albuminous radicals are bound together.

Kühne[1] found that tryptic digestion rapidly converts a portion of the albumin into crystalline products, amongst which leucin and tyrosin may soon be recognised. The remainder of the albumin which is not acted upon by trypsin still gives the biuret-reaction, *i.e.* is still a peptone, at a time, when the whole of the tyrosin has been removed from the albumin.

This remainder, which was not acted upon by trypsin, Kühne called anti-peptone, and that part of the albumin from which the anti-peptone was derived, the anti-group. The other half he termed the hemi-group, and the peptone to which it gave rise and which could not be isolated, the hemi-peptone.

[1] W. Kühne, *Verhandl. d. Heidelberger naturhist.-medizin. Vereins*, N. F. I. 236 (1876); Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **19**. 159 (1883); **22**. 423 (1885); see also R. Neumeister, *ibid.* **23**. 381 (1887); *Lehrbuch d. physiol. u. pathol. Chem.*, 2. Aufl., Jena, 1897, p. 228.

Amongst the products of peptic digestion Neumeister distinguished also the anti- and the hemi-albumoses. This question was then taken up in Hofmeister's Laboratory by Pick,[1] who made out that of the two primary albumoses, the protalbumose belongs to the hemi-group, while the hetero-albumose belongs to the anti-group. He also established other chemical differences; the hemi-group contains the tyrosin and tryptophane, while the anti-group includes the glycocoll and phenylalanin. According to Pick's definition, casein seemed to be pure hemi-proteid, while gelatine was pure anti-proteid. Subsequently Kutscher[2] succeeded by very prolonged autodigestion of the pancreas to reach a stage where the biuret-reaction either failed altogether or was only very slightly marked, and the conclusion was arrived at that Kühne's classification was only a relative one. Kühne's view has, however, been confirmed quite recently by E. Fischer and Abderhalden,[3] who found that one or several polypeptids remain unacted upon by trypsin even after very prolonged digestion, but that they can be converted into amino-acids by treatment with acids. The substances in question do not, however, give the biuret-reaction, which is not very material, as has already been explained. In their composition they agree with anti-albumin, for they contain glycocoll and phenylalanin, further α-pyrrolidin-carboxylic acid, while tyrosin and tryptophane are completely split off. Leucin, alanin, aspartic and glutaminic acids are present both in the hemi- and in the anti-groups. Cystin, serin, and the di-amino-acids have not been especially investigated, but Kutscher's[4] previous work shows that they are liberated, at least partly, by trypsin. Ammonia is also liberated by tryptic digestion, according to Hirschler,[5] Stadelmann,[6] and Dzierzgowski and Salaskin.[7] Fischer and Abderhalden have observed that tyrosin is especially readily liberated, which was also noticed when Fischer and Bergell[8] subjected fibroin to tryptic digestion. Leucin, alanin, etc., are liberated later than is tyrosin.

Siegfried[9] arrived at identical results: he found "that by the

[1] E. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899).

[2] F. Kutscher, *ibid.* **28**. 88 (1899); *Endprodukte der Trypsinverdauung*, Marburger Habilitationsschrift, Strassburg, 1899.

[3] E. Fischer and E. Abderhalden, *Zeitschr. f. physiol. Chem.* **39**. 81 (1903).

[4] F. Kutscher, *Endprodukte der Trypsinverdauung*, Marburg, 1899.

[5] A. Hirschler, *Zeitschr. f. physiol. Chem.* **10**. 302 (1886).

[6] E. Stadelmann, *Zeitschr. f. Biolog.* **24**. 261 (1888).

[7] S. Dzierzgowski and S. Salaskin, *Zentralbl. f. Physiol.* **15**. 249 (1901).

[8] E. Fischer, *Chemikerzeitung*, 1902, II. p. 939. (Karlsbader Naturforscherversammlung.)

[9] M. Siegfried, *Zeitschr. f. physiol. Chem.* **38**. 259 (1903); F. Müller, *ibid.* **38**. 265 (1903); M. Siegfried, *Ber. d. sächs. Ges. d. Wissensch. zu Leipzig, math.-phys. Kl.*, 1903, p. 63.

action of trypsin on albumin a part of the latter is readily dissociated into amino-acids and bases, and that thereby peptones are formed which do not contain the tyrosin group and which vigorously withstand a further dissociation by means of trypsin."

By tryptic digestion fibroin yields two acid anti-peptones, which on dissociation give rise to lysin, arginin, glutaminic acid, ammonia, and perhaps serin and aspartic acid; by careful dissociation with acids there is formed either directly from gelatine or from an acid gelatine-peptone the substance glutokyrin. This kyrin is a crystalline peptone (see p. 200); it contains lysin, arginin, glutaminic acid, and glycocoll, and is a fairly strong base because of the preponderance of the basic constituents.

How the polypeptids of Fischer and Abderhalden and this kyrin are related to one another is not yet clear. Both contain glutaminic acid and glycocoll; the di-amino-acids, which make up the greater bulk of the kyrin, have so far not been investigated in the polypeptid, but attention is drawn to E. Fischer's[1] statement that pyrrolidin-carboxylic acid and the hexone bases usually occur together. One thing is certain, namely, that we have to do with a very considerable fraction of the proteid-molecule linked together in some particular way. Erepsin acts on anti-peptone in such a way that it no longer gives the biuret-reaction.[2]

It seems to be a common phenomenon, that after the first splitting off of a part of the albumin there remain behind substances which apparently do not differ much from the material we started with, substances which still bear the chemical character of albumins. That such a result is produced by trypsin and that kyrin is formed has been explained above, but on dissociating natural albumins with dilute hydrochloric acid, Goldschmidt[3] saw that different albumoses and peptones appeared simultaneously, even before acid-albumin was formed; during peptic digestion Umber,[4] Zunz,[5] and others noticed acid-albumin together with peptones and abiuretic dissociation-products; analogous observations have been made by Maas,[6] who dissociated albumins by means of alkalies. By oxidising albumins with permanganate in alkaline solutions, Bernert[7] found a certain percentage of the albumins to dissociate very rapidly into albumoses,

[1] E. Fischer, *Zeitschr. f. physiol. Chem.* **39**. 155 (1903).

[2] O. Cohnheim, *ibid.* **35**. 134 (1902).

[3] F. Goldschmidt, *Einwirkung von Säuren auf Eiweissstoffe*, Dissertation. Strassburg, 1898.

[4] F. Umber, *Zeitschr. f. physiol. Chem.* **25**. 258 (1898).

[5] E. Zunz, *ibid.* **28**. 132 (1899); *Hofmeister's Beitr.* **2**. 435 (1902), **3**. 339 (1902).

[6] O. Maas, *Zeitschr. f. physiol. Chem.* **30**. 61 (1900).

[7] R. Bernert, *ibid.* **26**. 272 (1898).

peptones, amino-acids and their secondary dissociation-products, while another part remained unchanged and became oxidised. Oxidation by means of nitric acid showed v. Fürth [1] that only a portion of the albumin becomes nitrated, while the remainder is dissociated.

The difference between the hemi- and the anti-groups is also of importance for the metabolism of animals, as the readily dissociable albumins, namely, casein and protalbumose, offer more favourable conditions for absorption and transformation than do gelatine and hetero-albumose.[2]

The difference in the readiness with which the two groups become dissociated may be explained in a twofold manner. We may assume, firstly: that one or several nuclei exist in an albumin-molecule, and that these nuclei completely resist the action of trypsin, and that they are only with difficulty attacked by boiling acids, and that attached to these nuclei is a smaller or greater number of other amino-acids, which may be split off readily. The other possibility is the following: An albumin may be built up of a number of co-ordinated peptones and peptids and may dissociate into these groups in the first instance; subsequently some of these groups may break up at once under the influence of trypsin and of acids, while others may show greater resisting power. The first view, held by Zunz, Goldschmidt, Bernert, v. Fürth, E. Fischer, and Bergell, is supported by the fact that the different amino-acids appear successively, but this sequence may perhaps only be an apparent one, owing to the slight solubility of tyrosin. The second view has in its favour that the nuclei of Siegfried and E. Fischer differ from one another constitutionally, but further research may show that these differences are only apparent.

The two views need not necessarily exclude one another, for we may assume with Kossel that each larger albumose-complex possesses its own nucleus. That the second view is the correct one will be proved as soon as an albumin is discovered which does not possess a 'nucleus' at all, which, therefore, to use Kühne-Pick's expression, is a pure hemi-albumin, devoid of those dissociation-products which are characteristic of the anti-group. Casein, which seems to be a hemi-albumin because of the absence of glycocoll, is in reality not a hemi-albumin, and the quickness with which the biuret-reaction disappears in the case of protalbumose proves nothing, as a peptid

[1] O. v. Fürth, *Einwirkung von Salpetersäure auf Eiweissstoffe*, Habilitationsschrift, Strassburg, 1899.

[2] L. Blum, *Zeitschr. f. physiol. Chem.* **30**. 15 (1900); O. Krummacher, *Zeitschr. f. Biolog.* **42**. 242 (1901); E. Bendix, *Arch. f.* (*Anat. u.*) *Physiol.* 1900, Suppl. p. 309; W. Falta, *Verhandl. der naturforschenden Ges. zu Basel*, **15**. Heft 2 (1903).

may remain behind which does not give the biuret-reaction. Therefore at present both views have an equal right to be considered.

Why some of the complexes dissociate so much more readily than do others we, as yet, do not know. Whatever the reason may be, there cannot be any doubt that some relationship exists between the ease with which dissociation occurs and the presence of definite dissociation-products in a given albumin. This view is borne out by a study of Pick's albumoses, and also by comparing different albumins with one another. Casein and globin, which are very readily digested, do not contain any glycocoll, but much tyrosin and tryptophane, while serum-globulin contains much glycocoll and is not readily digested, according to E. Fischer and Abderhalden and Umber;[1] gelatine contains the largest amount of glycocoll, no tyrosin and no tryptophane, and yields, according to Reich-Herzberge,[2] mere traces of leucin on being digested with trypsin. Thus the chemical character of an albumin is partly determined by the quantitative amounts of amino-acids present, and partly by the manner in which the amino-acids are distributed over the anti- and the hemi-groups.

The whole of the nitrogen is present as amide, and none in the form of nitro-, nitroso-, or azo-nitrogen, as is proved by the fact that proteids[3] and their dissociation-products give 'approximately' the same nitrogen value when they are examined by either Kjeldahl's method or by that of Dumas.[4]

The carbon is contained partly in fatty and partly in aromatic compounds. In both it is arranged in the same manner. The heterocyclic groups are represented by α-pyrrolidin-carboxylic acid, and by oxy-α-pyrrolidin-carboxylic acid. That histidin also contains an imido-azol nucleus has now been proved.

Diacipiperazin is also almost certainly a primary product according to E. Fischer[5] (see p. 55).

Hydroxyl-groups are present in serin, in tetra-oxy-amino-caproic-, in trioxy-di-amino-dodecanoic-, in oxy-α-pyrrolidin-carboxylic-, in oxy-amino-suberic-, in oxy-amino-succinic-acids, and in tyrosin.

Aldehyde and ketone groups are absent in albumins, according to Löw[6] and v. Lorenz,[7] and so are the groups $O-CH_3$ and $O-C_2H_5$, according to v. Lorenz.

[1] F. Umber, *Zeitschr. f. physiol. Chem.* **25**. 258 (1898).

[2] F. Reich-Herzberge, *ibid.* **34**. 119 (1901).

[3] J. Munk, *Arch. f.* (*Anat. u.*) *Physiol.* 1895, p. 551; F. Söldner and Camerer, *Zeitschr. f. Biol.* **33**. 66 (1896).

[4] See, however, note about Kjeldahl's method on pp. 81, 82.

[5] E. Fischer, *Ber. d. deutsch. chem. Ges.* **38**. 605 (1905).

[6] O. Löw, *Journ. f. prakt. Chem.* (2) **31**. 129 (1885.

[7] J. v. Lorenz, *Zeitschr. f. physiol. Chem.* **17**. 457 (1892).

Formerly it seemed as if albumins differed greatly from one another because of the way in which the individual groups were arranged in the molecule, but with the improvements in our methods of preparing dissociation-products these supposed differences have become less and less. Since Kossel made us acquainted with reliable methods for preparing lysin, arginin, and histidin, arginin has been found in all, and lysin and histidin in nearly all, albuminous substances. With E. Fischer's new methods only a few albumins have been examined, but these show a remarkable similarity. Even silk-fibroin with its entirely different configuration has been brought nearer to the other albumins, and the same holds good for gelatine and for keratin. Edestin, globin, and serum-albumin show even quantitatively the greatest similarity. Therefore differences in albumins are not so much dependent on differences in the building material as on the material being used in different amounts and in different arrangements. The amounts are very different, as most of the dissociation-products do not occur once but several times in the albumin-molecule. Comparing, for example, leucin and histidin with tyrosin, we find that globin must contain at least 32 leucin and 10 histidin molecules. Calculations based on analytical data show the molecular weight of hæmoglobin to be at least 16669; if this figure is correct, then hæmoglobin must contain at least 36 molecules of leucin and 12 molecules of histidin. Determinations of the amount of ammonia contained in gelatine show, analogously, that gelatine must contain 8 molecules of glycocoll, while the amount of histidin in edestin is equivalent to 12 molecules of leucin and 6 molecules of arginin. Kossel and Dakin [1] give the following figures for salmin: For every 10 molecules of di-amino-valerianic acid are found 10 molecules of urea, 2 molecules of serin, 1 molecule of mono-amino-valerianic acid, and 2 molecules of pyrrolidin-carboxylic acid.

That one and the same substance may occur in different combinations has been shown by E. Fischer and Abderhalden, who found leucin, alanin, glutaminic and aspartic acids both in the anti- and in the hemi-group.

Now arises the question: What grouping of atoms have we to consider as typical of albumins? a question which, if we can answer it, will define and accurately outline the group of albuminous substances.

The most important grouping, without doubt, is the union of α-amino-acids to form acid-imines, and therefore such bodies as glycyl-glycin and its homologues may be taken as the simplest of all albumins.

[1] Kossel, *Berliner klin. Wochenschrift*, No. 41, October 1904, p. 1065.

It is perhaps more correct to follow Kossel,[1] and also to consider the second mode of union, such as we see it in arginin, as essential for the conception of an albumin. According to this view we would have to define albumins as acid-imines composed of α-amino-acids and including also arginin.

This definition will cover, without doubt, all peptones, and also the more complex peptids; further, the protamins, which latter ought not to be put into a separate class, if we consider what broad chemical and genetic transitions exist between them and the other albumins. A classification on a physiological basis, as has been attempted by Löw[2] and by Hofmeister,[3] has, according to Cohnheim, great disadvantages, and is not permissible, since it has become probable that the animal body can build up its proteids from all nitrogenous compounds, provided that these can be acted upon by its ferments. Kossel[4] believes, however, and the author thinks rightly, that from the physiological standpoint we are, nowadays, not justified in believing that meat-albumin possesses the same nutritive value as milk-albumin or the albumin of maize. These substances differ in their chemical constitution, and therefore they must also play different parts in nutrition. Kossel has looked on this matter from still another point of view. He draws attention to the fact that arginin up till now is the only dissociation-product which has been found in all albumins, and that certain albumins exist, namely, the protamins, in which arginin forms the main bulk of the dissociation-products, and that the protamins appear relatively simple, because of the smaller number of compounds composing them. Wheresoever the other amino-acids increase in number and complexity, arginin diminishes in amount. He therefore considers arginin as the nucleus of the albumin-molecule, or rather as the nucleus round which the individual complexes of albumoses group themselves in building up the albumin-molecule. Attention is drawn once more to the fact that the most resisting element in albumins, which Siegfried succeeded in isolating by means of careful treatment with trypsin and acids, namely, the kyrins, are bases, consisting for the greater part of arginin and lysin.

The Carbohydrate Radicals of Albumins

That either a carbohydrate, or some radical resembling a carbo-

[1] A. Kossel, *Ber. d. deutsch. chem. Ges.* **34**. III. 3214 (1901); *Bull. de la Soc. chim. de Paris*, 3ème sér., **1**. 29, Nr. 14, Juli 1903.

[2] O. Löw, *Maly's Jahresber.* 1900, p. 18.

[3] F. Hofmeister, *Ergebnisse der Physiologie*, I. **1**. 1902, p. 759.

[4] A. Kossel, *Berl. klin. Wochenschr.* **41**. 1065 (1904).

hydrate, must be contained in albumins, has been asserted for a long time. Berzelius[1] came to this conclusion because certain decomposition-products, such as humin, saccharic and oxalic acids, are common to both albumins and carbohydrates; subsequently, in confirmation of his view, he showed that the furfurol reaction is not only characteristic for carbohydrates, but for all albumins. Kossel, Blumenthal, and others found pentoses and other carbohydrates amongst the dissociation-products of the nucleo-proteids; these were derived, however, not from the albumin-moiety, but from the nucleic acid radical. The first definite proof as to the existence of carbohydrate-groups in proteids we owe to Eichwald,[2] who discovered a carbohydrate in mucin. This line of research was then taken up by Hammersten, who was, however, so little inclined to believe that the carbohydrate formed a part of the proteid-molecule, that he classed mucins and similar substances together as compound glycoproteids to distinguish them from ordinary proteids. Only after Pavy[3] had prepared from what he considered to be a pure albumin, namely, egg-white, the osazone of a hexose, was this whole question discussed with more interest. Within the last ten years much work has been done in this field, but one complication arose which made progress very difficult, for the formation of sugar from proteids during metabolism, and particularly in cases of diabetes mellitus, has repeatedly been mixed up with the simple problem, or at least has been connected with it (Cohnheim.)

Such confusion is, however, quite unjustifiable, as has been pointed out by Müller,[4] Seeman,[5] and Müller and Seemann,[6] for the amounts of the carbohydrates in albumin are far too small in proportion to the sugar so produced.

The most important fact, from the physiological standpoint, is the conversion of a great portion of the carbon of the albumin into dextrose or glycogen.[7] Leucin, which Fr. Müller,[8] Cohn,[9] and others have thought of in this respect, is a derivative of isocaproic acid, and therefore would have to undergo a preliminary and complicated

[1] According to N. Krakow, *Pflüger's Arch.* **65**. 282 (1897).

[2] Eichwald, *Ann. d. Chem. und Pharmac.* **134**. 177 (1865).

[3] Pavy, *Physiology of Carbohydrates*, 1895.

[4] Friedrich Müller, *Zeitschr. f. Biolog.* **42**. 468 (1901).

[5] Seemann, *Über d. reducierenden Substanzen, welche sich aus Hühnereiweiss abspalten lassen:* Inaugural Dissertation, Marburg, 1898.

[6] F. Müller and J. Seemann, 'Über die Abspaltung von Zucker aus Eiweiss,' *D. med. Woschenschr.*, 1899, Nr. 13, p. 209.

[7] M. Cremer, *Ergebnisse der Physiologie*, by Asher and Spiro, **1**. 803 (1902); Rolly *Deutsch. Arch. f. klin. Med.* **78**. 250 (1903).

[8] Fr. Müller, *Zeitschr. f. Biol.* **42**. 468 (1901).

[9] R. Cohn, *Zeitschr. f. physiol. Chem.* **28**. 211 (1899).

transformation. E. Fischer[1] believes the oxy-amino-acids to be especially concerned in this conversion into carbohydrates. How important in this connection di-amino-propionic acid is has been pointed out on p. 164.

During the transformation of amino-acids into dextrose, the elimination of the amino-group is of course essential, and it is very interesting to observe, firstly, how the grouping of atoms, as met with in the α-amino-acids, is readily attacked by bacteria (see p. 100) and by the oxidising forces within the animal body[2] (see also p. 106); and secondly, how the nitrogen and the carbon of the albumin pursue different paths in metabolism.[3] These observations are of the greatest biological interest, but as yet they throw but little light on the chemistry of proteids.

In the following pages only such præformed carbohydrate-groups will be discussed as may be obtained, analogously to tyrosin or arginin, by dissociating albumins without allowing any disintegration or secondary processes to come into play.

The first well-defined carbohydrate-compound isolated from an animal tissue was glucosamin, which Ledderhose[4] prepared by boiling the shells of lobster's claws in concentrated hydrochloric acid. Winterstein then obtained a similar body from fungus-cellulose.[5]

An identical carbohydrate group exists, without doubt, also in the mucins and mucoids, as the investigations of Landwehr,[6] Zanetti,[7] Hammarsten[8] and his pupils,[9] Mörner,[10] Löbisch,[11] and Friedrich

[1] E. Fischer and A. Skita, *Zeitschr. f. physiol. Chem.* **35**. 221 (1902); E. Fischer and H. Leuchs, *Bericht d. deutsch. chem. Ges.* **36**. I. 24 (1903).

[2] H. Weiske and B. Schulze, *Zeitschr. f. Biol.* **20**. 277 (1884); N. Zuntz (and Bahlmann), *Arch. f. (Anat. u.) Physiol.* 1882, p. 424.

[3] J. Frentzel (and N. Zuntz), *ibid.* 1899, p. 383; O. Frank and F. v. Gebhard, *Zeitschr. f. Biolog.* **43**. 117 (1902); O. Frank and R. Trommsdorf, *ibid.* **43**. 258 (1902).

[4] Ledderhose, *Ber. d. deutsch. chem. Ges.* **9**. 1200 (1878); *Zeitschr. f. physiol. Chem.* **2**. 213 (1878-79), and **4**. 139 (1880). See also Tiemann, *Ber. d. deutsch. chem. Ges.* **17**. 241 and **19**. 49.

[5] E. Winterstein, *Zeitschr. f. physiol. Chem.* **19**. 521 (1894).

[6] H. A. Landwehr, *ibid.* **5**. 371 (1881), **6**. 74 (1881), **8**. 114 (1883).

[7] C. U. Zanetti, *Ann. di Chim. e Fermac.* **12**. (1897); in Maly's *Jahresber. f. Tierchemie*, **27**. 31 (1897).

[8] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **6**. 194 (1882), **12**. 163 (1887); Pflüger's *Arch. f. d. ges. Physiol.* **36**. 373 (1885); *Zeitschr. f. physiol. Chem.* **15**. 203 (1891).

[9] E. A. Jernström, abstracted from the Swedish original by Hammarsten, Maly's *Jahresber. f. Tierchemie*, **10**. 34 (1880).

[10] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **18**. 61, 213, 233 (1893).

[11] W. F. Löbisch, *ibid.* **10**. 40 (1885).

Müller[1] and his pupils,[2] Fränkel,[3] Mitjukoff,[4] and others, have shown. Some other substances belong also to this group, namely, the glycoproteid of Helix and ichthulin, which Hammarsten classed together with the glycoproteids, and further, the mucilagenous envelope of frog's eggs,[5] the coverings of the eggs of Sepia and Loligo,[6] and the ground substance of gelatinous sponges.[6] Amongst invertebrates similar substances are probably widely distributed.

All we knew at first about the nature of this carbohydrate was that albumins, on being boiled with acids, split off a body which reduced cupric oxide in an alkaline solution, *i.e.* which gave Trommer's test, or one of the modifications of the latter. Subsequently all researches aimed at isolating an osazon, which could then be identified, by comparing its melting point and its composition with those of one of the known mono- or di-sacharides. The fact was established that the carbohydrate which could be isolated from mucins—and eventually from other proteids—was a hexose, and that in all probability it was dextrose. Eichholz,[7] Blumenthal,[8] and Mayer[9] prepared a glycosazone having the characteristic melting point of 202° to 204°.

The real state of matters was first discovered by Friedrich Müller.[1] There is contained in mucin a glucosamin, *i.e.* a nitrogen-containing derivative of dextrose. As this compound gives the same osazone as does the non-aminated hexose, all the older statements, made by different observers, retain their value. The existence of this glucosamin amongst the dissociation-products of the mucins and mucoids has been confirmed, in addition to Müller's pupils: Weydemann,[2] Seemann,[2]

[1] F. Müller, 'Schleim der Respirationsorgane,' *Sitzungsber. der Ges. z. Beförd. d. ges. Naturw. zu Marburg*, 1896, p. 53; 1898, p. 117; *Zeitschr. f. Biol.* **42**. 468 (1901). (This paper contains a complete summary of the work done by Müller and his pupils.)

[2] H. Weydemann, *Tierisches Gummi aus Eiweiss:* Dissert., Marburg, 1896; J. Seemann, *Reduzierende Substanzen aus Hühnereiweiss:* Dissert., Marburg, 1898; Zängerle, *Münch. medizin. Wochenschr.*, 1900, Nr. 13.

[3] S. Fränkel, 'Spaltungsprodukte des Eiweiss bei der Verdauung,' *Monatsh. f. Chem.* **19**. 747 (1898).

[4] K. Mitjukoff, Dissert., Bern; *Arch. f. Gynäkologie*, **49**. fas. 2, 1895.

[5] F. N. Schulz and F. Ditthorn, *Zeitschr. f. physiol. Chem.* **29**. 373 (1900), **32**. 428 (1901).

[6] O. v. Fürth, *Hofmeister's Beiträge*, **1**. 252 (1901).

[7] A. Eichholz, 'The Hydrolysis of Proteids,' *Journal of Physiology*, **23**. 163 (1898).

[8] F. Blumenthal and P. Mayer, *Berichte d. deutsch. chem. Gesellschaft*, **32**. I. 274 (1899).

[9] P. Mayer, *Deutsche med. Wochenschr.* 1899, Nr. 6, p. 95.

and Zängerle,[1] by Zanetti,[2] Fränkel,[3] and Jacewicz,[4] Steudel,[5] Panzer,[6] Leathes,[7] and Neuberg and Heymann.[8]

Baisch[9] discovered in 1894 an amino-hexose in urine, which is in all probability identical with the glucosamin which reacts with Ehrlich's test. Ehrlich's reaction with *p*-dimethyl-amino-benzaldehyde, which, according to Müller, is a glucosamin-reaction, is dealt with on p. 10.

Müller and his pupils have prepared glucosamin by benzoylising solutions containing glucosamin with benzoylchloride and caustic soda, and subsequently converting the resulting product, with hydrochloric acid under pressure, into a soap. Steudel coupled the glucosamin with phenyl-isocyanate, and then converted the newly-formed body by boiling with acetic acid into the very slightly soluble anhydride. Neuberg and Heymann[8] employed bromphenyl-hydrazin. According to E. Fischer and Leuchs,[10] glucosamin has the formula—

$$\begin{array}{c} \text{H H OH} \\ \text{CH}_2\text{(OH)} - \text{C} - \text{C} - \text{C} - \text{CH(NH}_2\text{)COH} \\ \text{OH OH H.} \end{array}$$

It is therefore a derivative of dextrose. Only the stereo-chemical position of the amino-group is as yet uncertain. It is identical with the above mentioned glucosamin, which Ledderhose[11] prepared from the chitin of arthropods. E. Fischer considers it to be a link between the carbohydrates and the oxy-*α*-amino-acids. "As the latter occur fairly abundantly in proteids, glucosamin forms to a certain extent a bridge between carbohydrates and proteids." Glucosamin is not fermentable with yeast, and is not, or only slightly, affected by the oxidising and dissociating ferments of mammals (Fabian,[12] Fränkel and Offer[13] and

[1] H. Weydemann, *Tierisches Gummi aus Eiweiss:* Dissert., Marburg, 1896; J. Seemann, *Reduzierende Substanzen aus Hühnereiweiss:* Dissert., Marburg, 1898; Zängerle, *Münch. medizin. Wochenschr.* 1990, Nr. 13.

[2] C. U. Zanetti, *Ann. di Chim. e Fermac.* **12.** (1897); in *Maly's Jahresbericht f. Tierchemie*, **27.** 31 (1897).

[3] S. Fränkel, 'Spaltungsprodukte des Eiweiss bei Verdauung,' *Monatsh. f. Chem.* **19.** 747 (1898).

[4] M. Jacewicz, Dissert. Petersburg (Russian), from *Maly's Jahresbericht f. Tierchemie*, **26.** 8 (1896).

[5] H. Steudel, *Zeitschr. f. physiol. Chem.* **33.** 223 (1901), **34.** 353 (1901).

[6] T. Panzer, *ibid.* **28.** 363 (1899).

[7] J. B. Leathes, *Arch. f. experiment. Path. u. Pharmakol.* **43.** 245 (1899).

[8] C. Neuberg and F. Heymann, *Hofmeister's Beiträge* **2.** 201 (1902).

[9] K. Baisch, *Zeitschr. f. physiol. Chem.* **19.** 339 (1894).

[10] E. Fischer and H. Leuchs, *Ber. d. deutsch. chem. Ges.* **36.** I. 24 (1903). Here also summary of literature.

[11] G. Ledderhose, *Zeitschr. f. physiol. Chem.* **2.** 213 (1878).

[12] R. Fabian, *ibid.* **27.** 167 (1899).

[13] S. Fränkel and Offer, *Zentralblatt f. Physiologie*, **13.** 489 (1899).

Cathgart [1]), which is a further proof that it has nothing to do with the formation of sugar out of albumins. The mucin obtained from frog's spawn contains, according to Schulze and Ditthorn, galactosamin instead of glucosamin.

This glucosamin is, however, not contained in the mucins as such, but in a more complex form. If mucin is boiled with strong acids, as in preparing amino-acids, only little or no carbohydrate is found; it is apparently destroyed, being entirely converted into humin or melanoidin, as Langstein [2] has shown. Müller obtained larger quantities of glucosamin, only, by boiling for a short time with dilute hydrochloric acid, the greatest yield being obtained by boiling for three to five hours with 2·5 per cent hydrochloric acid. The mucin is, however, not completely destroyed by this process, there being formed albumoses and peptones, which make the extraction of the carbohydrate very difficult. The carbohydrate is, however, not attached to any of the proteid-radicals, as these peptones and albumoses may be got rid off by phosphotungstic acid (Steudel), or iron acetate and tannic acid (Müller).

The largest yield is obtained, according to Seeman and Langstein,[3] when the proteid is allowed to swell up into a jelly with an alkali, and is then dissociated with an acid. Glucosamin obtained by dissociating glyco-proteids with acids, has these properties: It reduces, but it does not contain any free NH_2-groups, as Steudel failed to couple it with phenyl-cyanate till he had previously heated the compound with sulphuric acid under pressure. Leathes has therefore assumed that a second carbo-hydrate is attached to the nitrogen, analogous to the coupling of atoms in chondrosulphuric acid, as held by Schmiedeberg;[3] Neuberg and Heymann failed, however, to demonstrate a second carbohydrate, and they deny the correctness of Leathes' views; glycuronic acid they also excluded.

Ledderhose found in chitin, Müller in mucin, and Schmiedeberg[3] in chondrosulphuric acid, prepared from chondromucoid, acetic acid constantly in combination with glucosamin. As Müller further observed that diacetyl-glucosamins give Ehrlich's dimethyl-*p*-amino-benzaldehyde reaction (see p. 10), it is very well possible that the body which is liberated from mucins is at first a diacetyl-glucosamin, especially as penta- and mono-acetyl-glucosamins do not give the colour reaction of Ehrlich.

In egg-white, glucosamin is not contained as such, because, according to Steudel, it does not at first combine with phenylcyanate.

[1] Pr. Cathcart, *Zeitschr. f. physiol. Chem.* **39**. 423 (1903).

[2] L. Langstein, *ibid.* **31**. 49 (1900).

[3] O. Schmiedeberg, *Arch. f. experiment. Path. u. Pharmakol.* **28**. 355 (1891).

Glucose pentacetate	$C_{16}H_{22}O_{11} = C_6H_7O_6(C_2H_3O)_5$.
Acetyl glucosamin	$C_8H_{15}O_6N = C_6H_{12}O_5N(COCH_3)$.
Diacetyl glucosamin	$C_{10}H_{17}O_7N = C_6H_{11}O_5N(COCH_3)_2$.
Pentacetyl glucosamin	$C_{16}H_{23}O_{10}N = C_6H_8O_5N(COCH_3)_5$.

If further glucosamin is injected into the circulatory system, it is eliminated for the greater part, according to Fabian,[1] while proteids containing glucosamin are completely oxidised. Ellinger and Gentzen [2] have shown analogously that tryptophane is completely oxidised when administered subcutaneously, while it is excreted for the most part if it is first converted into indol by the bacteria of the alimentary canal. Glucosamin and indol, judging by these experiments, are therefore, not normal products of animal metabolism, and just as indol is linked up as indol-amino-propionic acid, so must glycosamin also be linked up normally with some other radical (Cohnheim).

In addition to glucosamin substances have repeatedly been discovered which do not reduce, but which give the reaction of Molisch (p. 8) and other reactions. For this reason the substances in question are probably polysaccharids, which must be converted, in the first instance, into monosaccharids before they can act as reducing bodies. Such a body has been found by Löbisch in the mucoid of tendon, and by Hammarsten in helicoproteid; the latter called it sinistrin because of its lævo-rotation. By treatment with pepsin, succeeded by treatment with barium hydrate, Fränkel obtained 'albamin,' which he considers to be a nitrogen-containing biose. After very prolonged peptic digestion Langstein [3] isolated from egg-albumin a body, the analysis of which points to its being a dihexosamin. This body gives rise to a reducing carbohydrate on being boiled for a short time with hydrochloric acid. Here must also be mentioned the so-called 'animal gum,' which Landwehr prepared by acting with alkalies on mucin, and which Fohn,[4] Müller, and Weydemann also prepared, according to Landwehr's directions, by boiling mucin in a Papin's pot with 10 per cent potassium hydrate, or by digestion with pepsin and trypsin. Animal gum is a light powder, soluble in water and in 70 per cent alcohol. It is precipitated by phosphotungstic acid and lead acetate and ammonia, and seems, according to Müller, to be the lime salt of an organic acid. It is not attacked by diastase, but by very short boiling is converted into glucosamin, yielding from

[1] R. Fabian, *Zeitschr. f. physiol. Chem.*, **27**. 167 (1899).
[2] A. Ellinger and M. Gentzen, *Hofmeister's Beiträge*, **4**. 171 (1903).
[3] L. Langstein, *Hofmeister's Beiträge*, **2**. 229 (1902).
[4] O. Folin, *Zeitschr. f. physiol. Chem.* **23**. 347 (1897).

50 to 80 per cent of the latter. Animal gum is not a uniform body but a mixture of the suspected polysaccharid with more or less of mucin-albumoses and perhaps also albuminates. Leo Langstein [1] believes, however, in the existence of a true animal gum.

On dissociating pseudo-mucins by strong mineral acids, Otori [2] has obtained laevulinic acid, showing the presence of a true carbohydrate radical in pseudo-mucin. Paramucin does not give rise to laevulinic acid according to Panzer.[3]

The percentage composition of animal gum is interesting, as, according to Weydemann it contains only 4 to 5 per cent of nitrogen, which means that some portion of the animal gum must contain less nitrogen than does glucosamin. As the carbon percentage is also low, the simplest assumption to make is that animal gum is an aminated polysaccharid. This conclusion has been arrived at by Schulz and Ditthorn ;[4] as the glucoproteid contains hardly more nitrogen than does the galactosamin which is prepared from it, there must be present either another non-nitrogenous body, or the NH_2-group of the galactosamin must be linked in some other manner to the proteid moiety. It is, of course, possible that individual mucins may be quite different in this respect (Cohnheim.)

No definite statements can be made as to the amount of glucosamin present in an albumin-molecule, as it is so readily destroyed. The highest values attained so far are 36·9 per cent for the mucin from the trachea (Müller) ; 34·9 per cent for ovomucoid (Seemann) ; 30 per cent for the pseudomucin of ovarial cysts (Zängerle). For pseudomucin much lower values have been obtained, namely, 12·5 per cent by Mitjukoff ; 10 per cent by Steudel, 2 per cent by Pfannenstiel ; we are therefore dealing in all probability with different bodies (Neuberg and Heymann). Schulz and Ditthorn [4] found in the mucin of frog's spawn a large amount of galactosamin, as already mentioned.

Egg-albumin behaves exactly as do the mucins. After osazones and reducing substances had repeatedly been prepared from it, Seemann and Langstein [5] succeeded in obtaining glucosamin from it. Seemann obtained 8·5 grammes glucosamin from 100 grammes albumin ; Hofmeister [6] obtained even 15 per cent.

Now the question arises : Shall we put the mucins and allied

[1] Langstein, *Ergebnisse d. Physiol.* **3**. Part 1, p. 453 (1904) ; and in *Zeitschr. f. physiol. Chem.* **42**. 171 (1904).

[2] J. Otori, *Zeitschr. f. physiol. Chem.* **42**. 453, **43**. 74 and 86.

[3] Panzer, *ibid.* **28**. 363 (1899).

[4] F. N. Schulz and F. Ditthorn, *ibid.* **32**. 428 (1901).

[5] L. Langstein, *ibid.* **31**. 49 (1900).

[6] F. Hoffmeister, *ibid.* **24**. 159 [p. 170] (1899) ; D. Kurajeff, *ibid.* **26**. 462 (1899).

substances into a special class, heading them glycoproteids, and separate them from the remaining proteids, as does Hammarsten,[1] or is this impossible because glucosamin is found also in other proteids if not in all albumins? The statement of Pavy that a reducing substance may be prepared from all the organs of the animal body, and usually as an osazone, is not convincing, because mucins and mucoids are much more widely distributed over the animal body than was known at one time. Mörner[2] found, for example, large amounts of ovomucoid in egg-white; Zanetti[3] discovered a similar substance in blood-serum; Hammarsten[4] found, further, in ascitic fluid and Mörner[5] in urine, bodies, the mucoid nature of which is, however, doubted by Langstein.[6] All these substances are purified only with great difficulty from the accompanying globulins and albumins, and therefore it is quite possible that in all those cases where only very small amounts of sugar, usually osazones, are obtained from genuine proteids we are dealing with admixtures of mucoids. Not taking into account the older researches, what has just been stated holds good also for the work of Krakow,[7] who found that fibrin, serum-albumin, serum-globulin, lactalbumin, and the proteid of peas, possess a slight reducing power, and that they yield an osazone, while no osazone was obtained from casein, vitellin, legumin, and gelatine; K. Mörner's[8] statements as to the existence of a carbohydrate in serum-globulin is also not convincing, and Blumenthal and Mayer[9] did not purify their yolk-albumin from mucoid or other proteids. Whenever the mucoid was removed, Eichholz[10] failed to find a carbohydrate in serum-globulin, serum-albumin, and casein (Cohnheim).

To settle the question as to whether a carbohydrate radical is contained in serum-albumin, Langstein[6] has employed thrice crystallised horse-albumin prepared by Krieger's method. He obtained results comparable to those got with egg-albumin; by dissociation with alkalies a non-reducing substance is obtained which gives an intense furfurol reaction, and which, after short boiling, yields a reducing carbohydrate, apparently glucosamin.

[1] O. Hammarsten, *Lehrbuch d. physiol. Chem.*, 4th edition, 1899, p. 388.
[2] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **18**. 525 (1893).
[3] C. U. Zanetti, *Maly's Jahresber. f. Tierchemie*, **27**. 31 (1897).
[4] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **15**. 203 (1891).
[5] K. A. H. Mörner, *Skandinavisches Arch. f. Physiol.* **7**. 332 (1895).
[6] L. Langstein, *Hofmeister's Beitr.* **1**. 259 (1901).
[7] A. Krakow, *Pflüger's Arch. f. d. ges. Physiol.* **65**. 281 (1897).
[8] K. A. H. Mörner, *Zentralbl. f. Physiol.* **7**. Nr. 20, p. 561 (1893).
[9] F. Blumenthal and P. Mayer, *Ber. d. deutsch. chem. Ges.* **32**. I. 274 (1899).
[10] A. Eichholz, *Journ. of Physiol.* **23**. 163 (1898).

He further suspects a carbohydrate-acid. In serum-globulin [1] he found grape-sugar; an aminated hexose, which was not glucosamin and still other substances resembling carbohydrates. But as serum-albumin contains only 0·5 per cent, and serum-globulin a little more than 1 per cent of these bodies, the proof is yet wanting that these substances are really derived from albumins and globulins, and that they are not simply admixtures, linked perhaps chemically to the albumin (compare with p. 222). Abderhalden, Bergell, and Dörpinghaus [2] state that they prepared serum-albumin which gave no trace of Molisch's reaction. Langstein [3] observes that he never had so pure a specimen, and that in his case the serum-albumin may 'perhaps' have contained traces of non-coagulable albumins, which according to Zanetti [4] always contain glucosamin. Langstein has certain evidence, not yet published, that pure serum-albumin does contain some glucosamin.

Although, then, no exact proof has been so far adduced in support of the view that, with the exception of egg-albumin, mucins, and other glycoproteids, a carbohydrate radical is contained in proteids, there is a whole series of other facts (apart from the results of Langstein, Krakow, and Fränkel discussed above) which show that a carbohydrate group is present in most albumins, for the following reasons:—

1. The reaction of Molisch (see p. 8) is a furfurol reaction, and is admitted by every one to demonstrate the presence of carbohydrate. Pick [5] has shown that Molisch's reaction is present only in some of the dissociation-products resulting from peptic digestion. He has isolated amongst the deutero-albumoses a gluco-albumose, and further a gluco-peptone or 'Peptone A.' These substances, in addition to giving the reaction of Molisch, are further characterised by a high oxygen and a low carbon and nitrogen percentage. Gluco-albumose has so far been prepared only from Witte's peptone, and as it is not known from what material Witte's peptone is made, the possibility of mucin albumoses being present must not be lost sight of. A glyco-peptone has, however, on the other hand, been prepared by Umber [6] from serum-albumin and serum-globulin.

[1] L. Langstein, *Sitzungsber. der Wiener Akad. d. Wissensch., math.-nat. Kl.*, Part 2[b], **112**. (May 1903).

[2] Emil Abderhalden, Peter Bergell, and Theodor Dörpinghaus, *Zeitschr. f. physiol. Chem.* **41**. 530 (1904).

[3] Leo Langstein, *ibid.* **42**. 171 (1904).

[4] Zanetti, *La Chim. Ital.* **1**. 160 (1903).

[5] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899); *Hofmeister's Beitr.* **2**. 481 (1902).

[6] F. Umber, *Zeitschr. f. physiol. Chem.* **25**. 258 (1898).

2. The second reason has already been given on p. 67. If, after deducting the water, one adds together the carbon, nitrogen, and oxygen values of the known dissociation-products of globin, figures are obtained with a higher value for carbon and nitrogen and a lower value for oxygen than are possessed by the mother-substance, namely globin. Therefore the most ready explanation seems to be that one or more carbohydrate groups are contained in the unknown radical (Cohnheim).

Mucins and egg-albumin are therefore not glyco-proteids in the sense that compounds of nucleic acid with albumins are nucleo-proteids, for they only contain one of the usually occurring dissociation-products in a larger or in a more readily accessible amount. Some albumins —*e.g.* casein, gelatine, and elastine—do not seem to possess any carbohydrate, and are in this respect analogous to other compounds in which, *e.g.*, the glycocoll or tyrosin radicals are absent.

In what form carbohydrates are contained in the albumins we therefore do not know; we can only say that glucosamin is derived from it secondarily.

Some Physiological Considerations

As in cases of diabetes mellitus, the amount of sugar found in the urine is far in excess of the amount of glucosamin occurring normally in the albumin-molecule (see p. 161). Müller, Kossel, Kraus,[1] and R. Cohn have developed the conception that carbohydrate may be formed out of certain atomic complexes which themselves do not possess a carbohydrate nature. These investigators have assumed that the hexone-bases (see p. 20) or that amino-acids, set free by the dissociating action of pepsin, trypsin, and erepsin may become des-aminated; that their carbon-chain may be broken up and then be reformed into carbohydrates.

That di-amino-propionic acid is of special interest in connection with the formation of carbo-hydrates out of albumins has been pointed out by Paul Mayer.[2] (See below.)

Klebs, the discoverer of di-amino-propionic acid,[3] showed that this acid is converted into *i*-glyceric acid on being acted upon by gaseous nitrous acid. With the view of removing only one NH_2-radical, Neuberg and Silbermann[4] added an equivalent amount of silver nitrite

[1] Fr. Kraus, *Berlin. klin. Wochensch.* No. 1 (1904). (Here is given a summary of the literature.)

[2] P. Mayer, *Zeitschr. f. physiol. Chem.* **42**. 59 (1904).

[3] O. Klebs, *ibid.* **19**. 301 (1894).

[4] C. Neuberg and M. Silbermann, *Ber. d. deutsch. chem. Ges.* **37**. 341 (1904).

to the hydrochloride of α-β-di-amino-propionic acid[1] and obtained iso-serin or α-oxy-β-amino-propionic acid.

$$CH_2(NH_2) . CH(NH_2)COOH \rightarrow CH_2(NH_2) . CH(OH) . COOH.$$

"In this nitrite reaction, as in the physiological des-amination of the di-amino-fatty-acids, the amino-group, NH_2, next the carboxyl-group (COOH) is more readily eliminated," for δ-amino-valerianic acid is formed during putrefaction out of the α-δ-di-amino-valerianic acid, or out of arginin, as shown by E. and H. Salkowski.[2]

Paul Mayer,[3] by injecting the hydrochloride of di-amino-propionic acid into rabbits, found this di-amino acid to become des-aminated:

$$\begin{array}{l} CH_2 . NH_2 \\ | \\ CH \ . NH_2 + 2H_2O \\ | \\ COOH \end{array} = \begin{array}{l} CH_2 . OH \\ | \\ CH \ . OH \ + 2NH_3 \\ | \\ COOH. \end{array}$$

Di-amino-propionic acid + water = glyceric acid + ammonia.

This transformation into glyceric acid is a new proof of the intimate physiological relationship between amino-compounds and hydroxyl-compounds, and between the latter and carbohydrates, because by the reduction of glyceric acid, $CH_2OH—CHOH—COOH$, into glycerine-aldehyde, $CH_2OH—CHOH—COH$, there is formed a true sugar. The glycerine-aldehyde being in itself a true sugar, by condensation of two molecules can readily give rise to hexoses, according to Wohl and Neuberg,[4] analogous to the two-carbon series, when, as Mayer[5] has shown, there is formed glycol-aldehyde.

The probable inter-relationship between *d*-glycuronic acid and *d*-glyceric acid has already been alluded to on p. 36 under the di-amino-propionic acid.

As in the fatty di-amino-acids the α-NH_2-radical is eliminated by the action of nitrites,

$$\text{lysin, } NH_2 . CH_2 . [CH_2]_3CH . NH_2 . COOH,$$

probably gives rise to ϵ-amino-α-oxy-caproic acid:

$$NH_2 . CH_2[CH_2]_3CH(OH) . COOH,$$

[1] They now advise the use of barium nitrite. *Ber. d. deutsch. chem. Ges.* **36**. 4384 (1903).

[2] E. and H. Salkowski, *ibid.* **16**. 1191 and 1802 (1883).

[3] P. Mayer, *Zeitschr. f. physiol. Chem.* **42**. 59 (1904).

[4] Wohl and Neuberg, *Ber. d. deutsch. chem. Ges.* **33**. 3095 (1900).

[5] P. Mayer, *Zeitschr. f. physiol. Chem.* **38**. 135 (1903).

which is isomeric with the oxy-amino-acids, $C_6H_{13}NO_3$, described by E. Fischer and Tiemann [1] and Neuberg and Wolff.[2]

In the ϵ-amino-α-oxy-caproic acid, the NH_2-radical may be substituted by a halogen (*e.g.* by Jochem's method), and the resulting compound be reduced to α-oxy-*n*-caproic acid, $CH_3 . [CH_2]_3CHOH . COOH$, and in this way a relationship be established to glucosamic acid and to glucose.

Analogous to the conversion of di-amino-propionic acid into glyceric acid is the change which alanin, or mono-amino-propionic acid, undergoes in passing through the body, for it gives rise to lactic acid,[3] which, as a tautomer of glyceric-aldehyde, is closely related to glucose. Embden and Salomon [4] have further shown that in dogs suffering from pancreatic diabetes, alanin produces a very marked and quickly occurring increase in the amount of sugar in the urine. The same authors in a later paper [5] show in addition that lactic acid, glycocoll, and asparagin increase the amount of sugar, while urea does not. That asparagin leads to sugar-formation was, however, first observed by Nebelthau.[6]

The two most promising observations made recently in connection with the formation of sugars from albumin, and reversely the conversion of sugars into aminated radicals occurring in the albumin molecule, are those of Skraup and Wohlgemuth who discovered the di-amino-poly-carboxylic acids. (See pp. 44 to 45.)

The two most promising observations made recently in connection with the formation of sugars from albumin, and reversely the conversion of sugars into aminated radicals occurring normally in the albumin, are the discovery of oxy-diamino-dicarboxylic acids by Skraup [7] (see p. 44), and the conversion of grape-sugar into methyl-imidoazol by Windaus and Knoop.[8]

The oxy-amino-acids—serin, oxy-prolin, tetraoxy-amino-caproic acid, oxy-amino-suberic acid, trioxy-diamino-dodecanoic acid, and oxy-amino-succinic acid—occupy a position midway between the sugars and the amino-acids. The following oxy-acids have been synthetised :

[1] Fischer and Tiemann, *Ber. d. deutsch. chem. Ges.* **27**. 144 (1894).

[2] Neuberg and Wolff, *ibid.* **35**. 4015 (1902).

[3] Neuberg and Langstein, *Verhand. physiol. Ges. Berlin*, 1903. See also Neuberg and Silbermann, *Ber. d. deutsch. chem. Ges.* **37**. 339 (1904).

[4] G. Embden and H. Salomon, *Hofmeister's Beiträge*, **5**. 507 (1904).

[5] *Ibid.* **6**. 63 (1904).

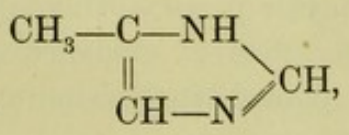
[6] Nebelthau, *Münchener mediz. Wochenschr.* 1902, p. 917.

[7] Zd. H. Skraup, *Zeitschr. f. physiol. Chem.* **42**. 274 (1904) ; and *Wiener Monatshefte*, **26**. 245 (1905). See also J. Wohlgemuth, *Ber. d. deutsch. chem. Ges.* **37**. 4362 (1904).

[8] A. Windaus and F. Knoop, *Ber. d. deutsch. chem. Ges.* **38**. 1166 (1905) ; and in *Hofmeister's Beiträge*, **6**. No. 8.

Serin by Fischer and Leuchs,[1] and Erlenmeyer;[2] oxy-amino-succinic acid by Neuberg and Silbermann;[3] diamino-succinic acid by Tafel;[4] diamino-adipic acid by Traube[5] and Köhl;[6] diamino-suberic and diamino-sebacic acids and their oxy-acids by Neuberg,[7] who has also proved experimentally that the diamino-acids by splitting off CO_2 become converted into the diamines met with during digestion,[8] and the ptomains[9] formed during putrefaction.

The transformation of grape-sugar into methyl-imido-azol is remarkable for the ease with which it occurs at the ordinary temperature, and also because the imido-azol forms the link between grape-sugar on the one hand, and histidin on the other hand, for it has been pointed out on p. 43 that histidin is an imido-azol compound. The (α- or β-) methyl-imido-azol (or methyl-glyoxalin)

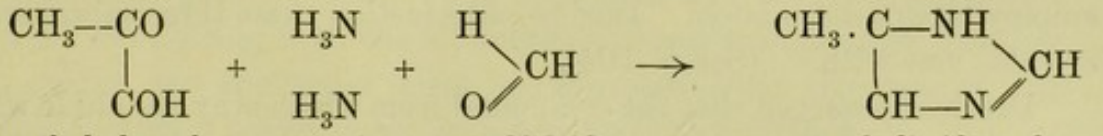


is obtained by letting a solution of zinc hydroxide in ammonia act on grape-sugar for six weeks.

The intermediate stages from grape-sugar to lactic acid are glyceric-aldehyde $\rightleftarrows$ methyl-glyoxal $\rightleftarrows$ lactic acid, and similarly methyl-glyoxal is formed as an intermediate product during the formation of methyl-imido-azol

$$\underset{\text{grape-sugar.}}{CH_2(OH)[CH(OH)]_4CHO} \longrightarrow \underset{\text{glyceric-aldehyde.}}{CH_2(OH).CH(OH).CHO} \longrightarrow$$

$$\underset{\text{methyl-glyoxal.}}{CH_3.CO.CHO} \longrightarrow \underset{\text{lactic acid.}}{CH_3.CH(OH).COOH}$$



methyl-glyoxal + ammonia + formaldehyde. methylimido-azol.

If the formaldehyde is a primary dissociation-product, and is not formed secondarily from methyl-glyoxal, we are dealing here with

[1] E. Fischer and Leuchs, *Ber. d. deutsch. chem. Ges.* **35**. 3790 (1902).
[2] E. Erlenmeyer, jun., *ibid.* **35**. 3769 (1902); and *Ann. d. Chem.* 337, 236 (1904).
[3] C. Neuberg and M. Silbermann, *Zeitschr. f. physiol. Chem.* **44**. 147 (1905).
[4] Tafel, *Ber. d. deutsch. chem. Ges.* **20**. 244 (1887); and **26**. 1890 (1893).
[5] W. Traube, *ibid.* **35**. 4121 (1902).
[6] W. Köhl, *ibid.* **36**. 172 (1903).
[7] C. Neuberg, *Zeitschr. f. physiol. Chem.* **45**. 92 (1905); and *ibid.* p. 110.
[8] A. Loewy and C. Neuberg, *ibid.* **43**. 338 (1904).
[9] A. Ellinger, *ibid.* **29**. 334 (1900); and *Ber. d. deutsch. chem. Ges.* **31**. 3183 (1899).

another example of a reversible process, for formaldehyde may be built up into hexoses, and the latter be reconverted into formaldehyde.

Many physiological data of the highest importance will also be found in the two papers by Neuberg,[1] who discusses the physiology of the pentoses and of glycuronic acid, and by Langstein,[2] who deals with the formation of carbohydrates out of albumins, and special attention is also drawn to the paper by Roux,[3] in which new amino-sugars are described.

The occurrence of alcohol in the tissues is discussed by Landsberg.[4]

The formation of fat from albumins has been fully discussed by Slosse.[5] The existence of long carbon chains in the albumin-molecule, as discovered by Skraup (see p. 45), brings us nearer the higher fatty acids; but there is a good deal of evidence against fat-formation from albumins in the case of phosphorus-poisoning, judging by the account Boruttau[6] gives.

The Sulphur-Radicals of Albumins

With the exception of the protamins, peptones, and mycoproteid (see p. 172), sulphur is contained in all albumins. The sulphur-containing dissociation-products cystin, cystein, and α-thiolactic acid have already been referred to (pp. 56 and 83). The following substances occur also: Ethyl-sulphide, found by Abel[7] in urine, and by Drechsel[8] after dissociation with acids; methyl- and ethyl-mercaptan and sulphuretted hydrogen, discovered by Sieber and Schoubenko after fusion with alkalies, and similarly by Rubner,[9] who also obtained them when employing dry distillation. During putrefaction these three substances are also met with.[5] (See p. 104).

Drechsel observed that the compound from which ethyl-sulphide was

[1] Neuberg, *Ergebnisse d. Physiol.* **3.** fasc. 1, p. 373 (1904).

[2] Leo Langstein, *ibid.* p. 453.

[3] M. Roux, *Ann. de chim. et de phys.* **8.** 72.

[4] Georg Landsberg, *Zeitschr. f. physiol. Chem.* **41.** 505 (1904).

[5] A Slosse, *Annales de la Soc. roy. des sciences médicales et naturelles de Bruxelles,* **13.** fasc. 2 (1904).

[6] H. Boruttau, *Arch. ital. de Biol.* **36.** 157 (1901); and in Fano's *Arch. d. Fisiol.* **2.** 26 (1904).

[7] J. J. Abel, *ibid.* **20.** 253 (1895).

[8] E. Drechsel, *Zentralbl. f. Physiol.* **10.** 529 (1896).

[9] N. Sieber and G. Schoubenko, *Arch. des Sciences biol. de St. Pétersbourg,* **1.** 314 (1892); M. Rubner, *Arch. f. Hygiene,* **19.** 136 (1893); E. and H. Salkowski, *Ber. d. deutsch. chem. Ges.* **12.** I. 648 (1879); E. Baumann, *Zeitschr. f. physiol. Chem.* **20** 583 (1895).

derived was precipitated by phosphotungstic acid, that it therefore was a base, and Müller and Seemann [1] and Blum and Vaubel [2] also described a sulphur-containing base of unknown constitution amongst the dissociation-products of egg-white. Amongst these substances, cystin (for full account see p. 56) must be considered to be the primary dissociation-product, as it has been found in large quantities by Mörner,[3] Embden,[4] and Patten [5] after dissociation with acids and after digestion with trypsin. Cystein is formed secondarily, according to Patten. Besides α-thiolactic acid, Friedmann [6] believes to have found its disulphide. Sulphuretted hydrogen and the mercaptanes may be derived directly from it, while there is some difficulty in deriving ethyl-sulphide. The interrelation between α-thiolactic acid and cystin is explained on p. 83. The explanations of Baumann [7] are based on the older formula, according to which cystein is α-amino-α-thiolactic acid, and are therefore antiquated. Mörner has found cystins so constantly and in such preponderating amounts, that by comparison thiolactic acid and Drechsel's base are of very subordinate importance.

Cystin splits off a part of its sulphur as sulphuretted hydrogen on being boiled with sodium hydrate, and therefore behaves exactly as do albumins (Baumann,[8] Schulz,[9] and Suter [10]). In testing for sulphur, albumins are boiled, as a rule, with sodium hydrate and lead acetate, when, owing to the formation of lead sulphide, a black precipitate, or at least a dark coloration, is produced. Schulz and Suter have shown that sulphur is split off very gradually, eight to nine hours being required to obtain the maximal splitting-off. During this process the sulphuretted hydrogen may become oxidised, for which reason Schulz made his determinations in an atmosphere of coal gas, substituting at the same time zinc for lead. The conditions are much more difficult when we are dealing with the albumins themselves instead of working with cystin, for the latter must be liberated from the rest of the albumin-molecule before it can be acted upon any further.

A great deal of importance used to be attached to the idea that albumins contained their two sulphur atoms bound up in different ways —the one in the form of sulphuretted hydrogen, the other in a non-dis-

[1] J. Seemann, Dissertation, Marburg, 1898.
[2] F. Blum and W. Vaubel, *Journ. f. prakt. Chem.* [2], **57**. 365 (1898).
[3] K. A. H. Mörner, *Zeitschr. f. physiol. Chem.* **28**. 595 (1899), **34**. 207 (1901).
[4] G. Embden, *ibid.* **32**. 94 (1900).
[5] F. A. Patten, *ibid.* **39**. 350 (1903).
[6] E. Friedmann, *Hofmeister's Beiträge*, **3**. 184 (1902), **4**. 486 (1903).
[7] E. Baumann, *Zeitschr. f. physiol. Chem.* **20**. 583 (1895).
[8] E. Baumann, *ibid.* **8**. 299 (1884).
[9] F. N. Schulz, *ibid.* **25**. 16 (1898).
[10] F. Suter, *ibid.* **20**. 564 (1895).

sociable, perhaps somewhat more highly oxidised form. But this whole conception is erroneous, apart from the existence of the A- and B-cystin which have been described on p. 56, since the symmetrically built cystin and the cystein containing only one atom of sulphur behave exactly as do albumins, and since Mörner has found cystin in sufficient amounts to account for the whole or for the greater part of the sulphur occurring in albumins. Mörner, however, still makes use of the old view, for he calculates from the amount of sulphur which is readily split off how much cystin is actually present, and thence concludes as to whether any given albumin contains only cystin or whether it is necessary to assume the existence of still another sulphur-containing dissociation-product. He arrives at the conception that the keratin of cow's horn and of hair, that serum-albumin and serum-globulin, contain only cystin, while the shell-membrane of hens' eggs, egg-albumin, and fibrinogen contain, besides cystin, yet other sulphur bodies. These calculations are, however, not very certain. That we do not yet understand all the ins and outs of this question is shown by the following considerations. According to Maly,[1] Löw,[2] and Bernert,[3] the lead sulphide reaction is not given by oxyprot-sulphonic acid, which is formed when albumins are oxidised with an alkaline solution of potassium permanganate; it contains, however, the whole of the sulphur, and will split off sulphuretted hydrogen if oxidation is prevented (Schulz[4]). An analogous behaviour is shown by the iodine-containing albumins (according to Hofmeister[5]), and by the salts which denaturalised albumins form with many heavy metals (Harnack[6]). Even when dissociating albumins slowly with dilute alkalies, some sulphur is split off quite early.[7] The authors assume a partial oxidation, but other explanations are possible.

Pick,[8] on the other hand, has shown that primary albumoses prepared from the cystin-containing fibrin give off the whole of their sulphur in the form of sulphuretted hydrogen, and that therefore the sulphur cannot be contained in these albumoses as cystin. Mörner[9] states further that the sulphur of glutin, which cannot be split off, is not even oxidised if aqua regia be used.

[1] R. Maly, *Monatshefte f. Chemie*, **6**. 107 (1885), **9**. 258 (1888).

[2] O. Löw, *Journ. f. prakt. Chem.* [2], **5**. 433 (1872), **31**. 129 (1885).

[3] R. Bernert, *Zeitschr. f. physiol. Chem.* **26**. 272 (1898).

[4] F. N. Schulz, *ibid.* **29**. 86 (1899).

[5] F. Hofmeister, *ibid.* **24**. 159 (1897).

[6] E. Harnack, *Ber. d. deutsch. chem. Ges.* **31**. II. 1938 (1898).

[7] N. Lieberkühn, *Arch. f. Anat., Physiol. u. wissenschaftl. Medizin*, 1848, pp. 285 and 323.

[8] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899).

[9] C. T. Mörner, *ibid.* **28**. 471 (1899).

	Sulphur Percentage.	Cystin Percentage.	Other Dissociation-Products.
Hair, human	4·95 to 5·34 [4]	13·92 [1]	α-Thiolactic acid [19]
Hair, animal	2·52 [3] to 4·35 [4]	...	...
Feathers, goose	2·59 to 3·16 [4]	...	α-Thiolactic acid [19]
Horn filings	3·39 [1]	6·8 [1]	α-Thiolactic acid [19]
Wool	...	...	α-Thiolactic acid [19]
Hoof	3·5 [4]	...	...
Egg-shell, hen	4·25 [7]	7·62 [1]	...
Gorgonin	2·32 [18]	...	...
Neurokeratin	2·93 [17]	...	...
Serum-albumin, horse	1·89 [2]	2·53 [1]	Ethylsulphide
Serum-albumin, human	2·31 [5]	...	...
Serum-globulin, horse	1·38 [2]	1·51 [1]	...
Egg-albumin	1·18 [2]	0·29 [1]	...
Fibrinogen	1·25 [8]	1·17 [1]	...
Myosin	1·26 [10]	...	...
Casein	0·758 [9]	Traces [1]	...
Globin	0·42 [2]	0·31 [20]	...
Edestin	0·884 [6]	0·25 [20]	...
Excelsin	1·088 [6]	...	...
Legumin	0·385 [6]	...	...
Vignin	0·426 [6]	...	...
Amandin	0·429 [6]	...	...
Gliadin	1·027 [6]	...	...
Zein	0·6 [6]	...	...
Mucin (salivary glands)	0·843 [12]	...	...
Mucin (snails)	1·71 to 1·6 [11]	...	...
Glutin	0·25 [13]	...	...
Elastin	0·55 [14]	...	...
Spongin	0·73 [15]	...	...
Amyloid	1·56 [16]	...	...

No sulphur is contained in the peptones, in the protamins, and

[1] K. A. H. Mörner, *Zeitschr. f. physiol. Chem.* **34**. 207 (1901).
[2] F. N. Schulz, *ibid.* **29**. 86 (1899).
[3] F. Suter, *ibid.* **20**. 564 (1895).
[4] P. Mohr, *ibid.* **20**. 403 (1894).
[5] K. V. Starke, *Maly's Jahresber. f. Tierchemie*, **11**. 17 (1881).
[6] T. B. Osborne, *Chem. Zentralbl.* 1902, I. p. 502.
[7] V. Lindwall, *Maly's Jahresber. f. Tierchemie*, **11**. 38 (1881).
[8] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **22**. 333 (1896).
[9] O. Hammarsten, *ibid.* **9**. 273 (1885).
[10] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **25**. 358 (1889).
[11] O. Hammarsten, *Pflüger's Arch. f. d. ges. Physiol.* **36**. 373 (1885).
[12] O. Hammarsten, *Ges. d. Wissensch. zu Upsala*, June 15, 1893.
[13] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **28**. 471 (1898).
[14] Ebbe Bergh, *ibid.* **25**. 337 (1898).
[15] E. Harnack, *ibid.* **24**. 412 (1898).
[16] A. Tschermak, *ibid.* **20**. 343 (1894).
[17] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **26**. 291 (1890).
[18] M. Henze, *Zeitschr. f. physiol. Chem.* **38**. 60 (1903).
[19] E. Friedmann, *Hofmeister's Beitr.* **3**. 184 (1902).
[20] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **37**. 499 (1903).

in the mycoproteid, which Nencki[1] prepared from anthrax bacilli. Whether fibroin and conchiolin contain sulphur is not stated.

On decomposing albumin with pepsin-hydrochloric acid, cystin is found only in certain complexes; proto- and hetero-albumose contain some sulphur giving the lead reaction,[2] but most of the sulphur is contained in one of the deutero-albumoses, which Pick[3] has therefore called thio-albumose.

[1] M. Nencki, *Ber. d. deutsch. chem. Ges.* **17**. II. 2605 (1884); Nencki and F. Schaffer, *Journ. f. prakt. Chem.* [2], **20**. 443 (1879).

[2] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899).

[3] E. P. Pick, *Hofmeister's Beitr.* **2**. 481 (1902).

CHAPTER V

ALBUMOSES AND PEPTONES

WHATEVER means we adopt for dissociating the naturally-occurring or 'native' albumins, we always find that, at first, they break up into albumoses and into peptones. These latter are still albumins, using this term in its wide sense, for they possess the same chemical structure and dissociate into the same radicals as do the albumins. For these reasons, too, they give the same chemical reactions, and, amongst the colour tests, particularly the biuret-reaction. They are further precipitated by acids and by bases. Albumoses and their salts are, however, much more soluble than are the albumins, and the further they are removed from the albumins the more difficult does it become to precipitate them and to obtain those reactions which are typical of the albumins. They also differ from albumins in those physical properties which depend on the size of molecules and on the colloidal state. For this reason albumoses and peptones have always been compared to the dextrines and to the di- and mono-saccharids, substances which are derivatives of the colloidal carbohydrates.

The transition from the 'native' albumin to the amino-acids is a very gradual one owing to the existence of a large number of intermediate substances. As only a few substances out of this large number of bodies have been isolated as distinct chemical individuals, any attempt at classification must as yet be difficult and arbitrary. Long ago all substances derived from albumin were classed together as peptones, but Kühne[1] introduced in 1885 the following nomenclature :—

Albumoses.—These are defined as dissociation-products of albumins, which cannot be coagulated by heat, but which can be salted out by certain salts, as, for example, by ammonium- or zinc-sulphate in acid solutions.

[1] W. Kühne, *Verh. des naturhistor.-medizin. Vereins zu Heidelberg*, N.F. III. 286 (1885); Pollitzer, *ibid.* III. 293 (1885); S. Wenz, *Zeitschr. f. Biolog.* **22**. 1 (1886); W. Kühne and R. H. Chittenden, *ibid.* **20**. 11 (1884).

Albumoses are again subdivided into primary and secondary albumoses. The primary may greatly resemble the albumins; they are precipitated, and thus separated from the secondary albumoses, by either completely saturating their solutions with sodium chloride, or half saturating them with ammonium sulphate. The primary albumoses are represented by the proto- and the hetero-albumose. Kühne also describes a dys-albumose, but it is now generally believed that the latter represents hetero-albumose which has become insoluble. The secondary or deutero-albumoses are in many instances only divided from the peptones by arbitrary definitions.

Beyond the stage of peptones we come to the peptids or substances built up of two or more amino-acids on exactly the same principles as are albumins, and which therefore, from the chemical standpoint, are doubtless albumins. A number of di- and poly-peptids have been prepared synthetically by Curtius and Fischer and their pupils, as stated in Chapter III. It is difficult to separate peptids from peptones; the most ready, but again arbitrary, method would be to make use of the biuret-reaction; thus peptones do, while peptids do not, give this reaction. The peptids are called by Hofmeister[1] peptoids.

Albumoses, when in the dry state, are white, dust-like, non-crystalline powders. With the exception of the hetero-albumoses, they are readily soluble in water; still more soluble are many of their salts. They are all precipitable by alcohol, the different albumoses being precipitated by different concentrations of alcohol. Albumoses give a red biuret-reaction with a tinge of violet. All albumoses give the xanthoproteic test, while the other colour tests are either negative or positive according to the radicals contained in the individual albumoses.

Albumoses show, according to Kühne,[2] Neumeister,[3] and Hofmeister's school,[4] the following precipitation tests:—Albumoses are precipitated by ferric chloride, neutral and basic lead acetate, mercuric chloride, platinum chloride, and other metallic salts, but the precipitates are more or less soluble in an excess of the precipitating agent. Copper sulphate, and the even more sensitive copper acetate, precipitate only the primary but not the deutero-albumoses, and are therefore used to separate the primary from the secondary albumoses.

[1] F. Hofmeister, *Ergebnisse der Physiologie v. Asher-Spiro*, I. **1**. 759 (1902).

[2] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **20**. 11 (1884).

[3] R. Neumeister, 'Über die Reaktionen der Albumosen und Peptone,' *ibid.* **26**. 324 (1890).

[4] E. P. Pick, *Zeitschr. f. physiol. Chem.* **24**. 246 (1897); E. Zunz, *ibid.* **27**. 219 (1899).

Ferrocyanic acid, used generally in the form of acetic acid + potassium ferrocyanide, precipitates all albumoses, but the presence of peptone may interfere with the reaction. On heating, the precipitate disappears, while it reappears on cooling. Nitric acid precipitates the primary albumoses even in the absence of salts, the deutero-albumoses only in the presence of sodium chloride, and the lowest members of the deutero-albumoses only if the solution be saturated with salt. The precipitate is soluble in an excess of nitric acid, especially on heating, but it returns on cooling. This last reaction Kühne held to be characteristic of albumoses, but it is also given by histones (see under Histone).

Albumoses are completely precipitated by the following alkaloidal reagents:—Phosphotungstic-, phosphomolybdic-, picric-, tannic-, trichlor-acetic-, and metaphosphoric acids. The tannic acid precipitate of prot-albumose is, however, soluble in an excess of the acid. Some of the precipitates dissolve on heating and re-form on cooling, while others are permanent when heated. Albumoses are also precipitated by bin-iodide of mercury dissolved in potassium iodide, bismuth iodide dissolved in potassium iodide and potassium iodide + hydrochloric acid, but the precipitates of the deutero-albumoses are partly soluble in an excess of hydrochloric acid. Bang[1] has further found amongst the albumoses certain substances which must be preponderatingly basic in their nature, because they are precipitated by alkaloidal reagents even if the reaction be neutral, and also by alkalies. As 'acro-albumose,' Kühne[2] and Folin[3] have described an acid albumose which is precipitable by acetic acid; it occurs occasionally in Witte's peptone, and belongs, if judged by its solubility, to the primary albumoses. Albumoses are lævorotatory, but so far no determinations have been made with chemically pure preparations.

The primary albumoses, but not the deutero-albumoses, give, according to Kossel,[4] precipitates with protamins and histones as do the natural albumins. Kutscher,[5] reversely, obtained precipitates when sodium salts of the acid albumins—globulin, myosin, syntonin, etc.—were allowed to drop into deutero-albumose solution. He explains this phenomenon by assuming the formation of the slightly soluble globulinates of deutero-albumose, but it is possible that the deutero-albumose withdraws the sodium from the normally very

[1] J. Bang, *Zeitschr. f. physiol. Chem.* **27**. 463 (1899).

[2] W. Kühne, *Zeitschr. f. Biol.* **30**. 221 (1894).

[3] O. Folin, *Zeitschr. f. physiol. Chem.* **25**. 152 (1898).

[4] A. Kossel, *Deutsche med. Wochenschr.* 1894, p. 146; *Zeitschr. f. physiol. Chem.* **22**. 176 (1896).

[5] F. Kutscher, *ibid.* **23**. 115 (1897).

slightly soluble globulins, and thereby causes the latter to become precipitated. Acetyl-derivatives of the albumoses have been prepared by Schrötter.[1]

Peptones.—All substances which cannot be salted out are called peptones, and these are further characterised by giving the precipitation-tests only to a limited extent. Of the various colour-tests, they constantly give the biuret-reaction, but only occasionally the other colour-tests.

Peptones are colourless, dry powders, according to Siegfried[2] and his pupils.[3] They are very soluble in water, also in glacial acetic acid, and in all salt solutions; partly soluble in 96 per cent alcohol; insoluble in all the other solvents in general use. All give an intense, pure red biuret-reaction even when greatly diluted, and also the xantho-proteic reaction; while the other colour-tests may or may not be given, according to the nature of the peptone under examination. Peptones contain no sulphur. The heavy metals produce no precipitation; amongst the alkaloidal reagents, phosphostungstic and picric acid when used in concentrated solutions give a precipitate which disappears on heating and reappears on cooling.

Tannic acid causes in concentrated solutions a precipitate, which is soluble in acetic acid. Bin-iodide of mercury, bin-iodide of bismuth, and iodine in potassium iodide solutions, and, further, trichloracetic acid,[4] do not precipitate in watery solutions, but do so in concentrated calcium chloride,[5] calcium nitrate,[5] and ammonium sulphate[6] solutions, according to Kühne,[4] Pick,[5] and Cohnheim and Krieger.[6] Mercuric chloride produces a slight turbidity. Ferrocyanic acid and metaphosphoric acid do not precipitate, nor does nitric acid even if the solution be saturated with salt. Peptones are lævo-rotatory; the rotation has been measured by Siegfried. All albumoses and peptones hitherto examined are dissociated by erepsin, according to Cohnheim.[7]

Albumoses and peptones form with acids and with bases salts, the hydrolytic dissociation of which is the same as that of the salts of albumins and the more complex proteids. The chlorides of albumoses have been frequently examined, as they occur during

[1] H. Schrötter, *Monatsh. f. Chem.* **14**. 612 (1893).

[2] M. Siegfried, *Zeitschr. f. physiol. Chem.* **35**. 164 (1902).

[3] F. Müller, *ibid.* **38**. 265 (1903); C. Borkel, *ibid.* **38**. 289 (1903); T. Krüger, *ibid.* **38**. 320 (1903); P. Mühle, *Amphopepton*, Dissertation, Leipzig, 1901.

[4] W. Kühne, *Zeitschr. f. Biol.* **29**. 320 (1892).

[5] E. P. Pick, *Zeitschr. f. physiol. Chem.* **24**. 246 (1897).

[6] O. Cohnheim and H. Krieger, *ibid.* **40**. 95 (1900).

[7] O. Cohnheim, *ibid.* **35**. 134 (1902).

gastric digestion; the chief investigators are Paal,[1] Sjöqvist,[2] Cohnheim,[3] Bugarszky and Liebermann,[4] Cohnheim and Krieger,[5] and v. Rhorer.[6] Erb,[7] however, is the only observer who has worked with a 'pure' albumose,[8] namely, hetero-albumose. The pure peptones prepared by Siegfried[9] are distinctly acid; while the kyrins of Siegfried[10] are well-marked bases. Albumoses are still pluri-acid and pluri-basic; hetero-albumose is according to Erb[7] at least 23-acid; Siegfried's[9] peptones are monobasic if calculations are based on the simple formula, but the results of dissociation by means of acids show that the formula of these peptones must be multiplied.

Albumoses have a smaller molecular weight than have the true albumins, but it is still very high. If we make, for example, deductions from the numbers obtained by analysis, primary albumoses must possess at least a molecular weight of 2600, which weight must probably be multiplied by 2, because of the cystin which contains two atoms of sulphur. Because of their smaller size albumoses pass through parchment, and they thus differ from the albumins, but their passage is a very slow one. The different albumoses diffuse with different velocities.[11]

Peptones possess a much lower molecular weight; Siegfried[12] calculated originally the molecular weight for anti-peptone as 273, but now[13] he believes this number to be too low; pepsin-peptone diffuses, according to Kühne,[11] only one half as quickly as does grape-sugar.

The question as to whether different albumins give rise to different albumoses and peptones, or whether the same albumoses and peptones, according to their arrangement, form different albumins, is still an open one. In all probability the albumoses and peptones differ as little from one another as do the amino-acids, for, with the exception of casein,[14] and perhaps also of globin,[15] which do not contain

[1] C. Paal, *Ber. d. deutsch. chem. Ges.* **25**. I. 1202 (1892); *ibid.* **27**. II. 1827 (1894).
[2] J. Sjöqvist, *Skandinav. Arch. f. Physiol.* **5**. 277 (1894).
[3] O. Cohnheim, *Zeitschr. f. Biolog.* **33**. 489 (1896).
[4] St. Bugarszky and L. Liebermann, *Pflüger's Arch. f. d. ges. Physiol.* **72**. 51 (1898).
[5] O. Cohnheim and H. Krieger, *Zeitschr. f. Biol.* **40**. 95 (1900).
[6] L. v. Rhorer, *Pflüger's Arch. f. d. ges. Physiol.* **90**. 368 (1902).
[7] W. Erb, *Zeitschr. f. Biol.* **41**. 309 (1901).
[8] See p. 184, where the results obtained by Haslam are discussed.
[9] M. Siegfried, *Zeitschr. f. physiol. Chem.* **27**. 335 (1899), **35**. 164 (1902), **38**. 259 (1903).
[10] M. Siegfried, *Sitzungsber. d. sächs. Ges. d. Wissenschaften zu Leipzig, math.-phys. Kl.*, 1903, p. 63, **43**. 44 and 46 (1904).
[11] W. Kühne, *Zeitschr. f. Biol.* **29**. 1 (1892).
[12] M. Siegfried, *Zeitschr. f. physiol. Chem.* **35**. 164 (1902).
[13] C. Borkel, *ibid.* **38**. 294 (1903).
[14] F. Alexander, *ibid.* **25**. 411 (1898). [15] F. N. Schulz, *ibid.* **24**. 449 (1898).

the hetero-albumose radical, all other albumins on being digested yield the same albumoses although in very different amounts.

For the examination of albumoses it is customary to use the 'peptonum siccum' of F. Witte in Rostock, which consists for the greater part of albumoses. It is said to be manufactured by digesting fibrin with artificial gastric juice, but nothing definite is known about it.[1] The first publications of Kühne[2] as well as those of Pick[3] and Haslam deal with Witte's peptone, while Siegfried prepared his own peptone directly from fibrin. According as to whether an albumose is prepared from globulin, vitellin, myosin, gelatine, fibrin, etc., it is called a globulose, vitellose, myosinose, gelatose, fibrinose, etc.; albumoses, according to this nomenclature, would be only the dissociation-products of egg- and of serum-albumin, but the term albumose is still generally used to include the products of the other albumins.

Chittenden has called the albumoses derived from serum- and egg-albumin by the name of proteoses.

I. THE ALBUMOSES AND PEPTONES OBTAINED BY PEPTIC DIGESTION

Albumoses

Albumoses formed by processes other than those of peptic and tryptic digestion have but rarely been examined. Tryptic digestion rapidly converts albumoses and peptones into simpler compounds, and therefore peptic digestion is more suitable for the study of the dissociation-products of albumins. Meissner[4] and Brücke[5] were the first to investigate gastric digestion; they were followed by Kühne[6] and his pupils,[7] in special by Neumeister.[8] Kühne's researches

[1] M. Siegfried, *Zeitschr. f. physiol. Chem.* **35**. 179 (1902).

[2] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **20**. 11 (1884).

[3] E. P. Pick, *Zeitschr. f. physiol. Chem.* **24**. 246 (1897), **28**. 219 (1899); *Hofmeister's Beiträge*, **2**. 481 (1902).

[4] G. Meissner, *Zeitschr. f. ration. Medizin*, **7**. 1 (1859), **8**. 280 (1860), **10**. 1. (1861), **12**. 46 (1861), **14**. 78 (1862), **14**. 303 (1862).

[5] E. Brücke, *Sitzungsber. d. Wiener Akad., math.-naturw. Kl.* **37**. 131 (1859).

[6] W. Kühne, *Verh. d. Heidelberger naturh.-med. Vereins* (N.F.) **3**. 286 (1885); W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **19**. 159 (1883); W. Kühne and R. H. Chittenden, *ibid.* **20**. 11 (1884), **22**. 423 (1885); W. Kühne, *ibid.* **29**. 1 (1892), **29**. 308 (1892).

[7] S. Wenz, *ibid.* **22**. 1 (1886); R. H. Chittenden and R. Goodwin, *Journ. of Physiol.* **12**. 34 (1891).

[8] R. Neumeister, *Zeitschr. f. Biol.* **23**. 381 (1887), **24**. 267 (1888), **26**. 324 (1890); *Lehrbuch der physiol. Chem.* 2nd edition, p. 228 ff. (1897).

dominated for a long time the chemistry of albumins, and they have laid the foundation-stone of our knowledge of albumoses, for he showed that pepsin breaks up albumins step by step. Acid albumins are formed in the first instance, and then the primary albumoses (proto- and hetero-albumoses), which may be salted out by saturation with sodium chloride. The hetero-albumose he separated from the proto-albumose by dialysis, whereby the hetero-albumose becomes insoluble; from the primary albumoses are derived the deutero-albumoses, which are only precipitated by saturated ammonium-sulphate solutions, and from these again, finally, the peptones or products which remain in solution.

An extraordinary advance on the teaching of Kühne was made by Hofmeister's school.[1] Hofmeister did not use different salts for the isolation of individual albumoses and peptones, as Kühne did, but introduced the principle of fractional precipitation by means of different concentrations of ammonium- and zinc-sulphate and different strengths of alcohol; the peptones were precipitated by means of iodine-potassium-iodide in saturated ammonium-sulphate solutions. Hofmeister also introduced the idea of working out in a systematic way, which of the dissociation-products contained, and which were devoid of, those radicals which give specific reactions. Hofmeister's school established the fact that different dissociation-products differ from one another, not only in respect of precipitability, but also in respect to other reactions, and therefore that marked differences do really exist between the different dissociation-products. The results obtained by Pick with Witte's peptone have been put together by Hofmeister[2] in the accompanying table, which has been somewhat extended by also including the peptones.

Pick separates the primary albumoses, namely, the proto- and the hetero-albumose, by means of adding to their solutions an equal bulk of a saturated ammonium sulphate solution, and then separates the proto-albumose from the hetero-albumose by the addition to their solution of an equal amount of alcohol. Folin[3] separates the primary from the secondary albumoses by means of copper acetate, and Schrötter[4] employs acetylation and benzoylation.

[1] E. P. Pick, *Zeitschr. f. physiol. Chem.* **24**. 246 (1897); F. Umber, *ibid.* **25**. 258 (1898); E. Zunz, *ibid.* **27**. 219 (1899); E. Zunz, *ibid.* **28**. 132 (1899); Fr. Alexander, *ibid.* **25**. 411 (1898); E. P. Pick, *ibid.* **28**. 219 (1899); E. P. Pick, *Hofmeister's Beiträge*, **2**. 481 (1902); E. Zunz, *ibid.* **2**. 435 (1902).

[2] F. Hofmeister, *Asher-Spiro, Ergebnisse der Physiol.* I. **1**. 759 (1902), table, p. 781.

[3] Folin, *Zeitschr. f. physiol. Chem.* **25**. 152 (1898).

[4] Schrötter, *Monatshefte f. Chem.* **14**. **16**. **17**. **19**. (1893–1896).

Hofmeister's Table of Albumoses obtained by Peptic Digestion of Witte's Peptone

Fractions.	Precipitation limits for Ammonium Sulphate expressed in percentage Saturations.	Albumoses.	Solubility in dilute Alcohols.	Percentage Composition.					Biuret Reaction.	Millon's Reaction.	Xantho-proteic Reaction.	Indol (Skatol) Formation when fused with Alkalies.	Furfurol (Carbohydrate) Reaction.	Lead Sulphide Reaction.
				C	H	N	S	O						
Hetero-prot-albumose	24 to 42	Hetero-albumose	insoluble in 32 %	55·12	6·61	17·98	1·22	19·07	positive	very feeble	positive	very feeble	absent	positive
		Protalbumose	soluble in 80 %	55·64	6·80	17·66	1·21	18·69	,,	strong	,,	very strong	,,	,,
Deutero-albumoses A	54 to 62	Thio-albumose	insoluble in 60-70 %	48·96	6·90	16·02	2·97	25·15	positive	positive	positive	positive	absent	very strong positive
		A-albumose poor in S	soluble in 70 %	53·11	7·16	17·86	0·8	21·07	,,	,,	,,	,,	,,	
Deutero-albumoses B	70 to 95	B I. albumose	insoluble in 35 %	...	...	16·94	...	...	positive	positive	positive	...	absent	positive
		Gluco-albumose = (B II. albumose)	insoluble in 60-70 %	48·72	7·03	13·76	30·49		,,	,,	,,	feeble	very strong	,,
		B III. α-albumose	soluble in 80 %	43·98	6·91	14·25	1·63	33·23	,,	,,	,,	strong	absent	absent
		B III. β-albumose	soluble in 80 %	52·32	7·32	15·36	1·21	23·79	,,	,,	,,	,,	,,	,,
		Pepto-melanin	soluble in 80-90 %	60·70	6·68	11·46	21·16		absent	absent	?	,,	,,	,,
Deutero-albumoses C	100% + Acid	C-albumose	soluble in 67-80 %	34·52	5·85	17·24	42·89		positive	not a trace	very strong	not a trace	absent	absent
		(Fibrin)		52·68	6·83	16·91	1·10	22·48						
Peptones	not precipitable	Peptone A	insoluble in 96 %	...	...	...	...	...	positive	not a trace	positive	absent	strong	absent
	not precipitable	Peptone B	soluble in 96 %	...	...	...	...	...	positive	positive	positive	...	absent	absent

Amongst albumoses, only the proto- and the hetero-albumoses, and perhaps also the gluco- and the thio-albumoses, offer some guarantee of purity, while the other albumoses are mixtures. But even the former may not be pure substances, because Pick points out that albumoses, apart from slight differences in their solubilities, have a tendency to unite with one another, forming salt-like compounds (see also the results obtained by Haslam on p. 184), a view which has also been adopted by Haslam.[1] Being amino-acids, albumoses possess both acid and basic characters; and therefore if several albumoses are present they will keep one another mutually in solution, instead of separating out, as they would do if only one amino-acid were present.

A glance at the table on p. 180 will show that the albumoses which have been isolated, so far, show very marked differences both as regards their percentage composition and their disintegration-compounds. We know further the following facts:

Prot-albumose contains, according to Pick,[2] both tyrosin and tryptophane and some leucin, but no glycocoll, while the hetero-albumose contains phenylalanin and glycocoll, much leucin, but no tryptophane or tyrosin. Hart[3] finds that dissociation with acids yields the following percentage weights :—

	Ammonia.	Lysin.	Histidin.	Arginin.
Hetero-albumose .	0·97	7·03	0·37	8·52
Prot-albumose .	0·76	3·08	3·35	4·55

Trypsin[3] and erepsin[4] rapidly produce such a change in prot-albumose that it no longer gives the biuret-reaction, while hetero-albumose is not acted upon by trypsin at all, and a slight biuret-reaction is still obtainable, even after four weeks' digestion with erepsin. The ease with which these albumoses dissociate is determined by their chemical constitution; if we adopt the nomenclature discussed on p. 174 we would class the hetero-albumose and perhaps also the deutero-albumose C under the heading of the anti-group, while the hemi-group would be represented by prot-albumose. A special group is formed by gluco-albumose and peptone A, which are both rich in carbohydrate.

As such great differences exist between the different albumoses, all the older attempts at determining the percentage composition of

[1] H. C. Haslam, *Journ. of Physiol.* **32**. 267 (1905).
[2] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899).
[3] E. Hart, *ibid.* **33**. 247 (1901). [4] O. Cohnheim, *ibid.* **35**. 134 (1902).

mixtures of deutero-albumoses or albumoses in general are no longer of any value. The numerous analyses of Kühne and Chittenden,[1] Kossel,[2] Herth,[3] Thierfelder,[4] and many others, have always yielded figures approaching more or less those of albumins proper; but no uniformity was to be expected in the figures of different authors, because in such mixtures the individual albumoses varied greatly in amount. The investigations of Haslam,[5] Goto,[6] and Levene[7] have also only shown that in mixtures of albumoses the dissociation-products are present in about the same concentration as in albumins.

The sequence in which the individual albumoses and peptones are liberated and how they are connected together was first investigated by Neumeister[8] and recently by Zunz.[9] According to the latter dissociation first results in the liberation of three co-ordinated primary products: the prot-albumose, the hetero-albumose, and the gluco-albumose. The peptones and the deutero-albumose C are present even after a long time, *i.e.* presumably at the end of the peptic digestion.

That the primary albumoses are converted by further peptic digestion into deutero-albumoses and peptones, Neumeister and Pick have proved, but whether the deutero-albumoses are intermediate products and whether they also give rise to peptone, and how many such intermediate substances exist, are questions not yet answered.

Peptone A seems to be derived from gluco-albumose, the deutero-albumose C from hetero-albumose, and peptone B from protalbumose. No other connection between albumoses is known. During the very first stages of digestion Zunz already observed abiuretic bodies (see p. 17), which were apparently peptids. It is possible that these substances along with one or the other of the peptones are liberated directly from the albumin-molecule, while as yet the larger cohering complexes form the primary albumoses. Another possibility is that the albumin as a whole passes through the same changes, namely, primary albumoses, deutero-albumoses, peptones and peptids, but with different rates of rapidity.

The following substances have been investigated up till now by Pick's method: Crystalline serum- and egg-albumin and serum-globulin

[1] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **20**. 11 (1884), **22**. 423 (1885).

[2] A. Kossel, *Pflüger's Arch. f. d. ges. Physiol.* **13**. 309 (1876); *Zeitschr. f. physiol. Chem.* **3**. 58 (1879).

[3] R. Herth, *ibid.* **1**. 277 (1877).

[4] H. Thierfelder, *ibid.* **10**. 577 (1886).

[5] H. C. Haslam, *ibid.* **32**. 54 (1900).

[6] M. Goto, *ibid.* **37**. 94 (1902).

[7] P. A. Levene, *ibid.* **37**. 81 (1902).

[8] R. Neumeister, *Zeitschr. f. Biol.* **24**. 267 (1888).

[9] E. Zunz, *Zeitschr. f. physiol. Chem.* **28**. 132 (1899); *Hofmeister's Beiträge*, **2**. 435 (1902).

by Umber [1] and Zunz ;[2] casein by Alexander [3] and Zunz ;[2] Bence-Jones' body by Magnus-Levy ;[4] bile-mucin by Brauer [5] and the nucleo-proteids of the pancreas, in part at least, by Umber.[6]

The power of withstanding the action of pepsin is possessed, according to Umber,[6] to a different degree by different albumins : serum-albumin is dissociated more readily than is egg-albumin ; the most difficult substance to digest is serum-globulin, according to Umber [6] and E. Fischer and Abderhalden,[7] but this may be due to an admixture of anti-ferment.[8] Smith [9] and Strohmer [10] state that the digestibility of albumins is considerably diminished by heating the dried albumin, and Roterski [11] says that the same effect is produced by boiling watery solutions.

Some other properties of albumoses, not recorded in the table on p. 180, are the following :—

The protalbumose is very soluble in water, according to Pick,[12] and even more soluble in dilute alcohol. Precipitation commences with 80 per cent alcohol, and is approximately complete only if alcohol-ether is used. They diffuse very quickly,[13] and are precipitated only with difficulty. Nitric acid, the alkaloidal reagents, especially tannic acid, all give precipitates, but these are soluble in an excess of the reagent, a phenomenon generally only met with amongst the peptones.

The hetero-albumose is very slightly soluble in water, more so in salt-solutions, and very soluble in dilute acids and alkalies. It is precipitated from salt solutions by dilution with water. It has a tendency to pass into the insoluble state, *i.e.* to coagulate (see p. 191, under Plasteins). It contains, according to Hart,[14] Pick,[15] and Friedmann,[16] many hexone-bases. Its other dissociation-products have already been enumerated on p. 74. Erb [17] has examined the chloride

[1] F. Umber, *Zeitschr. f. physiol. Chem.* **25**. 258 (1898).
[2] E. Zunz, *ibid.* **27**. 219 (1899).
[3] F. Alexander, *ibid.* **25**. 411 (1898).
[4] A. Magnus-Levy, *ibid.* **30**. 200 (1900).
[5] L. Brauer, *ibid.* **40**. (1903).
[6] F. Umber, *Zeitschr. f. klin. Med.* **43**. fasc. 3 and 4 (1901).
[7] E. Fischer and E. Abderhalden, *Zeitschr. f. physiol. Chem.* **39**. 81 (1903).
[8] K. Glässner, *Hofmeister's Beiträge*, **4**. 79. (1903).
[9] H. Smith, *Zeitschr. f. Biol.* **19**. 469 (1883).
[10] F. Strohmer, *Chem. Zentralbl.* 1902, II. 971.
[11] T. Rotarski, *Zeitschr. f. physiol. Chem.* **38**. 552 (1903).
[12] E. P. Pick, *ibid.* **28**. 219 (1899).
[13] R. Neumeister, *Lehrb. d. physiol. Chem.* 2nd edition, Jena, 1897, p. 231.
[14] E. Hart, *Zeitschr. f. physiol. Chem.* **33**. 347 (1901).
[15] E. P. Pick, *ibid.* **28**. 219 (1899).
[16] E. Friedmann, *ibid.* **29**. 50 (1899).
[17] W. Erb, *Zeitschr. f. Biol.* **41**. 309 (1901).

of hetero-albumose. The maximal capacity for binding hydrochloric acid, which was observed, amounted to 314 mgrms. for 1 grm., therefore its equivalent value is only 116. Looked upon as a base, it is at least 23-acid, but probably much more so. Its hydrolytic dissociation is great.

How very real the difficulty of preparing individual albumoses is, becomes evident on reading the recent paper of Haslam.[1] He points out the absolute necessity of ascertaining that each fraction is not only free from the succeeding, but also from the preceding fractions.

To show that a proteid-precipitate is freed from the substances of the filtrate, Haslam proceeds as follows: The precipitate is dissolved in water, and the whole made to a given volume; the requisite amount of salt or of alcohol to produce precipitation is added; the mixture is allowed to stand for twenty-four hours, and is then filtered. If no proteid, or other substance which is being got rid of, is found in the filtrate, then the precipitate is pure. If not, the amount of organic nitrogen in the filtrate is estimated by Kjeldahl's method. The precipitate is then redissolved, again made to the same volume, and treated in exactly the same way as at first. The second filtrate is now again examined for organic nitrogen. If the amount of nitrogen in the second filtrate is the same as that in the first, it is due to small amounts of the precipitate being soluble in that particular medium, and the precipitate is therefore free from all extraneous nitrogenous matter. If, however, the amount of nitrogen in the first filtrate is greater than that in the second, there is still nitrogenous impurity to be got rid of, and further precipitations are needed.

If, however, the substance to be purified is in the filtrate, and the proteids that are being got rid of are in the precipitate—if, for example, serum albumin is to be freed from globulin—the following procedure is necessary. As it is impossible to completely remove all the globulin from a solution containing also albumins and peptones by half saturating the solution with ammonium sulphate, as some of the globulin is kept in solution by the albumin, recourse must be had to 'fractional precipitation.' If to serum an equal volume of saturated ammonium sulphate is added, most of the globulin is precipitated, but some accompanies the albumin and is present in the clear filtrate; for if to the latter some saturated ammonium sulphate be added until a small precipitate appears, if this precipitate be filtered off and be re-dissolved in water, it is possible by the addition of an equal volume of saturated salt-solution to obtain a precipitate of globulin, demonstrating the presence of globulin in the original filtrate. But even if no second

[1] H. C. Haslam, *Journ. of Physiol.* **32**. 267 (1905).

precipitate of globulin had been obtained on adding an equal volume of a saturated solution, this would not necessarily prove that no globulin was present in the fraction; for on adding a further quantity of the saturated salt-solution beyond the half-saturation-point, a small precipitate appears, which is a sub-fraction. This sub-fraction-precipitate when dissolved in water may, on the addition of an equal bulk of saturated salt-solution, again show a precipitate consisting of globulin. This method of taking sub-fractions, if pushed far enough, is an extremely delicate test, for the more easily precipitable bodies must tend to come down before the more soluble ones.

The following table shows how, after repeated precipitation, the amount of nitrogen in the final filtrates becomes constant. For the experiment Witte's peptone was used, from which the greater part of the primary albumoses had been separated. The albumose-precipitate was by repeated precipitation completely freed from peptones.

Nitrogen in the original mixture = 177·13.

Filtrate.	Vol. in c.c.	N in filtrate in mg. N.	Calculated impurity in filtrate.	Calculated impurity retained in precipitate.	Calculated percentage impurity retained in precipitate.
1	14	4·55	3·29 mg. N.	5·92 mg. N.	64
2	11·5	3·15	1·89	4·03	68
3	12·5	3·08	1·82	2·21	54
4	11·5	2·17	0·91	1·30	59
5	12	2·0	0·74	0·56	43
6	12	1·54	0·28	0·28	50
7	13	1·54	0·28		
8	12	1·84	No. 8 is an obviously faulty observation.		
9	12	1·26	N in filtrate constant.		
10	13	1·33			
11	12	1·26			
12	12	1·26			

Amount of albumose found at end of experiment, 153·93 mg. N.

The results obtained in this table were obtained by using ammonium-sulphate as the precipitant, but sodium-sulphate at 37° has the same salting-out capacity as ammonium-sulphate, as shown by Pinkus;[1] and Haslam uses this salt in preference, as it renders Kjeldahl's determinations easier.[2]

The next table shows the results obtained when separating the mixed albumoses from a mixture obtained by the peptic digestion of commercial casein.

[1] Pinkus, *Journ. of Physiol.* **27**. 57 (1901).

[2] Haslam gives in his paper special directions for the estimation of organic nitrogen in the presence of ammonium-sulphate.

Amount of nitrogen in the original mixture = 238·96 mg.

Precipitations.	Vol. of filtrate in cc.	Vol. of wash-water in cc.	N in filtrates in mg.	N in wash-water in mg.	Calculated impurity in filtrate in mg. N.	Calculated impurity retained in precipitate in mg. N.	Calculated percentage impurity retained in precipitate.
1st	46	...	42·25	...	39·45	35·04	48
2nd	45	14	19·77	3·57	17·03	18·01	51
3rd	38	11	9·25	1·61	6·94	6·04	47
4th	40	9	6·93	...	...	...	...
5th	39	15	5·88	...	...	...	...
6th	40	...	3·29	...	...	...	...
...	...	41	...	2·8	...	...	...
...	...	24	...	1·05	...	...	...
7th	40	...	2·45	} N-value constant.			
8th	40	...	2·59				
9th	40	...	2·45				
Totals	368	114	103·89				

482 cc. total in filtrates and washings.

Albumose found at end of experiment 178·46 mg. N.
Total N in filtrates and washings 103·89 mg. N.

Total N found . . . 282·35
Loss of substance in experiment 6·61 mg. N = 2·3 per cent.

To more rapidly remove the peptones and other impurities from the albumose precipitates, the latter should be rubbed for twenty minutes each time with successive portions of saturated ammonium-sulphate solution, at a temperature from 50°-55°, as at this temperature the albumose is softer.[1] In the second table given above the albumoses were rubbed fourteen times and precipitated six times before the nitrogen in the filtrates became constant. Haslam also points out that the greater the dilution of a mixture of albuminous substances the smaller is the 'carrying-down' power of the insoluble fraction; and the author has found similarly that the slower the salt-solution is added—in other words, the longer the time which is taken in causing a precipitate, the less foreign bodies will be enclosed in any given fraction.[2] The greater the difference in the constitution of two albuminous substances the easier is it to separate them; it is therefore 'easy' to separate albumins from albumoses, but difficult to separate the various albumoses from one another. In every case the less soluble albumins are kept in solution by the more soluble ones owing to chemical interaction.

So far Haslam has isolated from his primary albumoses, which are

[1] It was shown experimentally that albumoses may be heated to 60° with saturated Na_2SO_4 without undergoing decomposition.

[2] Slow addition of the salting-out medium may postpone the complete separation of a fraction for days.—The AUTHOR.

free from all admixture, three albumoses, namely—hetero-albumose, α-proto- and β-proto-albumose. He found that pure primary albumoses are precipitated by saturating the solutions with sodium-chloride (Kühne), or half-saturating them with ammonium-sulphate (Hofmeister), but the second method is more effective. Pick's method of preparing hetero-albumose by three precipitations with ammonium-sulphate solutions and four precipitations with alcohol did not yield Haslam a pure fraction, free from other fractions. On adding an equal volume of alcohol to his primary albumose Haslam obtained two fractions :—

1. One insoluble in cold water, and nearly insoluble in water at 60°, which he for this reason calls hetero-albumose, after Kühne. It amounts to rather less than fifty per cent of the fraction insoluble in equal parts of alcohol and water.

2. Another fraction, fairly easily soluble in water, yielding solutions of a syrupy consistence, and being nearly completely precipitated from its solutions by rather less than an equal volume of alcohol, or by an equal volume of saturated ammonium-sulphate solution. This fraction is called α-proto-albumose.

The β-proto-albumose is characterised by being soluble in equal parts of alcohol and water, at the ordinary temperature, but precipitable by the addition of an equal volume of saturated ammonium-sulphate solution. Haslam's β-proto-albumose corresponds principally with Pick's proto-albumose. The primary albumoses, as represented by the hetero, the α- and the β-albumose, all contain tryptophane, for they give the glyoxylic reaction, but only the β-proto-albumose gives a well-marked red colour with Millon's reagent, while the hetero- and α-proto-albumose give only a deep yellow colour, showing that the tyrosin group is present to a much greater extent in the β-proto-albumose.

Peptic-Peptones

The peptones resulting from peptic digestion Kühne[1] called ampho-peptones, because he believed them to be composed of the two tryptic-peptones : the anti- and the hemi-peptone. This, however, is not the case, and therefore the term must no longer be used. Siegfried[2] and his pupils Mühle, Borkel, and Scheermesser were the first to prepare pure peptic-peptones by precipitating them from a saturated ammonium sulphate solution with iron-ammonia alum.

[1] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **22**. 423 (1885).

[2] M. Siegfried, *Ber. d. deutsch. chem. Ges.* **33**. III. 3564 (1900) ; *Zeitschr. f. physiol. Chem.* **38**. 259 (1903) ; P. Mühle, Dissertation, Leipzig, 1901 ; C. Borkel, *Zeitschr. f. physiol. Chem.* **38**. 289. (1903) (here is given a detailed description) ; W. Scheermesser, *ibid.* **37**. 363 (1903).

From fibrin two peptones may be prepared which differ from one another in 1 molecule of H_2O, and which may be converted into one another. From 11 kgrms. wet fibrin Borkėl obtained, after repeated purification, accompanied by great loss, 203 grms. peptone. The analyses gave these figures:—

Pepsin-peptone α	$C_{21}H_{34}N_6O_9$.
Pepsin-peptone β	$C_{21}H_{36}N_6O_{10}$.

Peptones are "pronounced acids, which redden litmus paper, and which form salts with carbonates after having driven out the carbonic acid." Siegfried is of the opinion that Kühne's peptones were ammonium salts. Borkel has analysed the zinc-salts of both peptones, and Mühle the silver and barium salts. Adopting the simplest formula, peptones are monobasic acids, but such a simple formula has to be multiplied. Peptones are lævo-rotatory. Borkel found for α-pepsin-peptone

$$\alpha_D^{20} = -36{\cdot}36.$$

The ammonium salt is more strongly lævo-rotatory. It reacts towards precipitating reagents as do other peptones. It gives the reactions of Millon and of Adamkiewicz-Hopkins, but not that of Molisch.

On being acted upon with trypsin, pepsin-peptone yields tyrosin, arginin, and the two trypsin-peptones (see below), which in their turn contain lysin, arginin, glutaminic and aspartic acids, and ammonia. As, further, the Adamkiewicz-Hopkins, or, to put it shorter, 'the glyoxylic acid,' reaction indicates tryptophane, the pepsin-peptone must be a very complicated substance—a fact which makes its constancy all the more remarkable.

From glutin Siegfried and Scheermesser prepared a pepsin-peptone having very similar properties:

$$C_{23}H_{39}N_7O_{10}.$$

If the simple formula be taken, it is a monobasic acid. Scheermesser has examined the zinc and the barium salts:

$$\alpha_D = -77{\cdot}5.$$

The furfurol- and the glyoxylic-acid reactions give negative results.

The relation of Pick's peptones to the pure peptones of Siegfried is not known, nor whether other peptones exist besides those enumerated above. Pfaundler[1] describes an ether-soluble peptone which was precipitated by metallic salts and the alkaloidal reagents, and

[1] M. Pfaundler, *Zeitschr. f. physiol. Chem.* **30**. 90 (1900).

which gave Millon's but not Molisch's reaction. Fränkel and Langstein [1] have broken up pepsin-peptone into four fractions.

Peptids

Kühne had assumed that peptic digestion only proceeded to the formation of peptone, but Zunz,[2] Pick,[3] Pfaundler,[4] and Reach [5] showed, after precipitating the albumoses and peptones, even if digestion had been going on for only a short time, that over one-half of the nitrogen was in solution in some non-proteid form, *i.e.* a form which did not give the biuret-reaction. It might have been thought that pepsin acted similarly to trypsin, but only more slowly, and that it also gave rise to amino-acids, and therefore a large number of attempts have been made to find amino-acids after very prolonged peptic digestion. The older experiments of Lubavin,[6] Möhlenfeld,[7] Lawrow,[8] and Langstein [9] did not settle this question; for they were made with the gastric mucous membrane, and we know through Salkowski,[10] Jacoby,[11] and Vernon [12] that all organs contain small amounts of ferments capable of dissociating albumins into amino-acids and even into more simple compounds. In purifying pepsin, the albumins and salts are got rid of; but traces of the ferments just alluded to are not removed, because they have the same solubility as pepsin. That tryptic ferments are present has been shown by Malfatti [13] and Zunz,[14] and the 'pseudo-pepsin,' the non-existence of which Klug [15] and Pawlow [16] have since proved, is probably also a trypsin-like body. That besides pepsin other ferments are also present is proved by the researches of Langstein and Lawrow, who find phenylethylamin, putrescin, and cadaverin. There

[1] S. Fränkel and L. Langstein, *Sitzungsber. d. Wiener Akad., math.-nat. Kl.* **110**. Abt. II^b. February 1901.

[2] E. Zunz, *Zeitschr. f. physiol. Chem.* **28**. 132 (1899); *Hofmeister's Beiträge*, **2**. 435 (1902), **3**. 339 (1902).

[3] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28**. 219 (1899).

[4] M. Pfaundler, *ibid.* **30**. 90 (1900).

[5] F. Reach, *Hofmeister's Beiträge*, **4**. 139 (1903).

[6] Lubavin, *Hoppe-Seyler's med.-chem. Untersuch.* p. 463 (1871).

[7] Möhlenfeld, *Pflüger's Arch.* **5**. 381 (1872).

[8] D. Lawrow, *Zeitschr. f. physiol. Chem.* **26**. 513 (1899), **33**. 312 (1901).

[9] L. Langstein, *Hofmeister's Beiträge*, **1**. 507 (1902), **2**. 229 (1902).

[10] E. Salkowski, *Zeitschr. f. klin. Medizin*, 1891, Suppl.

[11] M. Jacoby, *Zeitschr. f. physiol. Chem.* **30**. 149 (1900).

[12] H. M. Vernon, *Journ. of Physiol.* **32**. 33 (1904).

[13] H. Malfatti, *ibid.* **31**. 43 (1900).

[14] E. Zunz, *Hofmeister's Beiträge*, **2**. 435 (1902).

[15] F. Klug, *Pflüger's Arch.* **92**. 281 (1902).

[16] S. Salaskin and K. Kowalewsky, *Zeitschr. f. physiol. Chem.* **38**. 571 (1903).

seems to be, however, no ferment present which dissociates albumin hydrolytically, or which acts on the carboxyl-group of the amino-acids.

The only reliable researches are those of Salaskin,[1] Salaskin and Kowalewsky,[2] and Salaskin and Dzierzgowsky,[3] who worked with gastric juice obtained by Pawlow's method. They also obtained small amounts of ammonia,[3] leucinimide,[1] and amino-acids. Leucinimide being readily formed from the peptid leucylleucin, could therefore be accounted for. As regards the amino-acids, the objection might be raised that they are formed from peptones owing to the action of hydrochloric acid. Langstein[4] states that 1 per cent sulphuric acid does not act on albumin even if it be allowed to act for months; but he did not determine its action on peptones, which, according to Siegfried,[5] are very readily acted upon by acids.

It is therefore questionable whether pepsin gives rise to amino-acids: in any case only unappreciable amounts of these acids are formed even after digesting for one to two months, for Salaskin found that pepsin gave rise to only one-tenth the amount of amino-acids which could be obtained by the action of mineral acids. The large quantity of dissociation-products containing neither amino-acids nor peptones, judging by the biuret-reaction, Pfaundler has shown to be composed of substances intermediate between amino-acids and peptones. He isolated an alcohol-soluble product which is precipitable by mercuric sulphate but not by the alkaloidal reagents, and which dissociates into leucin and a di-amino-acid, probably histidin, on being treated with boiling hydrochloric acid. This substance is therefore a compound of several amino-acids, and occupies the same position as do the synthetic peptids of E. Fischer (Chapter III.), and as does glycyl-alanin, which E. Fischer and Bergell[6] prepared from the fibroin of silk by a combined action of acids, trypsin, and alkali. Pfaundler says justly, that the study of these products will give us the most important information regarding the chemistry of albumins. Another similar body is leucinimide, which Salaskin and Salaskin and Kowalewsky found in peptic digests, and which consists of two molecules of leucin. E. Fischer[7] and Abderhalden[8] believe it to be derived from leucyl-leucin, *i.e.* a peptid.

[1] S. Salaskin, *Zeitschr. f. physiol. Chem.* **32**. 592 (1901).
[2] S. Salaskin and K. Kowalewsky, *ibid.* **38**. 567 (1903).
[3] S. Dzierzgowsky and S. Salaskin, *Zentralbl. f. Physiol.* **15**. 249 (1901).
[4] L. Langstein, *Zeitschr. f. physiol. Chem.* **39**. 208 (1903).
[5] M. Siegfried, *Sitzungsber. d. sächs. Ges. d. Wissensch. zu Leipzig*, 1903, p. 63.
[6] E. Fischer, *Chemikerztg.* 1902, II. p. 939.
[7] E. Fischer and E. Fourneau, *Ber. d. deutsch. chem. Ges.* **34**. II. 2868 (1901).
[8] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **37**. 484 (1903).

Plasteines. Anti-albumids. Dysalbumose

Kühne[1] was the first to observe the formation of the so-called anti-albumids. If that portion of a proteid which is not acted upon by a prolonged digestion with pepsin-hydrochloric acid is subsequently subjected to tryptic digestion, a delicate jelly separates out, the 'anti-albumid coagulum,' which is also very slightly changed by trypsin. This resisting anti-albumid is remarkable for containing a much higher percentage of carbon than does ordinary albumin: Kühne and Chittenden found from 57 to 58 per cent carbon; it yields very little tyrosin and much anti-peptone.

Subsequently Okunew,[2] Lawrow,[3] Sawjalow,[4] and Kurajeff[5] found, in Danilewsky's laboratory, a similar fraction on adding lycopodium powder to a mixture of the products resulting from peptic digestion. They called the body which separated out 'plastein,' and were of the opinion that it had been regenerated out of the albumoses. Sawjalow pointed out the resemblance between plastein and anti-albumid; for they are closely related, judging by their chemical composition. Gelatine and keratine yield, however, no plastein or coagulose on being digested (Okunew, Kurajeff).

Pure gastric juice obtained by Pawlow's method, according to Lawrow and Salaskin,[6] acts like rennet; papayotin does the same, according to Kurajeff.[7] Umber[8] noticed a similar fraction if ammonium sulphate was present during peptic digestion. All these phenomena are based evidently on the common factor that every proteolytic enzyme possesses a rennet-like action.

Kurajeff[9] recently prepared plastein by digesting crystallised egg-albumin for three days with pepsin + hydrochloric acid, and then precipitating the plastein with a solution of rennet. To purify it he dissolved the plastein in one-tenth normal NaOH solution, and precipitated it with HCl. The plastein obtained after three days' digestion he called plastein A, while the plastein prepared similarly, but after eighteen days' digestion, is called plastein B. In both cases the amount of plastein was 7·3 per cent of the dry residue of the products

[1] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **19**. 159 (1883).

[2] Okunew, Dissertation, St. Petersburg, *Maly's Jahresber. f. Tierchemie*, 1895, p. 291.

[3] Lawrow, Dissertation, St. Petersburg, 1897, according to Sawjalow.

[4] W. W. Sawjalow, *Pflüger's Arch. f. d. ges. Physiol.* **85**. 171 (1901).

[5] D. Kurajeff, *Hofmeister's Beiträge*, **1**. 112 (1901).

[6] M. Lawrow and S. Salaskin, *Zeitschr. f. physiol. Chem.* **36**. 277 (1902).

[7] D. Kurajeff, *Hofmeister's Beiträge*, **1**. 121 (1901).

[8] F. Umber, *Zeitschr. f. physiol. Chem.* **25**. 258 (1898).

[9] D. Kurajeff, *Hofmeister's Beiträge*, **4**. 476 (1904).

of digestion. Both plasteins give the biuret and lead sulphide reaction, as also the tests of Molisch and Adamkiewicz. The percentage compositions of A and B plasteins is :

	C	H	N	S
A plastein .	58·8	7·3	14·4	1·24
B plastein .	58·9	7·2	14·3	not determined.

On adding papayotin to an albumose solution, which was prepared by digesting egg-albumin for three days, a small amount of 'coagulose was obtained, which was partly soluble in dilute soda-solution.

The precipitate in question, according to Lawrow and Salaskin, is not a restituted or reformed albumin, but an albumose which can be dissociated by erepsin. Umber found its amount to run parallel to the amount of hetero-albumose contained in any given albumin, and considered it to be a precursor of hetero-albumose. Sawjalow likewise obtained plastein in larger quantities only from the hetero-albumose ; while from the other albumoses, which were by no means pure, only traces could be got. The precipitate is therefore either hetero-albumose or that portion of the acid-albumin which belongs to the anti-group, and which during metabolism is converted into hetero-albumose.

The coagulation of albumose by means of extracts of various organs, which had undergone auto-digestion for sixteen hours, has been studied by Nürnberg.[1] The extracts acted in the following descending order : Extracts from the liver, stomach, lungs, pancreas, small intestine, large intestine, kidney, brain, eggs, and muscle. Milk is most acted upon by pancreas extracts, less by fresh gastric extract, and hardly at all by other extracts.

While all the observers mentioned so far have found what they term plastein to be an albumose-like substance, Bayer[2] has isolated a plasteinogenous compound which is a peptoid (peptid), *i.e.* one of those dissociation products of albumin which are formed in large amounts during the early stages of peptic digestion (Zunz), and which give no biuret-reaction.

To obtain this peptoidal plastein Bayer proceeded as follows : (1) a 10 per cent watery solution of Witte's peptone was precipitated with an equal bulk of 95 per cent alcohol ; (2) the alcoholic filtrate mixed with twice its volume of acetone ; (3) the alcohol-acetone filtrate precipitated with 80 per cent alcohol. The final filtrate contains the plastein, which gives neither the biuret, nor the xantho-proteic, lead-sulphide, Millon's and Molisch's reactions.

[1] A. Nürnberg, *Hofmeister's Beiträge*, **4**. 543 (1904).

[2] H. Bayer, *ibid.* **4**. 554 (1904).

Plastein in the alcohol-acetone solution differs greatly from other albumins, for on analysis it yielded these percentages—

C 38·43 H 7·01 N 8·05

Bäyer obtained no plastein on adding rennet-ferment ('pegnin' of the Höchst Manufactory) to proto-, hetero-, thio-, and gluco-albumoses, prepared from Witte's peptone according to Pick's method. (See p. 179.)

Kühne [1] has observed that hetero-albumose has a great tendency to become insoluble on being kept, or by being heated up to 55°. This insoluble form of hetero-albumose Kühne termed 'dys-albumose.' In all probability plastein and anti-albumid are one and the same body, and closely related to dys-albumose. We do not yet know whether the formation of this insoluble body depends on rennin, on salts, or on some other factor. The amount of the precipitate depends on the strength of the ferment, as active pepsin, *e.g.* pure gastric juice, dissolves the precipitate: it was obtained in largest amount if by adding ammonium sulphate the action of pepsin was interfered with. Rotarski's [2] statement that the anti-albumid coagulum can only be obtained from albumin which has been altered by heat is incorrect, as Umber obtained it also from raw egg-white. On being still further digested, anti-albumid yields the products of the anti-group; it is, however, unsuitable for preparing anti-peptone, for it yields only small quantities, according to Siegfried and Müller.[3]

II. THE PEPTONES FORMED BY TRYPTIC DIGESTION

Claude Bernard and Corvisart were the first to discover that pancreatic juice dissolves and dissociates proteids. Kühne [4] showed subsequently that this dissociation is a very pronounced one, as it leads to the formation of the same crystalline products as are obtained by acting on albumins with boiling mineral acids. He also showed that the pancreatic action depends on the presence of the ferment trypsin, which acts in alkaline, neutral, or acid, but best in feebly alkaline solutions.[5] Kühne discovered leucin, tyrosin, and 'tryptophane'; Salkowski and Radziejewski,[6] Knieriem,[7] Hirschler,[8] Stadel-

[1] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **20**. 11 (1884).

[2] T. Rotarski, *Zeitschr. f. physiol. Chem.* **38**. 552 (1903).

[3] F. Müller, *ibid.* **38**. 265 (1903).

[4] W. Kühne, *Virchow's Arch.* **39**. 130 (1867); *Verh. d. Heidelberger nat.-med. Vereins*, N.F. I. 236, III. 463 (1886).

[5] W. Kühne, *Verh. d. Heidelberger naturh.-med. Vereins*, N.F. I. 190 (1876); A. Dietze, Medizinische Dissertation, Leipzig, 1900.

[6] E. Salkowski and S. Radziejewski, *Ber. d. deutsch. chem. Ges.* **7**. II. 1050 (1874).

[7] Knieriem, *Zeitschr. f. Biol.* **11**. 199 (1875).

[8] A. Hirschler, *Zeitschr. f. physiol. Chem.* **10**. 302 (1886).

mann,[1] Hedin,[2] Kutscher,[3] Külz,[4] and E. Fischer and Abderhalden[5] added to these three products nearly all the other dissociation-products. How our knowledge about tryptic action was further developed has already been alluded to on p. 144. It is definitely known that trypsin can only dissociate one portion of the albumin-molecule, and that a proteid-residue remains even after trypsin has acted for months. This compound, according to E. Fischer and Abderhalden, is a polypeptid; it is precipitated by phosphotungstic acid, and, on being dissociated with acids, yields glycocoll, α-pyrrolidin-carboxylic acid, phenylalanin, alanin, leucin, and aspartic and glutaminic acids. The three compounds mentioned first are only found in the polypeptid, while the other substances are also found in the digested portion of the tryptic digest. The di-amino acids have not yet been determined.

In addition to the polypeptid just mentioned, peptone is also found. The latter gives the biuret-reaction, and has been called 'anti-peptone' by Kühne. According to Kutscher,[3] Siegfried,[6] Mays,[7] Löwi,[8] and Lawrow,[9] its resistance is only relative, as it is dissociated still further by intense tryptic digestion; but the difference, compared with the readily digested albumin, is very great. While, according to E. Fischer and Abderhalden,[5] tyrosin is split off with such rapidity as to suggest that this amino-acid simply crystallises out, it takes weeks and even months for the biuret-reaction of the anti-peptone to disappear. Erepsin disintegrates anti-peptone also, only very slowly[10] (Cohnheim).

The anti-peptones, for there are several, Siegfried[11] and his pupils[12] have prepared in a pure state by adding iron-ammonia-alum to saturated ammonium sulphate solutions. From fibrin they obtained

α Anti-peptone $C_{10}H_{17}N_3O_5$ (C 46·2; H 6·74; N 16·26 per cent).
β Anti-peptone $C_{11}H_{19}N_2O_5$ (C 48·23; H 7·12; N 15·41 „).

[1] E. Stadelmann, *Zeitschr. f. Biol.* **24**. 261 (1888).

[2] S. G. Hedin, *Arch. f. (Anat. und) Physiol.* 1891, p. 73.

[3] F. Kutscher, *Zeitschr. f. physiol. Chem.* **25**. 195 (1898), **26**. 110 (1898), **28**. 88 (1899); *Endprodukte der Trypsinverdauung*, Marburg, 1899 (here a full account of the literature).

[4] E. Külz, *Zeitschr. f. Biol.* **27**. 415 (1890).

[5] E. Fischer and E. Abderhalden, *Zeitschr. f. physiol. Chem.* **39**. 81 (1903).

[6] M. Siegfried, *Ber. d. deutsch. chem. Ges.* **33**. III. 3564 (1900).

[7] K. Mays, *Zeitschr. f. physiol. Chem.* **38**. 428 (1903).

[8] O. Löwi, *Schmiedeberg's Arch.* **48**. 303 (1902).

[9] D. Lawrow, *Zeitschr. f. physiol. Chem.* **26**. 513 (1899).

[10] O. Cohnheim, *ibid.* **35**. 134 (1902).

[11] M. Siegfried, *Zeitschr. f. physiol. Chem.* **27**. 335 (1899); *Ber. d. deutsch. chem. Ges.* **33**. III. 2851 (1900), **33**. III. 3564 (1900); *Zeitschr. f. physiol. Chem.* **35**. 164 (1902), **38**. 259 (1903).

[12] Fr. Müller, *ibid.* **38**. 265 (1903) (in this paper the methods are fully discussed); T. R. Krüger, *ibid.* **38**. 320 (1903).

These peptones resemble the pepsin-peptones in being pronounced acids; they are monobasic if we adopt the simple formula, but the latter must be multiplied because of the dissociation-compounds which are formed.

The zinc and barium salts of these anti-peptones have been analysed. Their behaviour towards precipitants is the usual one described above; amongst colour-tests, positive results are only obtained with the biuret and the xanthoproteic reactions. Anti-peptones are lævo-rotatory:

Anti-peptone α . . . . . $\alpha_D^{20} = -24{\cdot}5$.

Anti-peptone β . . . . . $\alpha_D^{20} = -32{\cdot}4$.

The salts are more strongly lævo-rotatory.

Anti-peptone α on being dissociated with acids gives rise to lysin, arginin, 4 per cent ammonia, 12 per cent glutaminic acid, aspartic and other amino-acids. Anti-peptone β yields lysin, arginin, 3 per cent ammonia, and glutaminic acid. The bases account for only one-quarter the amount of nitrogen, and therefore mono-amino-acids must be abundantly present.

Gelatine, on tryptic digestion, gives rise to an anti-peptone:

$C_{19}H_{30}N_6O_9$ (C 46·74; H 6·2; N 17·36 per cent)

$\alpha_D = -100{\cdot}8$.

In other respects it resembles the anti-peptones α and β; on being boiled with acids it yields lysin, arginin, glutaminic acid, and glycocoll. On being carefully dissociated with acids, a base is obtained which is perhaps identical with kyrin, see p. 200.

Krüger describes yet another peptone with $\alpha_D = -64{\cdot}3$.

The fibrin-anti-peptones are not only formed directly from albumin, but may also be obtained, along with tryptophane, tyrosin, and arginin, by the tryptic digestion of pepsin-peptones.

From sturin Kossel and Matthews[1] prepared a trypsin-peptone, having the composition $C_{18}H_{35}N_7O_5$, the silver salt of which crystallised well, and was therefore a protone (see p. 423).

From silk-fibroin E. Fischer and Bergell[2] obtained, by tryptic digestion, after a preliminary partial dissociation with acids, a lævo-rotatory peptone having the composition $C_{40{\cdot}6}H_{6{\cdot}6}N_{18{\cdot}4}$, which on being dissociated with acids yielded 40·1 per cent glycocoll, 28·5 per cent alanin, and no tyrosin. Treatment with alkali gave rise to the di-peptid glycyl-alanin.

The pancreas-peptones used to be called tryptones by Fano.[3]

[1] A. Kossel and A. Matthews, *Zeitschr. f. physiol. Chem.* **25**. 190 (1898).

[2] E. Fischer, *Chemikerztg.* 1902, II. p. 939.

[3] Fano, *Arch. f. (Anat. u.) Physiol.* 1881, p. 277.

As intermediary products of tryptic digestion, albumoses,[1] and in particular deutero-albumoses, are also formed.[2] According to Umber,[3] in many cases these deutero-albumoses have been mistaken for nucleic acids.

By subjecting egg-albumin and casein to the combined action of pancreatic and splenic extracts, or to the combined pulp of the organs, Levene and Stoky[4] found digestion to proceed much quicker than corresponded to the sum of the action of the two organs. They believe the spleen to facilitate the conversion of trypsinogen into trypsin, as the spleen does not augment the digesting power of the pancreas after the trypsinogen has become converted into trypsin.

In this connection may be mentioned the interesting results which Vernon[5] has obtained by studying the protective value of proteids and their decomposition-products on trypsin:—"Most proteids have practically the same protective value, about 45 per cent of the trypsin of an extract being destroyed per hour in presence of 0·4 per cent of proteid; 27 per cent in presence of 1 per cent; 12 per cent in presence of 2 per cent; 7 per cent in presence of 4 per cent of proteid. When no proteid was present 56 per cent of the ferment was destroyed. Hydrated proteids have a slightly greater protective value than native proteids, and the decomposition-products of proteid hydrolysis a slightly greater one still. . . . If the acid-radicals in the various substances be previously neutralised by the addition of an alkali they entirely lose their protective power over the ferment." As the difference in the protective value between the native proteids and their hydrolysed derivatives is but slight, 'it would seem as if most of the COOH-groupings which are present in the products of proteid-decomposition are likewise present as such when forming part of the complex proteid-molecule, and in either condition are capable of combining with any molecules of alkali brought in their neighbourhood. This conclusion is at variance with that of Hofmeister,[6] who considers that the amido-acid nuclei in the proteid-molecule are linked together by an NH_2-grouping of one nucleus uniting with a COOH-grouping of another to form a

$$>CH—NH—CO\text{-grouping.}$$

[1] R. Neumeister, *Zeitschr. f. Biol.* **23**. 381 (1887), **24**. 267 (1887); U. Biffi, *Virchow's Arch.* **152**. 130 (1898).

[2] R. Neumeister, *Zeitschr. f. Biol.* **23**. 381 (1887), **24**. 267 (1887).

[3] F. Umber, *Zeitschr. f. klin. Mediz.* **43**. Nos. 3 and 4 (1901).

[4] P. A. Levene and L. B. Stoky, *Amer. Journ. of Physiol.* **12**. 1, (1904).

[5] H. M. Vernon, *Journ. of Physiol.* **31**. 346 (1904).

[6] Hofmeister, *Ergebnisse d. Physiol.* **1**. 787 (1902).

If such were the case, then more than half of the COOH-groupings would be required for this purpose, and so lose their power of combining with alkali. Accordingly, the neutralising power of proteids should be less than half as great as that of their hydrolytic decomposition-products." Vernon's results may be explained on the legitimate assumption that the products of digestion form salt-like combinations. See Chapter VI. (The author.)

III. PRODUCTS FORMED BY OTHER PROTEOLYTIC ENZYMES

A ferment occurs in the kidney which, according to Dakin [1] gives the end-products: ammonia, alanin, α-amino-iso-valerianic acid, leucin, prolin, phenylalanin, tyrosin, lysin, histidin, cystin, hypoxanthine, indol-derivatives, and an insoluble residue of para-nuclein. Arginin and asparctic acid were only feebly represented. Otherwise the ferment resembles trypsin in its action. It acts in an acid medium, and is a typical example of an autolytic ferment. Arginase is discussed on p. 111.

Erepsin [2] is found in the animal organism, in addition to pepsin and trypsin. It dissociates albumoses and peptones into amino-acids, but it has no action on natural albumins except on casein. It destroys the biuret-reaction of anti-peptone, but whether it also dissociates the polypeptid not attacked by trypsin has not yet been determined.

Vernon has shown that erepsin is widely distributed throughout the whole animal kingdom. It occurs in every organ so far investigated; the tissues of mammals are, as a rule, richer in ferment than are those of the pigeon, and warm-blooded animals contain distinctly more erepsin than do cold-blooded ones. Of individual tissues the kidney was even richer in ferment than the intestinal mucous membrane. Next in order to these two tissues came the pancreas, spleen, and liver; then with a considerable drop came heart muscle, whilst skeletal muscle and brain tissue were poorest of all in ferment. Erepsin of mammalian tissue digests peptone about three times less rapidly in neutral than in alkaline solutions. while in acid solutions the digestion rate was thirty to seventy times slower than in alkaline solutions. The various tissue erepsins seem further to be to some extent specific.[3]

Autolytic ferments have been demonstrated by Salkowski,[4]

[1] H. D. Dakin, *Journ. of Physiol.* **30**. 84 (1903).

[2] O. Cohnheim, *Zeitschr. f. physiol. Chem.* **33**. 451 (1901), **35**. 134 (1902), **36**. 13 (1902); S. S. Salaskin, *ibid.* **35**. 419 (1902); F. Kutscher and J. Seemann, *ibid.* **35**. 432 (1902); J. H. Hamburger and J. Hekma, *Koninkl. Akad. van Wetenschapen te Amsterdam*, 1902, p. 733; H. M. Vernon, *Journ. of Physiol.* **32**. 33 (1904).

[3] H. M. Vernon, *ibid.* **32**. 33 (1904).

[4] E. Salkowski, *Zeitschr. f. klin. Med.* **17**. Suppl. p. 77 (1891); H. Schwiening Medizin. Dissertation, Berlin, 1893.

Jacoby,[1] Kutscher and Lohmann [2] (pancreas), Levene [3] (testis, spleen), Hildebrandt [4] (mammary gland), Lane, Claypon, and Schryver [5] (gastric and intestinal mucous membrane), Waldvogel [6] (autolysis in relation to fatty degeneration), Schulze and Castoro [7] (germinating plants), and many others in all the organs of the body. They dissociate the tissue-albumins into albumoses and amino-acids. It has not yet been settled whether the autolysis is induced by special ferments or only by traces of pepsin, trypsin, and erepsin. According to Hedin and Rowland [8] these autolytic ferments act best in acid media.

Ferments which dissociate albumins into amino-acids are very widely distributed in nature, and occur probably in all animals; as intermediate products peptones are usually formed. Mack [9] has prepared such peptones, under the direction of Siegfried, from the seeds of *Lupinus luteus*. These peptones resemble in their properties and dissociation-products the trypsin-peptones. The ferments under discussion must not be identified, without proper reasons, with trypsin, as many of them act only in acid or in acid and alkaline solutions.[10] Many bacteria contain these ferments,[11] as does also yeast.

The ferments in germinating plants have already been mentioned. In some portions of plants, Vines [12] and Czapek [13] have found erepsin-like ferments. A ferment which in acid media dissociates albumins up to the stage of amino-acids is the 'bromelin' [14] of pine-apples. The ferment 'papayotin' found in the juice of *Carica papaya* has been carefully examined by Neumeister,[15] Mendel,[16] Ingraham,[17] Kurajeff,[18]

[1] M. Jacoby, *Zeitschr. f. physiol. Chem.* **30**. 149 (1900); **33**. 126 (1901); *Hofmeister's Beiträge*, **3**. 446 (1903).

[2] Kutscher and Lohmann, *Zeitschr. f. physiol. Chem.* **41**. 332 (1904).

[3] P. A. Levene, *Amer. Journ. of Physiol.* **11**. 437 (1904).

[4] P. Hildebrandt, *Hofmeister's Breiträge*, **5**. 463 (1904).

[5] J. E. Lane, Claypon, and S. B. Schryver, *Journ. of Physiol.* **31**. 169 (1904).

[6] Waldvogel, *Virchow's Arch.* **177**. 1 (1904).

[7] E. Schulze and N. Castoro, *Zeitschr. f. physiol. Chem.* **43**. 170 (1904).

[8] S. G. Hedin and S. Rowland, *ibid.* **32**. 341 (1901), **32**. 531 (1901).

[9] W. R. Mack, Dissertation, Leipzig, 1903.

[10] W. Biedermann, 'Tenebrio molitor,' *Pflüger's Arch. f. d. ges. Physiol.* **72**. 105 (1898).

[11] M. Hahn and S. Geret, *Zeitschr. f. Biol.* **40**. 117 (1900); *Zeitschr. f. physiol. Chem.* **33**. 385 (1901); F. Kutscher, *ibid.* **32**. 59 (1900), **32**. 419 (1901), **34**. 517, 520 (1902); E. Salkowski, *ibid.* **13**. 506 (1889).

[12] S. H. Vines, *Annals of Botany*, **17**. 237 (1903), **17**. 597 (1903), **18**. 289 (1904).

[13] F. Czapek, *Chem. Zentralbl.* 1903, p. 178.

[14] R. H. Chittenden, *Journ. of Physiol.* **15**. 249 (1883).

[15] R. Neumeister, *Zeitschr. f. Biol.* **26**. 57 (1890).

[16] L. B. Mendel and F. P. Underhill, *Trans. of the Connecticut Academy of Arts and Sciences*, XI. October 1901; L. B. Mendel, *Amer. Journ. of Med. Sc.* **124**. 310 (1902).

[17] L. B. Mendel. See footnote, *ibid.* p. 318.

[18] D. Kurajeff, *Hofmeister's Beiträge*, **1**. 121 (1901).

Vines,[1] Emmerling,[2] and Siegfried.[3] The authors agree that papayotin is active whatever the reaction may be; Mendel found it to be most active in alkaline, and Vines in acid media. According to Mendel it forms the same albumoses as does pepsin according to Pick, while Siegfried says the peptones are acid peptones, somewhat resembling those obtained by peptic and by tryptic digestion. Whether it forms crystalline dissociation-products has not yet been settled. Mendel failed to find them, Vines found tryptophane, and Emmerling amino-acids, but in very minute quantities. The main bulk of the dissociation-products consists of albumoses and peptones. According to Kurajeff and Ingraham it produces in solutions of albumoses a precipitate, as do other proteolytic ferments.

Bromelin, precipitated from pine-apple juice with sodium-chloride requires 0.025 per cent HCl and 0.25 to 1 per cent acetic acid to develop its maximal digestive power (Hoyer).[4]

Antweiler's peptone[5] consists of meat digested with papayotin.

IV. ALBUMOSES AND PEPTONES PRODUCED BY ACTION OF ACIDS

When albumins are boiled with strong acids there are formed amino-acids, but if one allow dilute $\frac{1}{10}$- or $\frac{1}{4}$-normal hydrochloric or sulphuric acids to act at room- or at incubation-temperature on albumins, according to Goldschmidt,[6] exactly the same albumose- and peptone-fractions are obtained as after peptic digestion. Goldschmidt examined egg-albumin and crystallised serum-albumin; Pick and Spiro,[7] edestin and casein. Acid-albumin is formed first, but it soon breaks up into primary albumoses, deutero-albumoses, and peptones. Serum-albumin, which is readily digested by pepsin, was found to be attacked with great difficulty; the same holds good for edestin. After 0·8 per cent hydrochloric acid had acted for eight days, 90 per cent of acid-albumin was still present.

Langstein and Neuberg on the other hand find that weak acids have no effect on albumin, for Langstein[8] remarks: "1 per cent H_2SO_4 at 37° will not dissolve, even after months, crystallised egg-

[1] S. H. Vines, *Annals of Botany*, **17**. 597 (1903).

[2] Emmerling, *Ber. d. deutsch. chem. Ges.* **35**. I. 695 (1902).

[3] M. Siegfried (and Tittmann), *Zeitschr. für physiol. Chem.* **38**. 259 (1903).

[4] Hoyer, *Ber. d. deutsch. chem. Ges.* **37**. 1436 (1904).

[5] J. Munk, *Therapeutische Monatshefte*, 1888, p. 176; R. Neumeister, *Zeitschr. f. Biol.* **26**. 57 (1890).

[6] F. Goldschmidt, Medizinische Dissertation, Strassburg, 1898.

[7] E. P. Pick and K. Spiro, *Zeitschr. f. physiol. Chem.* **31**. 251 (1900).

[8] L. Langstein, *ibid.* **31**. 208 (1900).

albumin, which has been dried at 100°"; and Neuberg found that 1 per cent H_2SO_4, after having acted for a twelvemonth on gelatine, had given rise to no mono-amino-acids. Lawrow,[1] however, has found 3·7 to 5 per cent gelatine to be markedly affected by 0·5 per cent HCl if kept at 37-38°, there being always an excess of chloroform. After 60 days the rotary power of gelatine had been diminished to the extent of 23 per cent, and nitrogenous radicals had been split off which could not be precipitated by phospho-tungstic acid, and which, therefore, were probably mono-amino-acids. Twice crystallised hæmoglobin treated similarly with 0·5 per cent HCl gave, after 161 days, over 30 per cent mono-amino nitrogen, Kühne's amphopeptone and nitrogenous products, probably mono-amino-acids, which could not be precipitated by phospho-tungstic acid.

In considering the primary and secondary effects produced by the action of acids on albumins the same unsolved question arises as when studying the action of ferments, namely: Do albumoses and peptones represent smaller complexes, split off from the unchanged acid-albumin, or does the albumin-molecule, as a whole, change into acid-albumin, and then into albumoses, but with different rapidities? The results of Goto[2] seem to show that the albumin, as a whole, is first divided into large complexes of the same size, and that therefore the second of the two conjectures is the right one.

Kyrins

By acting with 12·5 per cent hydrochloric acid on gelatine at 38°, Siegfried[3] prepared "gluto-kyrin," the first known crystalline peptone. It is a base having the composition—

$$C_{21}H_{39}N_9O_8.$$

The only difficulty with this formula is that Siegfried[4] obtained in recent experiments much less glutaminic acid than corresponds to one molecule of glutaminic acid to one molecule of kyrin. This discrepancy he explains as due to dissociation taking place in different ways, or due to the formation of secondary products such as pyrrolidin-carboxylic acid, or due to the formation of compounds or intermediate products containing the glutaminic acid radical. O. Pilz is engaged in Siegfried's laboratory in elucidating this question.

Of its salts, the chloride, the sulphate, which is almost insoluble in alcohol, the phosphotungstate, which crystallises, and the β-naphthalin

[1] D. Lawrow, *Zeitschr. f. physiol. Chem.* **44**. 447 (1905).

[2] M. Goto, *ibid.* **37**. 94 (1903).

[3] M. Siegfried, *Ber. d. sächs. Ges. d. Wissensch. zu Leipzig, math.-phys. Kl.*, 1903, p. 63.

[4] M. Siegfried, *Zeitschr. f. physiol. Chem.* **43**. 44 (1904).

sulpho-compound have been examined. Kyrin is also obtained from glutin anti-peptone, and its importance as the basic 'nucleus of the proteid' has been discussed already in connection with the anti-group, on p. 195; there it was also pointed out that Kossel's doctrine as to the existence of a basic nucleus is supported by the discovery of kyrin. The latter gives the biuret-reaction; on being dissociated with acids it yields lysin, arginin, glycocoll, and glutaminic acid. As two-thirds of the nitrogen is basic, and of this again two-thirds is contained in arginin, the composition seems to be as follows:—

1 molecule arginin . .	$C_6 H_{14} N_4 O_2$
1 ,, lysin . . .	$C_6 H_{14} N_2 O_2$
1 ,, glutaminic acid .	$C_5 H_9 N O_4$
2 ,, glycocoll . .	$C_4 H_{10} N_2 O_4$
	$C_{21} H_{47} N_9 O_{12}$
minus 4 molecules of water .	$H_8 O_4$
Gluto-kyrin	$C_{21} H_{39} N_9 O_8$

Siegfried[1] has prepared a quite similar basic nucleus, the caseino-kyrin, from casein. Its nitrogen is in the form of basic-nitrogen (arginin and lysin) to the extent of 84-85 per cent, while the amino-acid-nitrogen (glutaminic acid) amounts from 15 to 16 per cent.

This base has the formula $C_{23} H_{47} N_9 O_8$.

Hydrolytic dissociation sets free arginin, lysin, and glutaminic acid, but no histidin or ammonia. Siegfried assigns to caseino-kyrin the formula—

1 molecule arginin . .	$C_6 H_{14} N_4 O_2$
2 ,, lysin . . .	$C_{12} H_{28} N_4 O_4$
1 ,, glutaminic acid .	$C_5 H_9 N O_4$
	$C_{23} H_{51} N_9 O_{10}$
minus 2 molecules of water .	$H_4 O_2$
Caseino-kyrin . . .	$C_{23} H_{47} N_9 O_8$

V. ALBUMOSES PRODUCED BY THE ACTION OF ALKALIES

Maas[2] has dissociated albumins with dilute alkalies. He found alkali-albuminates and albumoses, but no peptones, as the latter were evidently at once broken up still further. An alkali-albumose prepared from egg-albumin was specially investigated, but similar

[1] M. Siegfried, *Zeitschr. f. physiol. Chem.* **43**. 46 (1904). [2] O. Maas, *ibid.* **30**. 61 (1900).

products were also obtained from fibrin and from serum-albumin. The alkali-albumose has the composition—

C	H	N	S	O
53·57	7·09	13·62	2·13	23·49 per cent.

It is lævo-rotatory, $\alpha_D = -49{\cdot}4$. It gives the biuret, the lead-sulphide, and the xantho-proteic reactions, also those of Millon and Molisch, and is precipitated by the alkaloidal reagents. It is insoluble in water and salt solutions; readily soluble in acids and alkalies; soluble in hot alcohol of 50-60 per cent; insoluble in cold alcohol. The precipitation limits for ammonium sulphate are between 18 and 42. It is not digested by trypsin.

VI. COMPOUNDS FORMED BY MOIST HEAT

Atmid Albumoses

The action of steam under pressure on proteids has been investigated by Meissner, Krukenberg, Neumeister,[1] and Salkowski[2] (see also p. 92). If fibrin or any other coagulated albumin is placed in a large amount of water, and is heated for one hour in an autoclave to 160°, sulphuretted hydrogen and ammonia are given off, while the albumin passes entirely, or for the greater part, into solution; in the fluid will be found peptones and albumoses. If the solutions which were heated had an acid reaction, Neumeister obtained substances which resembled in every respect Kühne's peptic-albumoses; while if the reaction was neutral or alkaline, two new albumoses were formed, which Neumeister called atmid-albumin and atmid-albumose. The behaviour of these two bodies towards acids and towards salts is a very complicated one, probably because these substances do not possess a uniform constitution. Towards precipitants they behave as do the primary albumoses; they give a violet biuret-reaction and well-marked reactions with the tests of Molisch and Adamkiewicz, but only a slight reaction with Millon's reagent. They do not give the black lead-sulphide reaction, although they still contain traces of sulphur. Their nitrogen-content is low. Both atmid-albumoses are converted by boiling sulphuric acid into deutero-albumoses and peptones, but are attacked only with difficulty by pepsin and trypsin. In all probability they are des-aminated albumoses of the anti-group, mixed perhaps with some form of acid-albumin (Cohnheim).

[1] R. Neumeister, *Zeitschr. f. Biol.* **26**. 57 (1890), **36**. 420 (1898).

[2] E. Salkowski, action of superheated water on albumin, *ibid.* **34**. 190 (1896), **37**. 401 (1899

By prolonged heating to only 130°, Salkowski prepared from meat and fibrin products essentially resembling those of Neumeister; they differ in giving a well-marked Millon's reaction, and being more readily digested than are Neumeister's preparations. Salkowski believes his substance to be the ammonium-salt of an albumose, and not an albumose.

The papayotin-albumoses closely resemble the atmid-albumoses, according to Neumeister.[1]

Somatose is an atmid-albumose.

Carnic or Sarctic Acid

Kemmerich's meat-extract contains, as first stated by Kemmerich[2] and confirmed by Mays,[3] albumoses and peptones, which are probably formed by a transformation of the not readily coagulable muscle-albumins. There are found, however, in muscle also pre-formed non-coagulable albumins. According to Mays, one of these substances is carnic acid, which Siegfried[4] and his pupils[5] prepared from Kemmerich's meat extract by removing all phosphates, and then precipitating with ferric chloride. Carnic acid has the same composition and the same properties as has an anti-peptone. Its formula is

$$C_{10}H_{15}N_3O_5.$$

Adopting this formula, it is mono-basic. It gives an intense, red biuret-reaction, but no other colour-tests; it is precipitated by alkaloidal reagents. It is not salted out by ammonium sulphate. Its disintegration-products are ammonia, carbonic acid, lysin, arginin, leucin, and succinic acid. Of still greater importance are its two derivatives, phosphocarnic acid and carniferrin. The phosphocarnic acid contains large amounts of phosphorus, and is, according to Siegfried, a nucleone, while carniferrin contains iron as well as phosphorus. Carniferrin is found in small amounts in urine, in muscles, and in cow's milk; in

[1] R. Neumeister, *Deutsche medizinische Wochenschrift*, 1893, Nr. 36 and 46.

[2] E. Kemmerich, *ibid.* **18**. 409 (1893).

[3] K. Mays, *Zeitschr. f. Biol.* **34**. 268 (1896).

[4] M. Siegfried, 'Über Fleischsäure,' *Arch. f.* (*Anat. u.*) *Physiol.*, 1894, p. 401; 'Zur Kenntnis der Phosphorfleischsäure,' *Zeitschr. f. physiol. Chem.* **21**. 360 (1895), **22**. 575 (1897); 'Über Antipepton,' I. *ibid.* **27**. 335 (1899), **28**. 524 (1899).

[5] W. S. Hall, 'Resorption des Karniferrins,' *Archiv für* (*Anat. und*) *Physiol.*, 1894, 455; C. W. Rockwood, 'Sarctic Acid in Urine,' *ibid.* 1895, p. 1; P. Balke and Ide, 'Quantitative Estimation of Phosphosarctic Acid,' *Zeitschr. f. physiol. Chem.* **21**. 380 (1895); F. R. Krüger, *ibid.* **22**. 45 (1896); P. Balke, 'Dissociation-products of Carniferrin,' *ibid.* **22**. 248 (1896); Martin Müller, 'The Amount of Nucleon in Human Muscle,' *ibid.* **22**. 561 (1897); K. Wittmaack, 'Nucleon-content of Milk,' *ibid.* **22**. 567 (1897); R. T. Krüger, *ibid.* **28**. 535 (1899); I. J. R. Macleod, *ibid.* **28**. 535 (1899).

somewhat larger amounts in human milk. Siegfried attributes to these substances, especially in muscle, a considerable biological importance, but Folin [1] and Kutscher [2] throw doubt on the chemical uniformity of these derivatives.[3]

Physiology of Albumoses and Peptones

The part played by the hydrochloric acid in peptic digestion is as yet but little understood. Meissner [4] stated that 0·2 per cent hydrochloric acid, acting on non-coagulated egg-white, produces a water-soluble substance not coagulable by heat, which we now know to be an acid-albumin, while Goldschmidt and Lawrow, as already stated on p. 199, maintain that hydrochloric acid without pepsin will ultimately produce the same effect as if pepsin had been present. From this it would appear as if the chief function of the pepsin was to hasten the effect of the hydrochloric acid. If the author's view is correct that the greater part of an albumin molecule under normal conditions is in the pseudo-acid-pseudo-basic state, *i.e.* that the amino-acids in the albumin molecule become de-ionised owing to ring-formation occurring (see pp. 211 to 215), then the first stage in gastric digestion must be the opening up of these rings. This may be done, firstly, either by the action of the acid H-ion of the HCl of the gastric juice, or the basic OH'- radical of the sodium carbonate of the pancreatic juice; or, secondly, by the enzymes uniting primarily with the albumin. If we consider how firmly enzymes adhere to the albumins, it becomes necessary to explain this union as depending on chemical action, and it is legitimate to suppose that the new chemical compound is so constituted that it is readily split up by the H°- or OH'-ions. If ferments are able of reacting in both alkaline and acid media, then, according to the author's view, these ferments must be considered to be amphoteric (see p. 208).

Attention is also directed in this connection to the interesting observation of Schwarz [5] that trypsin cannot act on albumin which has been treated with form- or acet-aldehyde, while pepsin + HCl has the power of acting on the acid methylene- and ethylene-albumins, which are formed by the action of the corresponding aldehydes.

For the combined action of pepsin and hydrochloric acid it is best to employ the pure gastric juice obtained by J. Pawlow's method, as

[1] O. Folin, 'Some Constituents of Witte's Peptone,' *Zeitschr. f. physiol. Chem.* **25**. 152 (1898).

[2] Fr. Kutscher, 'Antipepton,' I. *bis* III. *ibid.* **25**. 195 (1898), **26**. 110 (1898), **28**. 88 (1899). [3] Meissner, *Zeitschr. f. rationelle Medizin*, III. **8**. 280 (1860).

[4] Leo Schwarz, *Zeitschr. f. physiol. Chem.* **31**. 460 (1901).

[5] See *Zentralbl. f. Physiol.* 1904, under 'Nucleon.'

thereby it is possible to exclude all tissue ferments. The objection to gastric juice obtained by Pawlow's method is, however, that it does not represent both cardiac and pyloric secretions, and it may for this reason be less active than is the pepsin secreted from the whole stomach. The first to point out that peptic digestion liberates mono-amino-acids, such as leucin-imide, was Salaskin,[1] and later Salaskin and Kowalewsky.[2] Lawrow[3] obtained from gelatine 35·2 per cent of the nitrogen as mono-amino-nitrogen, on dissolving 400 grammes of commercial gelatine in 4 litres of 0·5 per cent HCl, and then adding 900 ccm. of dog's gastric juice, obtained by Pawlow's method, and an excess of chloroform.

A stomach which was allowed to autodigest itself in the presence of 0·5 per cent HCl, gave rise to exactly the same products as other albumins did on being acted upon by gastric juice obtained by Pawlow's method.

While according to Salaskin, Kowalewsky, and Lawrow, mono-amino-acids are always formed by peptic digestion, Abderhalden and Rostoski,[4] on the other hand, failed to get any mono-amino-acids apart from traces of tyrosin, when they acted on edestin prepared from cotton-seed.

While native albumins which have escaped the action of peptic digestion may be absorbed as such according to Rosenberg and Oppenheimer,[5] it is quite different with albumoses and peptones, for these, according to Neumeister,[6] are found nowhere in the animal body except in the alimentary canal. The contradictory statements of other authors are to be explained on the ground of the great difficulty met with in completely separating out all albumins ; traces of albumin which have escaped coagulation have been taken for pre-existing albumoses. The more recent statements of Embden and Knoop[7] and Langstein[8] as to the occurrence of albumoses in blood do also not appear convincing. Under pathological conditions, however, as during pus-formation,[9] during the absorption of exudations[10] and similar processes, albumoses do occur in the animal body. Sometimes they are formed rapidly and abundantly

[1] S. Salaskin, *Zeitschr. f. physiol. Chem.* **32**. 592 (1901).
[2] S. Salaskin and K. Kowalewsky, *ibid.* **38**. 567 (1903).
[3] D. Lawrow, *ibid.* **44**. 447 (1905).
[4] E. Abderhalden and O. Rostoski, *ibid.* **44**. 265 (1905).
[5] S. Rosenberg and C. Oppenheimer, *Hofmeister's Beiträge*, **5**. 412 (1904).
[6] R. Neumeister, *ibid.* **24**. 272 (1888).
[7] G. Embden and F. Knoop, *ibid.* **3**. 120 (1902).
[8] L. Langstein, *ibid.* **3**. 373 (1902).
[9] F. Hofmeister, *Zeitschr. f. physiol. Chem.* **4**. 268 (1880).
[10] Fr. Müller, *Verh. der Naturf. Ges. zu Basel*, **13**. 308 (1901) ; O. Simon, *Deutsch. Arch. f. klin. Med.* **70**. 604 (1901).

as the result of autolytic processes immediately after death, *e.g.* after phosphorus-poisoning.[1] Krehl's[2] investigations have further brought to light a close connection between albumoses circulating in the blood and fever; the temperature-raising constituents of Koch's tuberculin are the albumoses of the nutritive medium.[3] Traces of albumoses have also been observed in sputum.[4] During all these pathological conditions albumoses are also met with in the urine, while peptones are always absent. The urinary albumose of Bence-Jones is not an albumose at all, but coagulated albumin (see p. 369). (Cohnheim.)

In connection with the demonstration of albumose in urine,[5] special attention is drawn to the observations of Stokvis[6] and Salkowski,[7] who found that urobilin produces with sodium hydrate and copper sulphate a colour which is undistinguishable from that of the biuret-reaction. Both authors point out the danger of mistaking urobilin for albumose when using the biuret-test.

Amongst invertebrates Henze[8] has found in the secretion of the salivary glands of the marine snail *Tritonum nodosum*, besides aspartic acid, a substance which seems to be a peptone.

Albumoses possess a number of poisonous properties, such as lowering of blood-pressure, rendering blood non-coagulable,[9] etc. Pick and Spiro[10] believe that pure albumoses are non-poisonous, and that the poisonous constituent, the peptozyme, adheres to the pepsin or to the fibrin, and that in this way it gets into the pepsin- and fibrin-albumoses, but Underhill[11] has shown Pick and Spiro to be wrong.

Simple albumins, namely, the protamins and histones, also lower

[1] F. Soetbeer (and O. Cohnheim), *Arch. f. experiment. Path. u. Pharm.* **50**. 294 (1903).

[2] L. Krehl, *ibid.* **35**. 222 (1893); L. Krehl and M. Matthes, *Deutsch. Arch. f. klin. Med.* **54**. 501 (1894); *Arch. f. experiment. Path. und Pharm.* **38**. 248 (1897); L. Krehl, *Pathol. Physiol.* Leipzig, 1898.

[3] M. Matthes, *Deutsch. Arch. f. klin. Med.* **54**. 39 (1894).

[4] H. Kossel, *Zeitschr. f. klin. Med.* **13**. 149 (1888); E. Stadelmann, *ibid.* **16**. 128 (1889); O. Simon, *Arch. f. experiment. Path. u. Pharm.* **49**. 449 (1903).

[5] F. Hofmeister, 'Über den Nachweis von Pepton im Harn,' *Zeitschr. f. physiol. Chem.* **4**. 251 (1880). W. D. Halliburton, 'The Proteids which may occur in Urine,' *Transactions of the Pathol. Soc.* London, **51**. Part II. 1900.

[6] H. B. J. Stokvis, *Zeitschr. f. Biol.* **34**. 466 (1896).

[7] E. Salkowski, *Berliner klin. Wochenschr.* 1897, Nr. 17.

[8] M. Henze, *Ber. d. deutsch. chem. Ges.* **34**. I. 348 (1901).

[9] Schmidt-Mülheim, *Arch. f.* (*Anat. u.*) *Physiol.* 1880, p. 33; Fano, *ibid.* 1881, p. 277; R. H. Chittenden, L. B. Mendel, and Y. Henderson, *Amer. Journ. of Physiol.* II. 142 (1899); W. H. Thompson, *Journ. of Physiol.* **20**. 455 (1896); **24**. 374 (1899); **25**. 1 (1899).

[10] E. P. Pick and K. Spiro, *Zeitschr. f. physiol. Chem.* **31**. 235 (1900).

[11] F. P. Underhill, *Amer. Journ. of Physiol.* **9**. 345 (1903).

blood-pressure, but antipeptone, protones (*i.e.* digestion-products of the protamins), histones, and arginin-carbonate, give negative results,[1] and the same holds good for the following substances:—Glycine or glycocoll, leucin, tyrosin, uracil, cytosin, indol, skatol, tryptophane, xanthin, hypoxanthin, guanin, thymin, glycin-ethylester, α-pyrrolidin carboxylic acid, α-methyl-pyrrolidin carboxylic acid, arginin, glutaminic acid, and glucothionic acids, according to Wolf.[2] Negative results were also obtained by Halliburton[3] on injecting E. Fischer's polypeptids: leucyl-glycin, leucyl-leucin, glycyl-asparagin, alanyl-leucyl-glycin. The effect produced on respiration by the repeated injection of peptone solutions, has been studied by Nolf.[4]

On injecting mono-amino acids into rabbits through a vein in the ear, and examining the urine by Pfaundler's method,[5] Stolte[6] found alanin, aspartic acid, glutaminic acid and cystin (Blum) to increase the amount of urea-N, and also that of the mono-amino acid N. Glycocoll and leucin, if given in very large amounts, give rise to a transient elimination of firmly bound N, and to a constant increase in the urea-N. The aromatic mono-amino-acids, tyrosin and phenyl-alanin, do not lead to an increase of urea formation in twenty-four hours.

Glässner[7] has found that the amino-acids, leucin and lysin, are only absorbed from the small and not from the large intestine, and that normally albumoses, amino- and diamino-acids, are only met with in the small intestine, while in the large intestine the dissociation-products of the different amino-acids, as well as xanthin bases and ammonia, are met with. See also pp. 108 to 114.

[1] W. H. Thompson, *Zeitschr. f. physiol. Chem.* **29**. 1 (1900), and **32**. 137 (1905).
[2] C. G. L. Wolf, *Journ. of Physiol.* **32**. 171 (1905).
[3] W. D. Halliburton, *ibid.* p. 174.
[4] P. Nolf, *Arch. de Biol.* **20**. 101 (1904).
[5] Pfaundler, *Zentralbl. f. Physiol.* **14**. 538 (1900).
[6] K. Stolte, *Hofmeister's Beiträge*, **5**. 15 (1904).
[7] K. Glässner, *Zeitschr. f. klin. Med.* **52**. p. 386.

CHAPTER VI

THE SALTS OF ALBUMINS

I. Theoretical Considerations

The behaviour of amino-acids under different conditions is to the biologist of very special interest, and therefore the physico-chemical aspect has been gone into somewhat more fully by the author.

Amino-acids in many respects resemble the pyrons,[1] for both possess the power of forming additive compounds with acids and with bases. Strecker[2] seems to have been the first to advance the view that amino-acids fix metals by their CO.OH radical, while they bind acids by the NH_2-group, and the same conclusion has been arrived at by Bredig,[3] Winkelblech,[4] and Walker,[5] who have studied amino-acids in the light of physical chemistry. Bredig uses the term 'amphoteric electrolyte' for any substance 'which may split off, or unite with, H° and OH′ ions,'[6] or, in other words, any substance which can play the part of an acid towards a base or that of a base towards an acid. According to this definition, water is an amphoteric electrolyte because its hydrogen-atom H and its hydroxyl-radical OH may be converted into the chemically active ions H° and OH′ whenever water comes into contact with certain salts, as will be shown more fully later. Alcohols $(C_nH_{2n+1})OH$ are also amphoteric electrolytes. Thus $(C_nH_{2n+1})OH$ can unite with sodium according to the

[1] R. Willstätter and R. Pummerer, *Ber. d. deutsch. chem. Ges.* **37**. 3740 (1904); and J. N. Collie, *Journ. Chem. Soc.* 1904, p. 971.

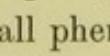
[2] F. A. Strecker, *Ann. d. Chem.* **148**. 87 (1886).

[3] G. Bredig, *Zeitschr. f. Elektrochemie*, 1899, No. 2.

[4] K. Winkelblech, *Über amphotere Elektrolyte und innere Salze*, Leipziger Dissertation, 1901; *Zeitschr. f. physikal. Chem.* **36**. 546 (1901).

[5] James Walker, *Zeitschr. f. physik. Chem.* **49**. 82 (1904).

[6] According to Ostwald's plan, a dot placed behind an atom or group of atoms signifies a positive electrical load, while a stroke stands for a negative load. In this book the dot has been replaced by a circle, thus H° stands for positive hydrogen-ion and Cl′ stands for negative chlorine-ion.

equation $(C_nH_{2n+1}O) - H + Na^\circ = C_nH_{2n+1}ONa + H$, when the alcohol remainder $[C_nH_{2n+1}O]'$ plays the part of an acid, the feeble kat-ion H° being replaced by the strong kat-ion sodium, Na°. On the other hand, the hydroxyl-group OH may be replaced by a stronger an-ionic radical, such as a chlorine-ion ; thus $[C_nH_{2n+1}]^\circ OH' + H^\circ Cl' = C_nH_{2n+1}Cl + H_2O$. This behaviour of alcohols depends on the presence of the OH radical, and it will readily be seen that other compounds which contain this OH radical will behave analogously. Such OH-compounds are, for example, serin (see p. 33) and all phenols,  OH (p. 49). Alcohols differ, however, from ordinary hydroxyl-compounds, as they only form alcohol-salts in the absence of water. These alcohol-salts on coming into contact with water dissociate hydrolytically, because water is hydrolysed by alcohol-salts. Hydrolysis is explained on p. 256.

The hydroxyl-compounds of most elements belonging to the middle regions of the periodic system may also play the part of either weak acids or weak bases (Winkelblech).

Thus, compounds having the constitution R·OH or H·R·OH may react in the following manner :—

1. Form simple kat-ions and an-ions. R·OH interacts with acids according to the scheme

$$ROH + H^\circ \ (+ Cl') \rightleftarrows R^\circ \ (+ Cl') + H_2O,$$

and with bases according to the equation

$$ROH + OH'(+ Na^\circ) \rightleftarrows RO'(+ Na^\circ) + H_2O.$$

An amphoteric electrolyte of this type will give off either a preponderating amount of OH′ or H° ions, according as to whether the remainder of the molecule has more basic or more acid properties ; thus—

$$ROH \rightleftarrows R^\circ + OH' \text{ or } ROH \rightleftarrows RO' + H^\circ.$$

Pluribasic acids such as aluminium hydroxide possess the following anions :—

$$Al(OH)_3 + 1Na^\circ + 1OH' \rightleftarrows Al(OH)_2O' + 1Na^\circ + 1H_2O$$
$$Al(OH)_3 + 2Na^\circ + 2OH' \rightleftarrows Al(OH)O_2'' + 2Na^\circ + 2H_2O$$
$$Al(OH)_3 + 3Na^\circ + 3OH' \rightleftarrows AlO_3''' + 3Na^\circ + 3H_2O.$$

Salt-formation, in water, is most complete in all pluribasic acids if one equivalent of base is present. With each additional basic radical the salt tends to hydrolyse more and more. The normal hydrate readily passes into the metahydrate AlO·OH.

2. Split off H° and OH′ ions, and then change into a non-electrolyte instead of becoming a real salt. This happens, for example, in the

case of As, Sb, Sn, and with the hydroxides of Titanium and Germanium.

With arsenic the following amphoteric reactions take place :—

$$As_2(OH)_6 + 6H^\circ + 6Cl' \rightleftarrows 2(AsCl_3) + 6H_2O,$$

and $$As_2(OH)_6 + Na^\circ + OH' \rightleftarrows As_2(OH)_5O' + Na^\circ + H_2O.$$

3. Attach H° and OH' ions to themselves and then form an-ions and kat-ions.

$$\begin{array}{l} R - NH_3 \\ | \quad | \\ COO \end{array} + Na^\circ + OH' \rightleftarrows \begin{array}{l} R - NH_3 . OH \\ | \\ COO' \end{array} + Na^\circ,$$

and

$$\begin{array}{l} R - NH_3 \\ | \quad | \\ COO \end{array} + H^\circ + Cl' \rightleftarrows \begin{array}{l} R - NH_3^\circ \\ | \\ COOH. \end{array} + Cl'$$

4. Give rise to ions which are simultaneously electro-positive and electro-negative (Bredig), and which Küster[1] calls 'Zwitter-ions,' *i.e* hermaphrodite-ions. Thus the 'Zwitter-ion' of glycocoll is the group $H_3N - CH_2 - COO$:

$$HO . H_3N . CH_2 . COOH \rightleftarrows \overset{-}{OH} + \overset{+}{H_3}N - CH_2 - \overset{-}{COO} + \overset{+}{H}.$$

5. Undergo a peculiar intramolecular change (Davidson and Hantzsch),[2] whereby there is formed the an-ion of a dibasic acid, as in the di-azonium compounds, represented by di-azonium-sulphanilic acid :

$$\begin{array}{l} C_6H_5—N : N \\ | \quad / \\ SO_3 \end{array} + 2Na^\circ + 2OH' \rightleftarrows \begin{array}{l} C_6H_4—N \\ | \quad \| \\ SO'_3 \; 'ON, \end{array} + 2Na + H_2O$$

and

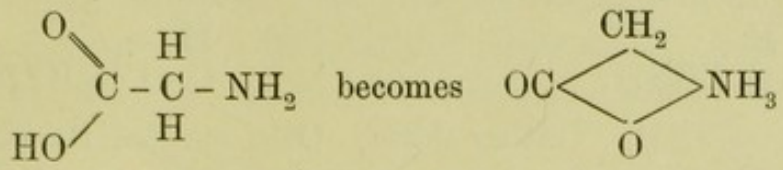
$$\begin{array}{l} C_6H_4—N : N \\ | \quad / \\ SO_3 \end{array} + H^\circ \quad + Cl' \quad \rightleftarrows \begin{array}{l} C_6H_4 - N^\circ \\ | \quad \| \\ SO_3H \; N. \end{array} + Cl'$$

The simultaneous presence of acid and of basic radicals in one and the same molecule must of necessity lead to a weakening of the acid or basic characters of the molecule towards other individual molecules, and must also set up within the amphoteric molecule a tendency towards 'internal salt-formation,' by which expression we mean that the acid and the basic radicals of an amphoteric electrolyte will tend to mutually satisfy one another. Whenever this tendency becomes an

[1] Küster, *Zeitschr. f. anorg. Chemie*, **13**. 136 (1897).

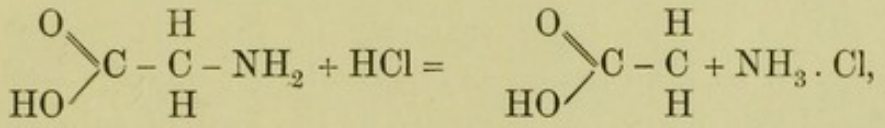
[2] W. B. Davidson and A. Hantzsch, *Ber. d. deutsch. chem. Ges.* **31**. 1612 (1898).

accomplished fact, then the previously open-chain compound is converted into a 'ring-compound.'[1] Thus

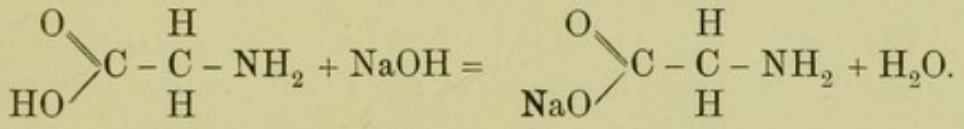


Chemically active glycocoll becomes chemically inactive glycocoll.

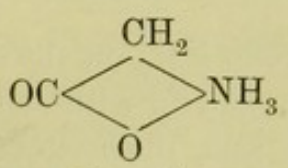
While glycocoll is in this inactive state it forms a true pseudo-acid-pseudo-basic compound; in other words, it cannot play the part of an an-ion or that of a kat-ion till the ring-like compound is reconverted into an open-chain. This change can only be brought about by subjecting the pseudo-acid pseudo-basic molecule to the influence of ions. If active or inactive glycocoll is brought into contact with a strong acid, such as hydrochloric acid, then glycocoll-hydrochloride is formed:



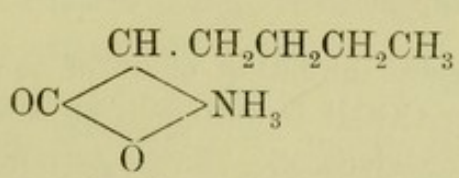
while with sodium hydrate it forms sodium glycocollate and water.



The proximity to or the remoteness from one another of the acid and basic radicals in the amphoteric amino-acid determines the ease with which an internal salt is made and is unmade. As most of the normally occurring mono-amino-acids are *α*-compounds (p. 21) in which the basic NH_2-group is as close to the acid COOH-group as possible, it follows that the length of the primary chain does not much interfere with the internal salt-formation as long as only one NH_2 radical and one COOH radical is present:



Glycocoll.

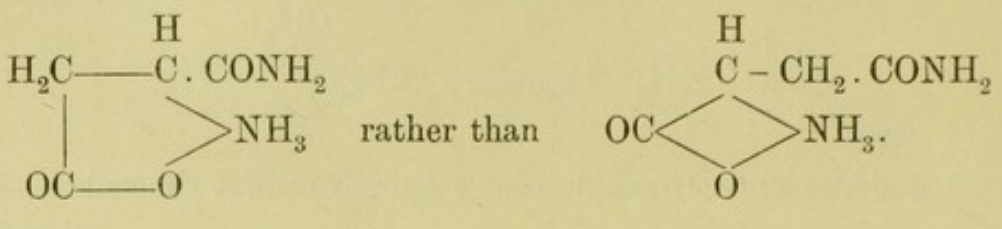


Leucin.

It is different, however, when two NH_2 and one COOH groups are present, or two COOH groups and one NH_2, as in the case of the mono-amino-di-carboxylic and di-amino-mono-carboxylic acids. From

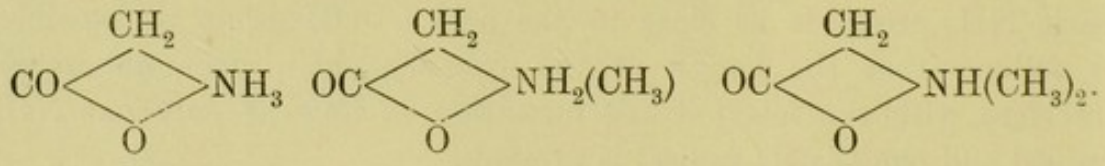
[1] Ring-formation by fusion of two molecules is described on p. 215. There are stereochemical difficulties in the way of one-molecule rings.

the fact that asparagin is a considerably stronger acid than is glycocoll, Winkelblech concludes that asparagin has the constitutional formula:



How the constitution of a molecule affects its acid and its basic character is not as yet fully understood. The author has for a considerable time investigated the problems of intramolecular coagulation in mono- and in di-amino acids, and hopes before long to be able to communicate definite facts bearing on this matter.

The last point to be considered is the relative strength of the acid and the basic radicals in the amphoteric amino-acids. If the carboxyl-group COOH is replaced by the much more strongly acid sulphonic radical SO_3, then the basic character of the NH_2-group may be diminished to such an extent as practically not to make itself felt at all. This holds good, for example, in the case of sulphanilic acid, $C_6H_4(NH_3)(SO_2O)$. On the other hand, the basic character of the NH_2-group may be strengthened by the introduction of alkyl-radicals (methyl, CH_3; ethyl, C_2H_5) till it is 10 to 20 times stronger than ammonia (Winkelblech). The acid character of the COOH radical may hereby be overcome so completely as to prevent the latter from acting as an acid radical, at least at the ordinary room-temperature. Thus betain develops acid characters only at zero-temperature (Davidson).[1] Glycocoll, sarcosin, and betain are in descending order less and less acid:



Glycocoll. Sarcosin. Betain.

The great inhibiting effect of the amino-group NH_2 on the carboxyl-group COOH is well seen by comparing acetic acid with amino-acetic acid or glycocoll. Thus $\frac{1}{3^{\frac{1}{2}}}$ normal acetic acid, dissociating only to the extent of 2 per cent, is 500,000 times more strongly acid than is amino-acetic acid.

As amino-acids are too feeble to allow of their dissociation-constant being determined at the ordinary temperature, *e.g.* by either measuring their electrical conductivity or their rate of sugar-inversion

[1] Davidson, *Über Diazonium und normale Diazotate*, Dissertation, Würzburg, 1898.

or splitting of esters, Winkelblech combined Walker's[1] principle of estimating the strength of feeble bases by studying the hydrolysis of their chlorides, with the analogous method of Shields,[2] who studied the hydrolysis of the sodium salts of feeble acids. In this way it was possible to determine for each amino-acid the relative strengths of its acid and basic radicals.

Winkelblech gives the following ratios of the acidity to the basicity of certain amino-acids :—

	Acidity to basicity.		Acidity to basicity.
Aspartic acid . . .	53 millions : 1	Asparagin	3000 : 1
o-amino-benzoic acid . .	4 millions : 1	Alanin .	240 : 1
p- „ „ „ . .	2·6 millions : 1	Glycocoll .	120 : 1
m- „ „ „ . .	0·5 millions : 1	Leucin .	115 : 1
		Sarcosin .	72 : 1

Further information on these complicated relationships, and particularly on the points in which amphoteric electrolytes differ from ordinary electrolytes, will be found in Walker's recent paper.[3]

The most remarkable property of amino-acids is their strong hydrolysis, which means that the salts which amino-acids form with other acids or bases are very readily broken up by the ions of water. If a strong base is linked to a strong acid,—if, for example, equivalent amounts of hydrochloric acid and of caustic soda are dissolved in water,—then the acid hydrogen-ion of the HCl unites with the alkaline hydroxyl-ion of the NaOH to form neutral water, while the sodium- and the chlorine-ions form a neutral salt. In this case the negative chlorine-ions and the positive sodium-ions possess a great electro-affinity for one another, and therefore they do not unite with either the feeble, acid hydrogen-ions or the feeble, alkaline hydroxyl-ions of the water. But if, instead of two such strong radicals as sodium and chlorine, there be present one feeble radical, *e.g.* an amino-acid, in combination with a strong acid or a strong base, then the strong radical will not join up with the amino-acid, which is a more feeble radical than are the ions of water, but will combine with one of the ions of the water.

Generally speaking, any amphoteric electrolyte H·R·OH will form, according to Walker, the ions H°, OH′, HR°, and ROH′, while the non-ionised portion must be either in the state of a hydrate H·R·OH or that of an anhydride R. Therefore an equilibrium in the solution depends on the factors

$$\overset{+}{H} \quad \overset{-}{OH} \quad \overset{-}{ROH} \quad \overset{+}{RH} \quad HROH \quad R.$$

[1] James Walker, *Zeitschr. f. physik. Chem.* **4**. 319 (1889).

[2] Shields, *ibid.* **12**. 167 (1893).

[3] James Walker, *ibid.* **49**. 82 (1904).

Walker's views on amphoteric electrolytes from the standpoint of the electrolytic dissociation-theory have been summarised by John Johnston[1] in the following way: If $H \cdot X \cdot OH$ is an amphoteric electrolyte with the acid-constant k_a and the base-constant k_b, and if K_w is the dissociation-constant of water:

$$[H+] = \sqrt{\frac{K_w + k_a \cdot [H \cdot X \cdot OH]}{1 + \frac{k_b}{K_w} \cdot [H \cdot X \cdot OH]}}$$

$$[OH-] = \frac{K_w}{[H+]}$$

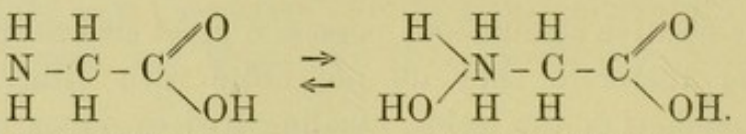
$$[HX+] = \frac{k_b}{K_w} \cdot [H^+] \cdot [H \cdot X \cdot OH]$$

$$[XOH-] = \frac{k_a \cdot [H \cdot X \cdot OH]}{[H+]}.$$

II. General Account of Salt-Formation

In working with amino-acids and with albumins it is necessary to constantly keep the salt-forming power of these substances before our mind's-eye, in addition to their physical characteristics, which will be explained later on. Glycocoll, being the simplest amino-acid, is therefore taken as a type.

Normal Glycocoll, $NH_2.CH_2.COOH$.—Winkelblech assumes that a watery solution of glycocoll contains 99·967 per cent of hydryated but non-dissociated molecules,



Normal glycocoll. Hydrated glycocoll.

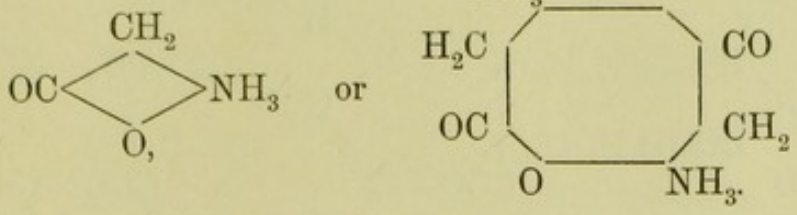
while the minimal remainder is made up of a few ions and of non-hydrated glycocoll molecules. He explains the neutral reaction of a watery solution of glycocoll as being due to the want of dissociation of the hydrated amino-acid. Although, then, the presence of glycocoll leads to a union of the water-ions with the amino-acid, there is no hydrolytic dissociation of the newly-formed compounds. A hydrated amino-acid does not dissociate, because both the acid and basic radicals are very feeble.

Walker[2] believes that the glycocoll molecules in watery solutions either form internal salts or that they unite in pairs, in such a way

[1] J. Johnston, *Ber. d. deutsch. chem. Ges.* **37.** 3625 (1904).

[2] James Walker, *Zeitschr. f. physik. Chem.* **49.** 82 (1904), and independently by the author, *Trans. Oxford Junior Scient. Soc.* 1904.

that the acid radical of one molecule links on to the basic radical of a second molecule : [1]



Whatever change an amino-acid undergoes, whether it form a ring-like compound on becoming an internal salt, or whether it form double molecules, or whether it become hydrated, or whether it unite with acids, the originally trivalent nitrogen always become pentavalent.

Glycocoll Hydrochloride, $ClH_3N.CH_2.COOH$, by hydrolysis sets free neutral glycocoll $H_2N.CH_2.COOH$ and hydrochloric acid, which then dissociates into the ions $H^\circ + Cl'$. The solution reacts strongly acid, owing to the hydrogen-ions, and it conducts the electric current mostly as hydrochloric acid, and to a very slight extent as the hydrochloride of glycocoll.

Glycocollate of Sodium, $H_2N.CH_2.COONa$: by hydrolysis neutral glycocoll and sodium hydrate are set free. The latter dissociates electrolytically into OH'- and Na°-ions. The solution has a strongly alkaline reaction owing to the hydroxyl-ions, and it conducts the current mostly as sodium hydrate, and to a very slight extent as sodium glycocollate.

In connection with the union of amino-acids with carbon-dioxide Siegfried [2] has made the following observation, which is of the greatest physiological importance :—

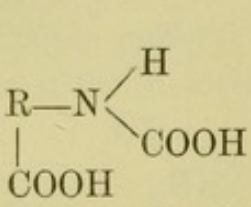
On saturating a mixture consisting of equal volumes of equinormal solutions of glycocoll and barium-hydroxide, an alkaline solution is obtained which remains clear when CO_2 is passed through it, and this continues to be the case till for each volume of glycocoll nearly two volumes of equivalent baryta water has been added. The solution so obtained gives off barium carbonate slowly on standing and quickly on boiling. Analogous results are obtained on substituting for glycocoll—i-alanin, l-leucin, sarcosin, phenyl-glycocoll, aspartic acid, glutaminic acid or asparagin, and on replacing barium-hydroxide by calcium or sodium hydroxide, and finally on substituting for CO_2 sodium carbonate.

Analyses of the compounds which glycocoll, i-alanin, l-leucin, sarcosin,

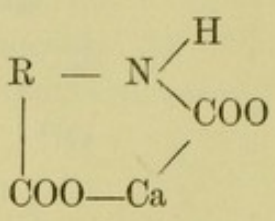
[1] The formation of anhydrides or di-acipiperazin rings is explained on pp. 55, 132.

[2] M. Siegfried *Zeitschr. f. physiol. Chem.* **44**. 85 (1905).

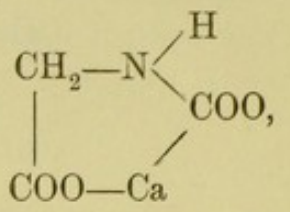
and phenyl-glycocoll form with calcium-hydroxide and CO_2 have shown that these amino-acids must contain the radical



Carbamino-acid radical,



Limesalt of same,



Calcium carbamino-acetate or calcium glycocoll-carbonate,

that is, the normal lime-salts of the hitherto unknown dibasic carbamino-acids of the glycocoll series. These compounds are therefore formed by the amphoteric amino-acids, simply adding CO_2, which thereby becomes de-ionised.

Quite analogous to the amino-acids behave the peptones, crystalline serum-albumin and dialysed horse-serum. Siegfried points out that the union of CO_2 in the blood appears in a new light, especially in connection with the hypothesis of Setschenow as to conversion of serum albumin into carbo-albumin by the action of CO_2, and also in connection with the carbo-hæmoglobin of Bohr.

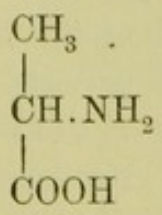
Siegfried assumes that during muscular activity, in addition to the view according to which muscle-proteid first takes up O_2 and then decomposes, CO_2 being given off, another view will have also to be considered, namely, that CO_2 is bound temporarily as a carbonate, which then undergoes hydrolysis secondarily and so sets free CO_2. This temporary binding of CO_2 would facilitate oxidative processes going on in the muscle.

In connection with chlorophyll, the CO_2 taken up may be converted into a carbamino-group, and therefore in future we have, in addition to the question of how CO_2 becomes reduced, also to keep in mind the question of how carbamino-acids are reduced.

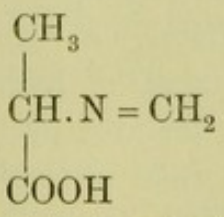
The double nature of amino-acids, *i.e.* to act either as acids or as bases, is interfered with as soon as one of the NH_2 or COOH radicals is bound up. Curtius and Göbel[1] have shown that glycin-ester, $H_2N.CH_2.COOC_2H_5$, and E. Fischer[2] that other amino-acid-esters are strong bases (as already mentioned in connection with sarcosin and betain, p. 212); Schiff,[3] on the other hand, found methylene-compounds to be acids, as the amino-radical is joined to formaldehyde;

[1] T. Curtius and F. Göbel, *Journ. f. prakt. Chem.* (2) **37**. 150 (1888).
[2] E. Fischer, *Ber. d. deutsch. chem. Ges.* **34**. I. 433 (1901).
[3] E. Schiff, *Liebig's Ann.* **310**. 25 (1899), **316**. 242 (1901), **319**. 59 (1901).

thus alanin is approximately neutral, while methylene-alanin is strongly acid :



Alanin.



Methylene-alanin.

The presence of a second NH_2 or COOH group does not alter the general character of an amino-acid, but the basic character predominates in lysin, and the acid character in glutaminic acid, but notwithstanding this the latter can act as a base, for it forms chlorides.

Albumins behave in exactly the same way as do the amino-acids. According to Sjöqvist,[1] Cohnheim,[2] Cohnheim and Krieger,[3] Erb,[4] Bugarszky and Liebermann,[5] and von Rhorer,[6] albumins react as bases towards acids, being in some cases even more basic than the amino-acids (see below). According to von Rhorer albumins are about 500 times more basic than is distilled water, and according to Sjöqvist about 74·2 times more feeble than is anilin. With acids they form salts which undergo great hydrolysis.

According to Erb the hydrolysis of the chlorides of albumins amounts to 88 per cent, and is probably even greater. Arrhenius and Ley[7] and others have further shown that the hydrolysis is not a constant factor, because, apart from temperature, it varies with the absolute and the relative amounts of the albumin and the acid present. Thus the greater the concentration of, for example, albumin-chloride, the less is its hydrolysis, so that dilute solutions contain more free hydrochloric acid and less albumin-chloride than do concentrated solutions. If, on the other hand, there is present more hydrochloric acid than is needed for rectifying the basic tendencies of an amino-acid or an albumin, hydrolysis is diminished ; with a large excess of hydrochloric acid hydrolytic dissociation is nearly absent, while hydrolysis amounts to 80-90 per cent if acid and albumin are in equivalent solutions. Erb has studied these phenomena most minutely on pure albumins : serum-albumin, egg-albumin, edestin, and hetero-albumose.

[1] J. Sjöqvist, *Skandinav. Arch. f. Physiol.* **5**. 277 (1894) (here the older literature is given), **6**. 255 (1895) ; *Zeitschr. f. klin. Med.* **32**. 451 (1896).

[2] O. Cohnheim, *Zeitschr. f. Biologie*, **33**. 489 (1896).

[3] O. Cohnheim and H. Krieger, *ibid.* **40**. 95 (1900).

[4] W. Erb, *ibid.* **41**. 309 (1901).

[5] St. Bugarszky and L. Liebermann, *Pflüger's Arch. f. d. ges. Phys.* **72**. 51 (1898).

[6] L. von Rhorer, *ibid.* **90**. 368 (1902).

[7] H. Ley, *Zeitschr. f. physikal. Chem.* **30**. 193 (1899).

He found that 1 gram of edestin is equivalent to 212 milligrams HCl if there be a large excess of hydrochloric acid, while it combines with only 26 milligrams, *i.e.* with only 12 per cent of the 212 milligrams, if there is no excess of hydrochloric acid; 88 per cent of the hydrochloric acid is liberated by hydrolysis. If one dissolve serum-albumin in one-tenth normal hydrochloric acid, then 1 gramme will bind 0·104 milligramme HCl; on diluting this solution with an equal bulk of 1:10 *n*-HCl, it binds 0·142 gramme; if it be diluted four times with 1:10 *n*-HCl it binds 0·204 gramme. By means of another method Sjöqvist found serum-albumin to bind 0·12 to 0·13 gramme.

Different albumins differ not only in their capacity for binding acids, but give different curves, when these are so constructed as to show that dissociation depends not only on the concentration but also on the excess of the acid. If a weaker acid be taken instead of hydrochloric acid, then the dissociation becomes even more marked.

Albumins behave quite analogously when they combine with bases; Bugarszky and Liebermann[1] and Spiro and Pemsel[2] have shown that sodium albuminate exhibits marked and varying hydrolysis.

One essential difference exists, however, between albumins and the simple amino-acids: the albumins are pluri-acid bases and pluri-basic acids. On p. 147 a comparison has already been made between the equivalent weights as determined by Erb, and the minimal molecular weight as calculated from the percentage composition; egg-albumin must be at least 35-acid and serum-albumin at least 56-acid. According to Sjöqvist egg-albumin is at least 19-acid. Regarded as acids they appear to be less basic, according to Spiro and Pemsel. According to Laqueur and Sackur[3] casein in its acid capacity is 4 to 6-basic.

Hydrolytic dissociation produces thus this effect: 1 gram of albumin neutralises according to its own concentration and that of the acid or base with which it comes into contact, and also according to the nature of the acids and bases, quite different amounts of these acids or bases, a phenomenon which for a long time made it very difficult to understand what was really taking place. Sjöqvist was the first to speak in clear terms of the chlorides of albumins and to deduce their properties from the laws formulated by Arrhenius. Then Spiro and Pemsel expressed the reaction between albumins, acids and bases, not as a salt-formation but as a process of distribution along physical lines. Cohnheim and Krieger described albumins as pseudo-bases and pseudo-

[1] St. Bugarszky and L. Liebermann, *Pflüger's Arch. f. d. ges. Phys.* **72**. 51 (1898).

[2] K. Spiro and W. Pemsel, *Zeitschr. f. physiol. Chem.* **26**. 233 (1898).

[3] E. Laqueur and O. Sackur, *Hofmeister's Beiträge*, **3**. 193 (1902).

acids in the sense of Hantzsch, to convey the idea that albumins in watery solutions are non-electrolytes, while in acid solutions they act as bases and in basic solutions as acids. Cohnheim now says: "Since Bredig and Winkelblech have shown that glycocoll and other simple amino-acids behave in exactly the same way, the hypothesis of Cohnheim and Krieger has become superfluous, and the riddle as to how albumins act has been solved by studying the behaviour of its constituent radicals."

The author is at a loss to understand this passage. In his *Physiological Histology* in 1902 he has made no difference between amino-acids and albumins, for he recognised them to be homologous compounds. To say the theory of albumins being pseudo-acids or pseudo-bases has become superfluous, because the simple amino-acids behave as do albumins, is not right. The correct and only conclusion to be drawn is that both albumins and amino-acids have a great tendency to be converted normally into pseudo-compounds. The author believes it very important to always remember that the waste in our body would be enormous if it were not for the ring-formation which amino-acids undergo when they assume the pseudo-acid—pseudo-basic state. He has held for years[1] "that so-called pure ash-free albumins (proteids) are chemically inert, and, in the true sense of the word, dead bodies." "What puts life into them is the presence of electrolytes, either unorganised or organised." "Thus tissues fixed in (neutral) absolute alcohol are potential acids and bases, or, as it is termed, are in the state of pseudo-acids and pseudo-bases, and are converted into real acids by the addition of bases, and into real bases by the addition of acids."

Let us therefore continue using Hantzsch's expressions 'pseudo-acid' or 'pseudo-base' to designate that ring-formation in amphoteric electrolytes by which chemically active compounds are converted into chemically inactive ones.

Pseudo-acids and pseudo-bases are only a special case of the amphoteric electrolytes discussed on p. 208, as shown by the investigations of Hantzsch into the nature of the alcoholic solutions of metallic hydrates, as in this class of amphoteric electrolytes a change in the ionic state goes hand in hand with a change in constitution. Zawidzki[2] also points out that amphoteric electrolytes, pseudo-acids, and pseudo-bases have this in common, that the intra-molecular change during the formation of ions may vary greatly in individual cases, but that in strong electrolytes it cannot occur by the addition, or the

[1] Mann, *Physiological Histology*, 1902, pp. 2, 25, 224, 338, 345, 348.

[2] Zawidzki, *Ber. d. deutsch. chem. Ges.* **37**. 153 (1904).

subtraction, of an oxygen-atom without changes in the constitution of the compounds. The theory of pseudo-acids is further discussed by Zawidzki in a later paper,[1] and the substitution of carbon-remainders for hydroxyl-groups in pseudo-bases by Martin Freund.[2]

Hydrolytic dissociation must always be taken into account under the following conditions:—

1. Between the water, the acids or bases, and the albumins a state of equilibrium exists for any given concentration, and this equilibrium is altered whenever one of the factors is changed. For this reason it is impossible to determine by titration the acidity or basicity of an albumin solution. Each cubic centimetre of NaOH solution added to a solution of an albumin-chloride diminishes the excess of hydrochloric acid, and thereby leads to an increase of hydrolytic dissociation, till ultimately nearly the whole of the hydrochloric acid is split off from the albumin and is thereby rendered free. Titration amounts, therefore, simply to an estimation of the whole of the acid with which the albumin was united, and the result obtained is the same as if the albumin had not been present at all. This method of titration is made use of in determining the acidity of gastric contents, in which albumin and albumose chlorides are present in addition to free hydrochloric acid. When titrating with rosolic acid or with phenolphthalein, the albumin chlorides are estimated as well as the free hydrochloric acid, and therefore one speaks of 'the total acidity.' As the state of equilibrium between albumin, salts, and water, is changed every time a substance is added, one ought never to use kinetic methods for determining the basicity of albumins, *i.e.* methods which depend on the removal or the using up of one of the components. This was pointed out by v. Rhorer. To overcome this difficulty Sjöqvist and Bugarszky and Liebermann determined the basicity of albumins by determining the electrical conductivity of albumin-chlorides, while Cohnheim estimated the free hydrochloric acid by its sugar-inverting power. Both methods are very complicated, and it has been shown that results of sufficient accuracy can usually be obtained by other methods. Cohnheim and Spiro and Pemsel have salted out the albumin-chlorides, which is quite as easy as salting out the albumins themselves, and have then determined in the filtrate the free hydrochloric acid. Still simpler is the method of Cohnheim and Krieger,[3] who precipitate the

[1] Zawidzki, *Ber. d. deutsch. chem. Ges.* **37**. 2298 (1904).

[2] Martin Freund, *ibid.* **37**. 4666 (1904).

[3] O. Cohnheim and H. Krieger, *Zeitschr. f. Biol.* **40**. 95 (1900); *Münchener medizin. Wochenschr.* 1900, p. 381.

chlorides of albumins by means of the neutral alkaloidal-precipitants, and then determine the hydrochloric acid in the filtrate. The reaction occurs according to the equation :

Calcium-phosphotungstate + albumin-hydrochloride =
Albumin-phosphotungstate + calcium-chloride.

The insoluble albumin-phosphotungstate is precipitated, and the filtrate may then be titrated without fear of hydrolytic dissociation. By means of this method Erb determined his figures. The same method may also be used for the albumoses of the gastric contents.[1]

A still simpler method is one which has been used for years for clinical purposes, namely, the titration of the albumin-chlorides by means of certain indicators 'for free hydrochloric acid,' such as phloroglucin vanillin, tropaeolin, Congo-red, methyl-violet, etc. Of these Günzburg's reagent (phloroglucin vanillin) and tropaeolin give, according to Cohnheim and Cohnheim and Krieger, approximately correct values.

The power albumins possess of combining with bases has been determined by Bugarszky and Liebermann, who measured the electrical conductivity, and by Spiro and Pemsel, who employed the salting-out method.

2. The alkaloidal reagents form with albumins insoluble salts, but these salts readily undergo hydrolysis and remain in solution if free acid be not present. Such weak bases as the albumins require, therefore, a certain excess of acid, *e.g.* phosphotungstic acid, to become precipitated. Neutral phosphotungstates, picrates, tannates, iodine, potassium iodide, biniodide of mercury in potassium iodide, potassium ferrocyanide, do not precipitate albumin—they only do so if the reaction be acid. The reagents generally used are therefore bin-iodide of mercury in potassium iodide + hydrochloric acid ; potassium ferrocyanide + acetic acid ; picric acid ; tannic acid ; and phosphotungstic acid. v. Rhorer also states that feeble acids, such as acetic or lactic acid, cannot take the place of hydrochloric acid. Only the histones are sufficiently strong bases to be precipitated if the reaction be neutral, while the still more strongly basic protamins are precipitated if the reaction be even alkaline, and in this respect the protamins resemble the alkaloids. The bases contained in albumin are likewise only completely precipitated by phosphotungstic and sulphuric acids. After precipitates have once been formed, they will remain permanent only if there be a certain excess of acid, for which reason

[1] O. Cohnheim and H. Krieger, *Zeitschr. f. Biol.* **40**. 95 (1900) ; *Münchener medizin. Wochenschr.* 1900, p. 381.

a precipitate made with phosphotungstic acid redissolves on being washed, for some time, with water. The objections which v. Rhorer has raised against Erb's estimations of hydrolysis by means of calcium phosphotungstate are not valid because the phosphotungstates undergo hydrolysis themselves.

That firm or solid salts of albumin may be dissociated by water should always be remembered when working with albumins.

Precipitates of albumins contain usually large quantities of other bodies, such as inorganic acids and bases, colouring matters, sugars, glycogen, lecithin, other albumins, ferments, etc. These may be removed more or less completely by prolonged washing, and therefore it was supposed that they were simply carried down mechanically and not bound chemically to the albumins. Jacoby[1] and others, who have worked at the purification of ferments, show, however, that this cannot be the case, as the admixtures are held by the albumins in chemical union, which latter is loosened whenever hydrolysis takes place.

In addition to the salts mentioned up till now, others have been described, in which the acid or the base is present in much smaller amount, and in which the acid or base cannot be removed so readily. Osborne[2] investigated the chlorides, dichlorides, sulphates, and nitrates of edestin, and the sodium and potassium salts of edestin. He estimates the equivalent weight of edestin at 14,300 to 7000, and states that the acid or base is very difficult to remove even after great dilution and prolonged washing. Harnack[3] describes similar salts of denaturalised egg-white with copper, Werigo[4] with sodium, and both agree in giving the equivalent weight of albumin as 4748. The equivalent weight of casein has been estimated, from calcium[5] and ammonium salts,[6] to be approximately 5100 by Hammarsten,[7] Söldner,[8] and Salkowski.[6] In the presence of an excess of alkali the equivalent weight of casein, according to Spiro and

[1] M. Jacoby, *Zeitschr. f. physiol. Chem.* **30**. 135, 166 (1900); *Arch. f. experiment. Path. u. Pharmakol.* **46**. 28 (1901); *Hofmeister's Beiträge*, **1**. 51 (1901).

[2] T. B. Osborne, *Journ. Amer. Chem. Soc.* **21**. 486 (1899); *Zeitschr. f. physiol. Chem.* **33**. 241 (1901).

[3] E. Harnack, *ibid.* **5**. 198 (1881); *Ber. d. deutsch. chem. Ges.* **23**. 3745 (1890); *Zeitschr. f. physiol. Chem.* **19**. 299 (1894).

[4] B. Werigo, *Pflüger's Arch. f. d. ges. Physiol.* **48**. 127 (1891).

[5] O. Hammarsten, *Ges. d. Wiss. zu Upsala*, 1877; F. Söldner, Dissertation, Erlangen, 1888.

[6] E. Salkowski, *Zeitschr. f. Biol.* **37**. 401 (1899).

[7] O. Hammarsten, *Ges. d. Wiss. zu Upsala*, 1877.

[8] F. Söldner, Dissertation, Erlangen, 1888.

Pemsel, is not more than 1178. The numbers given by Bülow[1] for the chlorides, and by Chittenden and Whitehouse[2] for the salts of the heavy metals of egg-white, differ more widely.

These salts differ from those previously described in possessing an approximately neutral reaction, a fact which also holds good for the casein salts, although casein is distinctly acid in its free state. Besides the above-mentioned salts, the authors just named describe other salts containing a higher percentage of metal and possessing a distinctly alkaline reaction. In dealing with these salts we have to keep in mind two possibilities: the small amounts of acid or of base which are found represent either a last remnant which has escaped hydrolysis, in which case the relative constancy of the values obtained has to be explained on the ground that the solubility of these salts remains fairly constant under the conditions of experimentation, or there are differences between the acid or the basic groups in the albumin-molecule, some giving rise to stable salts, while others undergo extensive hydrolysis. It has already been mentioned that it is possible for such differences to exist amongst amino-acids, even if the presence of sulpho-radicals is left out of account.

Both amino-acids and albumins may be deprived of their basicity by means of formaldehyde, there being formed, according to Blum,[3] Benedicenti,[4] Schiff,[5] and Schwarz,[6] methylene-albumins with markedly acid properties.

Albumins and all their derivatives resembling albumin possess, without exception, the power of uniting with acids and with bases as kations and as anions; but as in the case of the amino-acids so here either the basic or the acid character may predominate.

The albumins proper seem to possess a neutral, if anything a slightly alkaline, reaction.

Nucleo-albumins and *mucins* are strongly acid, as they displace carbonic acid from carbonates and as they redden litmus paper; they are precipitated by acids and dissolved by alkalies. The same holds good, although to a lesser degree, for the nucleo-proteids, with their nucleic acid radical, and holds good also for the globulins.

Histones, on the other hand, are basic substances which are precipitated by alkalies and dissolved by acids. Still more strongly basic

[1] K. Bülow, *Pflüger's Arch. f. d. ges. Physiol.* **58**. 207 (1894).

[2] R. H. Chittenden and H. H. Whitehouse, *Maly's Jahresber. f. Tierchem.* **17**. 11 (1887).

[3] F. Blum, *Zeitschr. f. physiol. Chem.* **22**. 127 (1896).

[4] A. Benedicenti, *Arch. f. (Anat. u.) Physiol.* 1897, p. 217.

[5] H. Schiff, *Liebig's Annalen*, **319**. 287 (1901).

[6] L. Schwarz, *Zeitschr. f. physiol. Chem.* **31**. 460 (1900).

are the protamins, which possess, however, a different constitution. Both histones and protamins form salts with nucleic acid, which in some cases are called nucleo-proteids.

Albumoses contain acid as well as basic substances, and react as a rule slightly alkaline.

Siegfried's peptones are strong acids. On being dissociated with acids they liberate the strong base kyrin (see p. 200).

Special attention is drawn to the fact that any albumin or its derivative maintains its amphoteric character (see p. 208), however acid or however basic it may be, although of course either the acid or the basic character will predominate.

III. The Individual Salts

The chlorides, nitrates, and sulphates of all albumins are much more soluble in water than are the pure albumins; they are also soluble in fairly strong alcohol, and differ in this respect from neutral albumins. According to Paal,[1] peptone-chlorides are soluble in absolute methyl alcohol, and in mixtures of methyl alcohol and glacial acetic acid. According to Mörner,[2] the crystalline serum-albumin, prepared by Krieger's method,[3] is the sulphate, while the crystalline egg-albumin of Hopkins and Pinkus[4] is the acetate. That gastric digestion gives rise to chlorides of the albumoses and peptones has already been mentioned. The salts of albumins with phosphotungstic acid and other alkaloidal reagents are insoluble, for which reason these reagents are used as precipitating agents. The peptone-phosphotungstates are soluble in alcohol according to Cremer.[5] The metaphosphates described by Lorenz[6] and Fuld[7] show distinct hydrolysis, and they have already been mentioned in connection with the nucleo-proteids.

The salts which albumins form with ammonia, with fixed alkalies, and with the alkaline earths, are still more important, because the acid albumins, nucleo-albumins, mucins, etc., occur in nature as such readily soluble salts. According to Friedrich Müller,[8] normal mucin is an alkali salt of mucin, while, according to Hammarsten,[9]

[1] C. Paal, *Bericht. d. deutschen chem. Ges.* **25**. II. 1202 (1892).

[2] K. A. H. Mörner, *Zeitschr. f. physiol. Chem.* **34**. 207 (1901).

[3] H. Krieger, *Strassburger*, Dissertat. 1899.

[4] F. G. Hopkins and S. N. Pinkus, *Journ. of Physiology*, **23**. 130 (1898).

[5] M. Cremer, *Münchener Ges. f. Morphol. u. Physiol.* 1898, fasciculus III.

[6] R. Lorenz, *Pflüger's Arch. f. d. gesamte Physiol.* **47**. 189 (1890).

[7] E. Fuld, *Hofmeister's Beiträge*, **2**. 155 (1902).

[8] Fr. Müller, *Schleim der Respirationsorgane; Sitzungsber. d. Ges. z. Bef. d. ges. Naturwissensch. zu Marburg*, 1896, p. 53.

[9] O. Hammarsten, *Kgl. Gesellsch. d. Wissensch. zu Upsala*, 1877, Reprint.

Lehmann,[1] and others, casein is calcium caseinogenate. The reserve-material in the seeds of plants, the crystalline phyto-vitellines, seem to occur always in the form of their calcium and magnesium salts,[2] and as such they also crystallise most readily under artificial conditions. Osborne[3] rightly points out that observers have frequently examined these albumin-salts under the belief that they were dealing with pure albumins.

Albumins form insoluble salts with the heavy metals, and hence ferric chloride, copper sulphate, etc., are used for precipitating albumins. (See p. 303.)

Organic bases will also combine with albumins to form salts: Spiro[4] showed that the denaturalised albumin 'albuminic acid,' combined with cholin, pyridin, anilin; further, with such basic substances as urethane, urea, thiourea; finally, also with oil of mustard. The importance of these bases, of the calcium salts, etc., is discussed under the heading of Coagulation in Chapter VIII.

IV. The Salts formed by the Union of Albumins with Aniline Dyes[5]

That histological staining reactions are microchemical reactions has been held for a long time by Miescher,[6] Ehrlich,[7] Knecht,[8] Knecht and Appleyard,[9] Malfatti,[10] Mann,[11] E. Zacharias,[12] Lilienfeld,[13]

[1] W. Hempel and J. Lehmann, *Pflüger's Arch. f. d. ges. Physiol.* **56**. 558 (1894).

[2] O. Schmiedeberg, *Zeitschr. f. physiol. Chem.* I. 205 (1877); G. Grübler, *Journ. f. prakt. Chem.* **131** (2 F. 32), 97 (1881).

[3] T. W. Osborne, *Journ. of the Amer. Chem. Soc.* **21**. 486 (1899).

[4] K. Spiro, *Zeitschr. f. physiol. Chem.* **30**. 182 (1900).

[5] A full account will be found in Mann's *Physiological Histology*, 1902, pp. 327-370.

[6] Miescher, *Verh. d. naturf. Ges. in Basel*, **6**. 138-208 (1874).

[7] Ehrlich, *Farbenanalytische Untersuchungen z. Hist. u. Klinik. d. Blutes*, Berlin, 1891, Hirschwald.

[8] Knecht, *Ber. d. deutsch. chem. Ges.* **21**. 1556 (1888); *Rév. Gén. des Mat. Col.* **4**. 251 (1900); *Ber. d. deutsch. chem. Ges.* **35**. 1022 (1902).

[9] Knecht and Appleyard, *ibid.* **22**. 1120 (1889). See also Knecht, Rawson, and Loewenthal, *Handbuch der Färberei d. Spinnfasern*, Berlin, 1895.

[10] Malfatti, *Ber. d. naturw. med. Vereins in Innsbruck* (1891-92).

[11] G. Mann, 'The Embryo-sac of *Myosurus minimus*: a Cell Study,' *Trans. and Proceed. of Bot. Soc. Edinburgh*, 1892, p. 351; 'The Functions, Staining Reactions, and Structure of Nuclei,' *Brit. Assoc. for the Advancement of Science*, 1892, p. 753; *Physiological Histology*, 1902, pp. 365-369.

[12] Zacharias, *Ber. d. deutsch. bot. Ges.* 11 (1893).

[13] Lilienfeld, *Arch. f. Anat. u. Physiol.* (1893); and in *Zeitschr. f. physiol. Chem.* **18**. (1894).

Weber,[1] Heine,[2] Walker and Appleyard,[3] Albert Mathews,[4] Vignon,[5] Prud'homme,[6] Wichmann,[7] Gillet,[8] Nietzki,[9] Hallit,[10] Macallum,[11] Martin Heidenhain,[12] Bethe, and others.

Albert Mathews in 1898 pointed out that albumins and albumoses, being weak bases, readily unite with free acids to form salts which may be either soluble or insoluble. Thus, on adding to albumin or albumose a solution of the free picric acid, metaphosphoric, molybdic, tungstic, phosphotungstic, tauric, stearic, or chromic acids, a coagulum is formed at once. Should these acids be employed in the form of their salts, no precipitate is formed till by the addition of a few drops of acetic or hydrochloric acid the solution containing, for example, the mixture of albumose and sodium picrate has been rendered slightly acid. Whenever the acid is added the albumose picrate is thrown down at once, "probably because the acetic acid sets free the picric acid." All the colour acids used by histologists react in exactly the same manner. These dyes are employed generally in the form of neutral salts, and require the addition of some acidifier, when they at once combine with the albumin, forming a dense coagulum Mathews obtained in this way albumin and albumose precipitates with acid-fuchsin, acid-green, nigrosin, aniline-blue-black, erythrosin, Congo-red, methyl-blue, sodium carminate, and indigo-carmine by using acid (or alkaline, see later) solutions of albumins and albumoses. "This reaction of the acid stains indicates beyond doubt that these stains, when in acidulated solutions, will enter into chemical combination with the albumose- or albumin-molecule like any other acid. Inasmuch as it is possible that the free acids enter one or more of the basic NH_2-groups of the albumin-molecule, the acid stains also probably enter this group." Colour-bases can also be made to unite with albumin and albumoses: if lead acetate is brought into a neutral solution of albumin or albumose nothing happens,

[1] Weber, *Journ. of Soc. Chem. Industr.* **13**. 120 (1894).

[2] Heine, *Zeitschr. f. physiol. Chem.* **21**. (1895).

[3] Walker and Appleyard, *Trans. Chem. Soc.* (1896), p. 1334.

[4] Matthews, *Amer. Journ. of Physiol.* 445-454, July 1898.

[5] Vignon, *Compt. Rend.* 357-360 (1897); abstract in *J. Soc. Chem. Indus.* 1014 (1897). [6] Prud'homme, *Rev. Gén. des. Mat. Col.* **2**. 213 and **4**. 72.

[7] A. Wichmann, *Zeitschr. f. physiol. Chem.* **27**. 575 (1899).

[8] C. Gillet, *Rev. Gén. des Mat. Col.* **4**. 183-9 (1900).

[9] Nietzki, *Chemie d. organisch. Farbstoffe*, 1901.

[10] Hallit, *Journ. Soc. Dyers and Colourists*, **15**. 30 (1899); abstract in *J. Soc. Chem. Indust.* (1899), pp. 368-70.

[11] Macallum, *Journ. of Physiol.* **22**. 92-98 (1897). A complete abstract is given in Mann's *Physiological Histology* (1902), pp. 290, 291, 294.

[12] M. Heidenhain, *Pflüger's Arch. f. d. ges. Physiol.* **90**. 115 *bis*, 230 (1902), **96**. 440 (1903); *Münchener Medizin. Wochenschr.* 1902, No. 11; *Zeitschr. f. wissenschaftl. Mikroskop. u. mikrosk. Technik*, **19**. 431 (1902).

but as soon as the reaction of the fluid is rendered slightly alkaline with sodium carbonate, then an insoluble lead-albuminate is thrown down. As gelatine and protamin-solutions are not precipitated under the same conditions, and as they are deficient in the phenol group, it follows that in albumin and similarly constituted proteids the basic lead radical must join on to the 'acid hydroxyl' radical of the phenol. Colour-bases used by histologists are neutral salts, being generally the chlorides, hydrochlorates, etc., and react as does lead acetate. Thus, on bringing together a solution of basic-fuchsin, methyl-green, thionin, toluidin-blue, safranin, or other basic stains ("with the possible exception of vesuvin," which contains a mixture of colour acids and colour bases) with a solution of an albumose, nothing happens; but if a similar albumose solution be rendered slightly alkaline with sodium carbonate, then a "flocculent, coloured precipitate, consisting of the albumose in combination with the dye, is thrown down."

"These experiments prove that many of the basic dyes enter into chemical combination with the albumose molecule when in alkaline solutions, forming insoluble coloured compounds," and "reacting in this respect like basic lead acetate, protamin, histone, or other organic bases. . . . Basic dyes in alkaline solutions may thus be used for the detection of albumins in the cell, and indeed of albumins possessing a phenol or tyrosin group." It is thus beyond all doubt that ordinary colour acids and colour bases will combine under suitable conditions with albumins and albumoses to form definite salt-like compounds. The behaviour of dyes towards coagulated proteid has been also carefully studied by Mathews, but the reader is asked to consult either the original paper by Mathews or the abstract in the author's *Physiological Histology*, 1902, pp. 349-51.

Nietzki, in the last edition of his *Chemistry of Organic Dyes*, says in 1901 : "Certain facts speak for the view that the union of dyes with fibres is a salt-like union, in which the fibre, analogously to an amino-acid, plays in the one case the part of an acid, in the other case that of a base. Thus rosaniline in the form of its free base (carbinol base) is colourless while its salts are coloured. If a skein of wool is placed in the colourless solution of the colour-base and the solution is then heated, the skein will be stained as intense a red as if the corresponding amount of rosaniline hydrochlorate or other rosaniline salt had been used." "The fibre in this union plays the part of an acid." "That the fibre may play the part of a base towards a colour acid is shown in a very instructive manner by the quinoid-ethylether of tetrabrome-phenol-phthalein. This ether in the free state is pale yellow, and dilute solutions appear almost colourless, while its

alkali salts are of an intense blue colour. If one acidify the blue solution of this salt with acetic acid till it has become colourless, and if one place a skein of silk in this colourless solution, it will stain an intense blue. Many free colour acids, *e.g.* the sulphonic acids of the amino-azo-compounds, differ in colour from their alkali salts, and as the free colour acids do not stain fibres with their own colour but that of their alkali salts, it follows again that the fibre must play the part of a base. As a rule the fibre is unable to decompose the alkali salts of strong colour acids, and therefore, the latter stain, only if by the addition of a stronger acid the colour-acid has been liberated."

Martin Heidenhain (1902) has also carried out a very extended series of experiments in which he showed that most of the dyes used for microscopical work form coloured salts with the tissue-albumins. Salts are formed whenever soluble or coagulated albumin, either in the form of a suspension or in the form of microscopical sections, is brought into contact with various dyes. He observed chemical changes which were quite as marked as when silver-oxide forms the insoluble silver-chloride on coming into contact with hydrochloric acid. He also noticed the amphoteric character of proteids, and gives the following examples: The salts of the Nile-blue-base are blue while the free acid is red; on bringing together the red base with a solution of an albumin there is formed the blue Nile-blue-albuminate in exactly the same way as if an acid had been added to the Nile-blue-base. Reversely the free Congo-acid is blue, but it becomes at once red when a solution of albumin is added. Solid proteids, *e.g.* microscopical sections, also become red on being placed in a solution containing the blue acid.

The laws of hydrolytic dissociation hold, of course, good for coloured salts also. The acid dye-stuffs act best in acid media, for in neutral solutions the albumin-dye-compound undergoes hydrolysis, and therefore, according to the strength of the colour acid, no or only very little staining takes place. An excess of acid, by preventing hydrolysis, allows the albumin-dye-compound to remain insoluble, and therefore the albumin appears coloured. Here again the behaviour of an albumin towards Congo-red is very instructive, for the Congo-red-albuminate will show the bright red salt colour, even if the solution has been rendered distinctly acid by the addition of acetic or of hydrochloric acid. The converse holds also good, for albumin coagula stain blue in an alkaline, red solution of Nile-blue.

In Heidenhain's papers will be found many examples illustrating the laws of dissociation. Some of the aniline dyes, particularly the acid ones, form with albumins insoluble salts, and therefore precipitate albumins as do alkaloidal reagents. (See above, p. 226, under Mathews.)

As these precipitates are deeply coloured they serve as delicate tests for the detection of albumins.

Attention has already been drawn to the fact that albumins differ from one another chemically. As all albumins are both basic and acid in their character, they must unite with whatever colour is offered to form coloured salts, and one finds indeed in most cases that tissues are stained at first in a diffuse manner, but that the stain is not uniformly resistant to after-treatment. If, for example, we have used a basic colouring matter, such as methylene-blue, then on washing the tissue, the salts which methylene-blue has formed with the feebly acid (more basic) albumins will become hydrolysed to a greater extent than will the salts which have been formed with decidedly acid albumins. The more basic albumins becoming hydrolysed part with the methylene-blue, and so become colourless, while the acid albumins form insoluble, 'permanent' compounds. If flocculi of coagulated neutral egg-albumin and of acid casein are suspended in alcohol, if Congo-red and Nile-blue are added simultaneously, if the solution is rendered alternately acid and alkaline, and if the coagula are well washed out, the casein will be found to have stained blue with Nile-blue, while the egg-albumin is stained red by the Congo-red. Fixation coagulates the tissues, but does not alter their chemical character (see Chapter VII.); if anything the differences between various proteids are accentuated by the use of acid fixatives or of formaldehyde, which weakens the basic character. Maschke [1] in 1859 was the first to employ carmine and indigo carmine, besides iodine, for staining albuminous substances.

The tissue constituents which stain most readily with basic dyes are the nuclei, mucus, cartilage, amyloid, and yolk constituents, for they contain strongly acid radicals in the nucleic acids, nucleo-proteids, mucins, mucoids, and vitellins.

Space forbids to discuss the chemistry of the hardening and staining methods employed in histological research. These questions have been dealt with for the first time in a systematic manner in the author's book,[2] after previous attempts (Heine [3]) had met with difficulties. There cannot be any doubt that the elective staining of tissues depends on differences in the basicity and acidity of the proteid substances.

To say that staining depends on differences in the coefficient of distribution means nothing, for the question we have to answer is what causes the difference in the coefficient, and this is undoubtedly due to differences in the chemical nature of the two media, amongst which the dye distributes itself. (The author's *Physiological Histology.*)

[1] O. Maschke, *Botanikerzeitung*, **17**. 21 (1859).

[2] Gustav Mann, *Physiological Histology, Methods and Theory*, Oxford, 1902.

[3] L. Heine, *Zeitschr. f. physiol. Chem.* **21**. 494 (1896).

CHAPTER VII

HALOGEN-ALBUMINS AND PRODUCTS OF OXIDATION

ALDEHYDE- AND IRON-COMPOUNDS

FOLLOWING up the older observations of Böhm and Berg,[1] halogen-compounds of albumins have been prepared by Blum,[2] Hofmeister,[3] and his pupils Kurajeff,[4] Liebrecht,[5] Hopkins,[6] Schmidt,[7] and Oswald.[8] Baumann,[9] Drechsel,[10] and Harnack[11] have shown iodine-albumins or iodo-albumins to occur also in nature: in the thyroid gland of vertebrates and in the supporting framework of sponges and corals.

Halogen-albumins arise, as Blum and Hofmeister have shown, by the replacement of one or of several of the hydrogen-atoms in one or several of the aromatic radicals of an albumin, by fluorine, chlorine, bromine, or iodine. Products formed in this way behave as do the halogen-substituted benzoles: they do not give a precipitate with silver nitrate, and the halogen can only be demonstrated chemically by incinerating the compound.

Iodo-Albumins

Blum and Hofmeister iodated albumins by allowing a mixture of potassium iodate, KIO_3, and potassium iodide, KI, to act on albumin

[1] R. Böhm and F. Berg, *Arch. f. experiment. Pathol. u. Pharm.* **5**. 329 (1876).

[2] F. Blum and W. Vaubel, *Journ. f. prakt. Chem.* [2] **56**. 393 (1897), [2] **57**. 365 (1898); F. Blum, *Zeitschr. f. physiol. Chem.* **28**. 288 (1899).

[3] F. Hofmeister, *ibid.* **24**. 158 (1897).

[4] D. Kurajeff, *ibid.* **26**. 462 (1899), **31**. 527 (1901).

[5] A. Liebrecht, *Ber. d. deutsch. chem. Ges.* **30**. II. 1824 (1897).

[6] F. G. Hopkins, *ibid.* **30**. II. 1860 (1897); F. G. Hopkins and S. N. Pinkus, *ibid.* **31**. II. 1311 (1898).

[7] C. H. L. Schmidt, *Zeitschr. f. physiol. Chem.* **34**. 55 and 194 (1901), **35**. 386 (1902), **36**. 343 (1902).

[8] A. Oswald, *Hofmeister's Beiträge*, **3**. 391 (1903), **3**. 514 (1903).

[9] E. Baumann, *Zeitschr. f. physiol. Chem.* **21**. 319 (1895); E. Baumann and E. Roos, *ibid.* **21**. 481 (1896); E. Baumann, *ibid.* **22**. 1 (1896).

[10] Drechsel, *Zeitschr. f. Biol.* **33**. 84 (1896).

[11] E. Harnack, *Zeitschr. f. physiol. Chem.* **24**. 412 (1898).

at a temperature of 40-50°. One portion of the iodine is substituted for the hydrogen in the aromatic group as already explained, while another portion is converted into hydrogen iodide, HI. To prevent the latter from dissociating the albumin, Hofmeister adds magnesium carbonate, $MgCO_3$. Blum adds, from the very commencement, an excess of sodium bicarbonate, $NaHCO_3$.

The iodo-albumins are brown loose powders, not soluble in water, alcohol, or acids, but very soluble in fixed alkalies, ammonia, and alkali-carbonates; they are precipitated from their solutions by acids, but pass again into solution if too much acid be added. They are precipitated in the same way as are other albumins; as to colour reactions they give positive results with the biuret, the xantho-proteic, and Molisch's tests, but negative results with the tests of Millon and Adamkiewicz, and also with the lead sulphide reaction. Apart from the iodine, these proteids do not differ essentially from other albumins in their percentage composition; the sulphur content is not altered. Iodo-casein contains phosphorus, according to Liebrecht; the iodo-hæmoglobin, according to Böhm and Berg and Hopkins and Pinkus, also iron.

The amount of iodine contained in albumins varies. For approximately pure albumins, the following numbers are given :—

	Per cent.
Serum-albumin (Kurajeff)	12
Serum-globulin (Hopkins)	13–14
Hæmoglobin (Kurajeff)	11–12
Egg-albumin (Hofmeister)	8·93
Egg-white (Blum)	7·1
Muscle-albumin (Blum)	10–11
„ „ (Kurajeff)	11
Caseinogen (Blum)	7–7·5
„ (Liebrecht)	5·7–8·7
„ (Oswald)	11–13
Gelatin (Oswald)	1·3–2·0
Thyro-globulin (Blum)	6–6·6
Nucleo-histone (Blum)	11·22

The iodo-egg-albumin possesses according to Hofmeister the composition :—

C	H	N	S	I
47·92	6·6	14·27	1·26	8·93

From this percentage composition Hofmeister calculates the formula :—

$$C_{227}H_{370}I_4N_{48}S_2O_{75}$$

In addition to the above, other albumins with a much higher iodine-content also exist, the halogen being partly in the above described firm union and partly in a very loose combination. To this class belongs the periodo-casein of Liebrecht with 17·8 per cent of iodine.

The iodine-compounds are believed to be formed in the following manner: In the aromatic groups of the albumin-molecule, *i.e.* in the tyrosin, tryptophane, and phenylalanin radicals, iodine is substituted for several of the hydrogen atoms. By employing the same method of iodising, as described above, Oswald prepared from tyrosin a tri-iodo-tyrosin. That phenylalanin takes up iodine as well as does tyrosin, Oswald proved by iodising hetero-albumose and gelatine, for these two substances do not contain either tyrosin or tryptophane. He proposes to make use of the iodine-number, which is readily determined, for estimating the amount of aromatic groups present in an albumin. This is permissible, because, as far as we know, no other groups but the aromatic ones will unite with iodine. The negative results obtained with the reactions of Millon and Adamkiewicz are not to be explained on the ground that the hydroxyl-group in tyrosin has been substituted, for Blum and Vaubel have shown that tyrosin and other substances with similar constitution do not give Millon's reaction, if halogens have been substituted in both ortho- or in both meta-positions. Millon's reaction is obtained again if the halogen is split off under a pressure of 5 to 6 atmospheres. The iodo-albumins behave in exactly the same way.

Little is known as to the other changes which are produced in proteids by iodisation, but distinct differences are induced according to the temperature used, and according to the duration of iodisation. A certain amount of disintegration of albumin is, however, unavoidable when using the methods of Blum and Hofmeister, even if the greatest care is taken by iodising at 40°, and by keeping the alkaline reaction as feeble as possible. The formation of hydriodic acid, $HI + H_2O$, speaks in itself for an oxidation of albumins; according to Vaubel[1] and Schmidt the amounts of iodic and bromic acids formed give iodine- and bromine-numbers, which are quite characteristic for the different albumins. The negative result obtained with the lead sulphide reaction shows that the sulphur must have become oxidised or have been changed somehow. Schmidt observed that the splitting off of amino-groups differed with individual albumins; he also noticed the formation of iodoform, formic, acetic, and carbonic acids. The ratio C : N is unchanged in serum-albumin, while it is greatly

[1] W. Vaubel, *Zeitschr. f. analyt. Chem.* **40**. 470 (1901); according to *Chem. Zentralbl.* 1901, II. p. 711.

diminished in egg-albumin. Iodo-albumins are affected very slightly by acids. When working with haemoglobin, Kurajeff noticed that haematin is also iodised.

By peptic and tryptic digestion a portion of the iodine is split off, according to Hofmeister and Kurajeff, and there are formed iodo-albumoses and iodo-peptones. Oswald succeeded in iodising all the albumoses of Pick, and in addition peptones and an abiuretic body, which was probably a peptid.

Hetero-albumose contains 10·27 per cent iodine, and prot-albumose 12·48 per cent. This slight difference is very remarkable, as the prot-albumose contains the whole of the tyrosin and tryptophane, while the hetero-albumose contains only phenylalanin. Amongst the products of tryptic digestion, besides tyrosin, yet another substance could be iodised.

During metabolism the iodine is completely split off and excreted as alkali-iodides. Only if very large amounts of iodised proteid are administered, does some pass unchanged into the urine.

From the physiological standpoint iodised albumins are indifferent inasmuch as they produce only such effects as can be obtained with the salts of iodine, but iodised albumins are metabolised more slowly than are simple albumins, according to Falta.[1] Dissociation with acids liberates iodine as does ferment action; on dissociating a chlorine-casein compound by means of fuming nitric acid, Panzer[2] observed chlorinated fatty acids besides and in place of the usual amino-acids. There is also formed according to Hofmeister and Oswald an iodised body with the properties of a peptone, which could not however be obtained in a pure form. Liebrecht has prepared caseo-iodine from iodised casein; both resemble one another in their reactions. The 'iodalbacid' of Blum belongs, according to Oswald, also to this group of substances.

Amongst the iodo-albumins occurring in nature, the one of the greatest importance to us is the thyro-globulin of the thyroid gland, which was discovered by Baumann, and more fully studied by Oswald[3] in Hofmeister's Institute. It contains only 1·75 per cent of iodine, therefore much less than do the artificial iodine-proteids, but it is capable of being still further iodised according to Blum. Being less iodised it is less acid than are the completely iodised albumins, but otherwise it has the same reactions. The peculiar physiological action of the thyroid gland is connected with the thyro-

[1] W. Falta, *Naturf. Ges. zu Basel*, XV., No. 2 (1903).

[2] T. Panzer, *Zeitschr. f. physiol. Chem.* **33**. 131 (1901).

[3] A. Oswald, *ibid.* **27**. 14 (1899), **32**. 121 (1901); (here also the literature).

globulin, but Roos[1] has shown, in opposition to Blum,[2] that the physiological activity is independent of the iodine-content of the thyroid gland.

Iodothyrin is a dissociation-product of the thyro-globulin, and is comparable to the caseo-iodine mentioned above. It was prepared by Baumann directly from the thyroid gland, and by Oswald by dissociating thyro-globulin by means of acids; it contains 14·2 per cent iodine, is insoluble in water and acids, but soluble in alkalies. It still possesses the physiological properties of thyro-globulin.

Drechsel[3] found in the framework of a coral, the *Gorgonia carolinii*, an iodised keratin, the gorgonin. Mendell[4] has found iodine also in other West Indian species of corals. Gorgonin possesses the properties of keratin, but contains nearly 8 per cent of organically bound iodine, while probably an even much higher percentage will be found in the older, firmer portions of the framework. The crystalline dissociation-products of gorgonin are given in the table on p. 73; besides these there is also formed, when gorgonin is dissociated with acids, the iodo-gorgonic acid, which Drechsel believed to be iodo-amino-butyric acid, a view which, however, is not confirmed by Henze.[5]

Sponges also contain an iodo-albumin according to Baumann,[6] Harnack,[6] and Hundeshagen.[7] From this iodo-albumin Harnack prepared iodo-spongin having this percentage composition:—

C	H	N	S	I	O
47·66	6·17	9·93	4·54	9·01	22·69

It is not a proper iodo-albumin, but a product split off from the original substance, which explains the, even for a keratin, high percentage of sulphur, the low N-value, and the absence of the biuret-reaction. The lead sulphide reaction is positive, but no other albumin reactions are obtainable. It is worthy of notice that the ratio I : S is the same for the whole sponge as it is for the iodo-spongin—"that therefore in sponges the iodine is only absorbed by the sulphur containing radicals of the organic matter." By weight iodo-spongin forms about one-sixth of the total unaltered molecule.

[1] E. Roos, *Zeitschr. f. physiol. Chem.* **28**. 40 (1899).

[2] F. Blum, *Pflüger's Arch. f. d. ges. Phys.* **77**. 70 (1899); [see also A. Oswald, *ibid.* **79**. 450 (1900)].

[3] E. Drechsel, *Zeitschr. f. Biol.* **33**. 84 (1896).

[4] L. B. Mendel, *Amer. Journ. of Physiol.* **4**. 243 (1900).

[5] M. Henze, *Zeitschr. f. physiol. Chem.* **38**. 60 (1903).

[6] E. Harnack, *ibid.* **24**. 412 (1898).

[7] F. Hundeshagen, *Zeitschr. f. angew. Chem.* 1895, p. 473; according to *Chem. Zentralbl.* 1895, p. 570.

From the biological point of view it is interesting that sponges, corals, and the thyroid glands of mammals possess the power of de-ionising and of storing in large amounts iodine, which is offered them in minimal quantities and in the state of an ion. That gorgonin and iodo-spongin are by no means examples of maximal iodisation is shown by the observations of Hundeshagen,[1] who found in tropical horny sponges 8 to 14 per cent of iodine, while the ordinary bath sponge contains on the whole only 1·5 to 1·6 per cent. Young sponges and corals, and the thyroid glands of young animals, contain considerably less iodine.

Other Halogen-Albumins

Blum and Vaubel[2] and Hopkins[3] have shown that the other halogens—bromine, chlorine, and fluorine—may be introduced into the albumin-molecule quite analogously to iodine. Generally speaking, the method was the same as in iodising; chlorination was performed at room-temperature; Blum has introduced chlorine also electrolytically. The halogen-content of albumins prepared in this way corresponds with that of iodine; for egg-albumin were found by—

Hopkins.			Blum.		
6·2	per cent	Iodine.	6-7	per cent	Iodine.
3·84	„	Bromine.	4-5	„	Bromine.
1·93	„	Chlorine.	2	„	Chlorine.
			1·2	„	Fluorine.

A number of fluorine compounds have been prepared by Gans.[4] In addition to the bromine-albumins just mentioned, Hopkins and Pinkus have also prepared more highly halogenated bodies with more loosely bound bromine, corresponding to the per-iodo-casein. They found the following maximal percentage values—

Egg-albumin, crystallised . .	12·6–16·43
Serum-albumin . . .	12·15–12·94
Serum-globulin . . .	13·53–14·03
Casein	11·17
Albumoses	16·3–17·63

According to Harnack many of the technically prepared halogen

[1] F. Hundeshagen, *Zeitschr. f. angew. Chem.* 1895, p. 473; according to *Chem. Zentralbl.* 1895, p. 570.

[2] F. Blum and W. Vaubel, *Journ. f. prakt. Chem.* [2] **56**. 393 (1897), **57**. 365 (1898).

[3] F. G. Hopkins, *Ber. d. deutsch. chem. Ges.* **30**. II. 1860 (1897); F. G. Hopkins and S. N. Pinkus, *ibid.* **31**. II. 1311 (1898).

[4] L. W. Gans, *Patentschrift, Chem. Zentralbl.* 1901, I. p. 148.

albumins are similar bodies containing a large amount of halogen, which is, however, readily split off.

Habermann and Ehrenfeld[1] and Panzer[2] have allowed nascent chlorine to act on albumins, when one part of the albumin is dissociated, while another portion, still albuminous in nature, has chlorine substituted for hydrogen. This substituted product contains no sulphur, is strongly acid, and of a reddish brown colour. (See also p. 96.)

The brominated and chlorinated albumins are, also, brown or greyish powders resembling the corresponding iodine-compounds as regards solubility, precipitation, and colour reactions. The same holds good for their behaviour during metabolism; physiologically they are indifferent or produce the same effect as would the corresponding chlorine and bromine salts. Nothing is known regarding their occurrence in nature, apart from a remark of Drechsel's, that a chlorinated albumin exists along with an iodo-albumin in the skeleton of *Gorgonia*.

Nitro-substitution Products

Just as it is possible to introduce halogen-radicals into the albumin-molecule, so is it possible to substitute nitro-groups, as was done first by Löw,[3] and quite recently in a very thorough manner by v. Fürth.[4] Löw[3] calls his products tri-nitro-albumin and hexanitro-albumin-sulphonic acid. By adding urea to the nitrous acid and so preventing the formation of nitric acid, v. Fürth obtained a nitro-casein having the following percentage composition :—

C	H	N	NO_2	S	P	O
52·6	6·69	15·87	1·78	0·64	0·56	23·64

Without the addition of urea a progressive dissociation takes place, xanthoprotein being formed, besides large amounts of albumoses and peptones, which generally are also nitrated. We therefore meet with the same changes as when we studied all the other dissociation-products on p. 94. The xanthoprotein and the other nitro-substitution products are acid in character and possess a yellow colour which, on adding a fixed alkali, is converted into a reddish-brown. The xanthoproteic reaction depends therefore on the formation of nitro-

[1] J. Habermann and R. Ehrenfeld, *Zeitschr. f. physiol. Chem.* **32**. 467 (1901); R. Ehrenfeld, *ibid.* **34**. 566 (1902).

[2] T. Panzer, *ibid.* **33**. 131 (1901), **33**. 595 (1901), **34**. 66 (1901), **35**. 84 (1902).

[3] O. Löw, *Journ. f. prakt. Chem.* [2] **3**. 180 (1871), [2] **5**. 433 (1872).

[4] O. v. Fürth, *Einwirkung von Salpetersäure auf Eiweissstoffe*, Habilitationsschrift, Strassburg, 1899 (here also the older literature).

substitution products. When nitrating albumins, the sulphur is also oxidised, and therefore no lead sulphide reaction is obtained, although both xanthoprotein and the nitro-albumoses retain the whole of their sulphur. The reaction of Millon gives negative results, because the nitration takes place in the tyrosin group.[1] In other respects the nitro-substitution products give the usual proteid-reactions.

Xanthoprotein on being dissociated with acids yields leucin, glutaminic and aspartic acids, and the nitro-substitution product xanthomelanin (see p. 95), derived from the original tyrosin. By peptic and tryptic digestion are liberated nitrated albumoses and peptones.

Physiologically, xanthoprotein is not indifferent, because 50 g. administered to a dog by the mouth produce symptoms of poisoning, which apparently are due to the xanthoprotein as a whole and not simply due to the nitro-groups. In the urine, xanthomelanin, or an allied body, is found.

Oxyproteic Acid or Oxyprot-sulphonic Acid

Béchamp[3] was the first to study the effect of potassium permanganate on albuminous substances, and to point out that oxidation gives rise to urea. This statement, after a great deal of controversy, has been now definitely settled by Kutscher and Zickgraf.[2] Béchamp[3] was followed by Subbotin[4] and Pott,[5] of whom the latter isolated from the conglutin of lupines an acid having the following percentage compositions:—

C	H	N	O
45·44–45·53	5·84–5·88	13·06–13·31	35·32–35·62

Chandelon[6] used for oxidising albumin, barium peroxide, which was suspended in the solution and then decomposed by CO_2. The nascent hydrogen-peroxide changed the albumin into an acid substance, which could be precipitated from alkaline solutions with acids. He further obtained propeptone and peptones.

Brücke[7] also discovered a peculiar acid giving the biuret reaction,

[1] That pure tryptophane gives Millon's reaction has been pointed out previously.

[2] Kutscher and Zickgraf, *Sitzb. d. kgl. preuss. Akad. d. Wiss.* 26th May 1903; Zickgraf, Inaugural Dissertation, Marburg, 1904; *Zeitschr. f. physiol. Chem.* **41**. 259 (1904).

[3] Béchamp, *Leibig's Ann. der Chem. und Pharm.* **100**. 247 (1856).

[4] Subbotin, *Chem. Centralbl.* 1865, p. 594.

[5] Pott, *Journ. f. prakt. Chem.* [2] **5**. 355 (1872).

[6] Chandelon, *Ber. d. deutsch. chem. Ges.* **17**. 2143 (1884).

[7] E. Brücke, *Sitzungsber. d. Wien. Akad., Math.-naturw. Kl.*, III. Abteil., **83**. (1881), January and February.

but not the xantho-proteic test nor the tests of Adamkiewicz, Millon, or Liebermann.

Maly[1] then showed that Brücke's acid, which he called oxy-prot-sulphonic acid or oxyproteic acid, was obtained from egg-white by oxidising the latter at room-temperature with half its weight of $KMnO_4$; precipitating the filtrate with acid, and drying the precipitate at a low temperature.

This oxy-prot-sulphonic acid had the percentage composition—

C	H	N	S	O
51·21	6·89	14·59	1·77	25·54

In the pure condition it is a loose, white, non-hygroscopic powder with strongly acid properties,—insoluble in water and salt solutions, but very soluble in alkalies, and precipitated from its solutions by acids. The property last mentioned is used for purifying this acid, but excess of strong acid must be avoided as otherwise the precipitate re-dissolves. In faintly alkaline solutions the precipitation-limits for oxyprot-sulphonic acid, prepared from crystalline serum-albumin, lie between 2·8 and 4·2 per cent saturated ammonium sulphate, and there seems to be another substance, present in small amounts, having precipitation limits between 4·8 and 6·4 per cent.

Further oxidation of oxyprot-sulphonic acid with $KMnO_4$ resulted in the formation of a pluri-basic acid rich in oxygen, which was called peroxyproteic acid, with the percentage composition—

C	H	N	S	O
46·22	6·43	12·30	0·96	34·09

Maly believed this compound to be formed by albumin taking up oxygen without undergoing any other change. Calculation showed each sulphur-atom to be associated with 71 O-atoms and 20–22 CO-groups.

Peroxyproteic acid gives a strong biuret reaction, and is not precipitated by alkaloidal reagents such as phospho-tungstic acid, mercury, potassium-iodide, or tannic acid. When treated with baryta water at a gentle heat, it gives rise to large quantities of barium oxalate, and traces of barium sulphate. On being boiled for several days with baryta water there were obtained—ammonia, glutaminic acid, leucin, formic- acetic- and benzoic-acids, and a compound which Maly believed to be isoglyceric acid. This last compound was not obtained from gelatine, on oxidising the latter with double its weight of $KMnO_4$, while all the other oxidation-products were got.

[1] R. Maly, *Untersuchungen über die Oxydation des Eiweiss mittels Kaliumpermanganat*, *Monatsh. f. Chem.* **6**. 107 (1885), **9**. 258 (1888), **10**. 26 (1889).

Siegfried,[1] on hydrolysing oxyprot-sulphonic acid with HCl, obtained bases which could be precipitated with phospho-tungstic acid. The platinum salt had the formula $C_8H_{22}N_2O_3Cl_6Pt$, while a silver salt consisted of $C_6H_{13}N_3O_2 . NHO_3 . AgNO_3$.

Subsequently Bondzynski and Zoja,[2] working under the direction of Bunge, used purer material and obtained for oxyproteic- or oxyprot-sulphonic acid the following percentage figures :—

	C	H	N	S
Crystallised egg-albumin .	50·73	7·02	14·70	—
Hæmoglobin of the horse .	52·32	6·96	16·04	—
Caseinogen (Hammarsten) .	49·11–52·07	6·39–7·10	14·63–14·99	0·71–0·76

Bernert,[3] Ehrmann,[4] and v. Fürth[5] have continued the investigation of Maly's products in Hofmeister's Laboratory.

Bernert has split up oxyprot-sulphonic, prepared from egg-albumin by means of fractional precipitation with ammonium sulphate, into two fractions, both of which gave negative results with the xanthoproteic test and the reactions of Millon and Adamkiewicz. When they are dissociated by means of HCl they gave rise to leucin and aspartic acid, but not to tyrosin. When fused with KOH, free fatty acids and pyrrol, but neither indol nor skatol, were obtained. Maly's view that oxyprot-sulphonic acid consists of albumin which has simply become oxidised is disproved by the fact that after precipitating and filtering off the oxyprot-sulphonic acid, Bernert found albumoses, peptones, fatty acids and basic products in the filtrate.

The sulphur seems to be in a peculiar state; Maly and Bernert failed to obtain the lead sulphide reaction, and assumed therefore that the whole of the sulphur which is usually split off as the sulphide was oxidised. Schulz[6] found, however, that oxyprot-sulphonic acid still contains 0·33 per cent of detachable sulphur. This amount is less than that usually split off, and therefore it would appear that that sulphur which under ordinary conditions is most readily split off is exactly the sulphur which is changed by oxidation, and hence the negative results

[1] Siegfried, *Ber. d. deutsch. chem. Ges.* **24**. 418 (1891).

[2] St. Bondzynski and L. Zoja, *Über die Oxydation der Eiweissstoffe mit Kaliumpermanganat, Zeitschr. f. physiol. Chem.* **19**. 225 (1894).

[3] J. Bernert, *Über Oxydation von Eiweiss mit Kaliumpermanganat, ibid.* **26**. 272 (1898).

[4] Ehrman, *Über die Peroxyprotsäuren*, Inaugural Dissertation, Strassburg (1903).

[5] O. v. Fürth, *Zeitschr. f. physiol. Chem.* **44**. 279 (1905).

[6] F. N. Schulz, *ibid.* **29**. 86 (1899).

with the lead sulphide test. The percentage composition of sulphur is not altered by oxidation.

By oxidising oxyprot-sulphonic acid with $KMnO_4$ at room-temperature, neutralising the resulting fluid with acetic acid and then precipitating firstly with lead acetate and subsequently with mercuric acetate, Bernert obtained two fractions, which he called peroxyproteic acids A and B. The compound B gives with phosphomolybdic and phosphotungstic acids dense precipitates soluble in an excess of HCl; mercury-potassium iodide, picric acid, and tannin do not cause a precipitate; with silver nitrate a precipitate is obtained which is soluble in both ammonia and in nitric acid. Boiling with baryta water yields with the B-fraction leucin, acetic and butyric acids, and probably pyridin, while the A-fraction gives in addition glutaminic and benzoic acids and benzaldehyde.

Ehrmann, using the same method as Bernert, obtained from serum albumin and casein also two peroxyproteic acids, neither of which could be precipitated by such alkaloidal reagents as phosphotungstic acid, or by means of mercuric acetate or nitrate.

The ratio of C : H : N : O was the same in Ehrmann's acid as in Maly's acid.

	C	H	N	O
Peroxyproteic acid of Maly .	4·4	7·3	1	2·4
Ehrmann's silver salt .	4·2	7·0	1	2·3

O. v. Fürth has made the most thorough examination of Maly's compounds. Peroxyproteic acids were prepared from defatted casein, which in the course of some weeks was gradually oxidised by $KMnO_4$ in an alkaline solution. When oxidation was complete, the clear yellow filtrate was treated with glacial acetic acid till it was just-alkaline, and was then precipitated with an excess of lead acetate. The heavy precipitate, consisting for the greater part of lead oxalate, was suspended in water and decomposed while warm with H_2S. As the peroxyproteic acid adheres firmly to the lead sulphide, the latter had to be extracted with hot water 6 to 13 times, the sulphide after each extraction being suspended in water, and again saturated with H_2S. The combined filtrates were freed from H_2S by passing air through the solution; the oxalic acid was removed by means of barium hydrate, and the excess of barium with CO_2. Then the solution was precipitated with silver nitrate; the silver precipitate suspended in water and decomposed with H_2S; the filtrate inspissated at 50°, and then dried in vacuo over H_2SO_4. The resulting mass, looking like varnish, was called 'Peroxyproteic acid A.' The filtrate from the

$AgNO_3$ precipitate is freed from silver with H_2S, the latter removed by a current of air; the solution is then neutralised with NaOH and precipitated with mercuric acetate. By decomposing the mercury precipitate with H_2S there is obtained the 'Peroxyproteic acid B.'

The filtrate remaining from the first lead precipitate was then precipitated with mercury acetate. On decomposing the voluminous precipitate with H_2S, there was obtained 'Peroxyproteic acid C.'

All peroxyacids are readily soluble in water, slightly soluble in dilute alcohol, insoluble in acetone and ether. The watery solution gives an intense biuret reaction, but no xantho-proteic reaction, nor the tests of Millon or Hopkins or the lead-sulphide reaction.

Phospho-tungstic acid in the presence of a trace of HCl gives a voluminous precipitate; if too much HCl is present no precipitate is formed, and if no HCl is present the precipitate first formed dissolves in an excess of the phospho-tungstic acid. Phosphomolybdic acid precipitates to a lesser degree, and the other alkaloidal reagents do not precipitate at all. Mercuric acetate and nitrate give a voluminous precipitate insoluble on heating and in acetic acid, but readily soluble in HCl. Mercuric chloride does not precipitate. Silver nitrate added to the free acid causes no precipitate, but on adding baryta water, drop by drop, a white precipitate, soluble in acetic acid and ammonia, is formed. A neutral salt of a peroxyproteic acid is precipitated directly with $AgNO_3$. Lead acetate causes a precipitate soluble on warming and in acetic acid. When heated with copper-acetate green flocculi are separated out, which are soluble in NH_3, with a deep blue, and in NOH with a biuret-colour. Ferric chloride gives a gelatinous precipitate insoluble in acetic acid, but readily soluble in HCl. Zinc- and tin-chlorides do not precipitate.

With calcium and barium carbonate, magnesium and zinc oxides, or by neutralisation with sodium carbonate and ammonia readily soluble, non-crystallisable salts are formed. No insoluble compounds were obtained with benzoyl-chloride, benzoyl-sulpho-chloride, and naphthol-sulpho-chloride and alkali.

The peroxyproteic acids B and C differ from A in their behaviour towards neutral and basic-lead acetate and silver nitrate, as C is not precipitated by either of these, while B is precipitated by lead-acetate but not by neutral lead-acetate and $AgNO_3$.

Peroxyproteic acid esters were obtained by boiling the dry peroxyproteic acids with alcoholic HCl (1 part of alcohol saturated with gaseous HCl to 10 parts of absolute alcohol) for one to two hours in a reflux boiler. The alcohol was removed by distillation in a vacuum under 30-40 mm. pressure, and the syrup-like residue kneaded with

water. The esters are readily soluble in chloroform; all traces of water were removed with freshly calcined $CuSO_4$, and the esters precipitated from the chloroform solution with ether. These esters are light-brown light powders, readily soluble in alcohol, acetone, chloroform, glacial acetic acid, hardly soluble in ether and petroleum-ether, and quite insoluble in water.

The esters are very readily saponified with NOH, but only very slowly by boiling water. Warm ammonia was used for this saponification, which might change $-COOC_2H_5$ groups into $-CONH_2$ compounds.

On being boiled for several hours with baryta water the peroxy-proteic acids lose all the oxalic-acid groups (totalling nearly $\frac{1}{3}$ of the molecule) and the basic complexes, and also a considerable portion of N. The resulting biuret-compounds are called desamino-proteic acids. These, by hydrolytic dissociation, give rise to glutaminic acid, leucin, benzoic acid, and ammonia.

While peroxyproteic acids in an alkaline solution are not attacked by $KMnO_4$, or only to a slight extent, the desamino-proteic acids are readily attacked, and by further oxidation give rise to kyro-proteic acids, which contain about half the nitrogen in loose, acid-amide combination. On treatment with nitrous acid they yield about ten times more N than is obtained from casein.

The gradual dissociation of the albumin-molecule is readily seen by comparing the mean percentage composition of the acids formed by oxidation and the ratios of the oxygen to the nitrogen:

	C	H	N	O	N : O
Casein . . .	53·0	7·0	15·7	22·65	1 : 1·25
Maly's oxyprot-sulphonic acid . .	51·21	6·89	14·59	25·54	1 : 1·53
Peroxyproteic acids A and B . .	45·74	6·08	13·97	33·06	1 : 2·07
Kyro-proteic acid A	42·24	6·42	11·08	38·68	1 : 3·06
Peroxyproteic acid B	42·33	5·88	8·96	41·80	1 : 4·08

Desamino-proteic acid and kyro-proteic acid are only collective names, to be used in the same sense as "albumose" or "peptone."

When peroxyproteic acids A and C are converted into desamino-proteic acids they lose all their oxalic-acid radicals, amounting to $\frac{1}{3}$ of their total weight, and simultaneously nearly $\frac{1}{3}$ of the total N is lost as NH_3. As peroxyproteic acid A contains only 6 per cent of its N in loose combination, it is not only the acid-amide N which is eliminated during the formation of desamino-proteic acid.

On comparing the ratio of loosely-bound acid-amide-nitrogen and the oxalic acid of the kyro-proteic acid A:

Total nitrogen	:	acid-amide-N	:	oxalic acid.
1	:	$\frac{1}{2}$	:	$\frac{1}{4}$

In kyro-proteic acid is sufficient loosely-bound N to allow us to assume that the whole of the oxalic acid is contained in kyro-proteic acid as oxamide groups—NH . CO . CONH . . . NH . CO . CO . NH.

On treating egg-white or other albuminous solutions with large amounts of potassium permanganate in strongly alkaline solutions at room temperature, a part of the albumin is very quickly dissociated. Albumoses and peptones are formed which, by fractional precipitation with ammonium sulphate, can be shown to be the same fractions as are obtained by peptic digestion. They show towards precipitants the same behaviour as do such albumoses and peptones as are obtained by digesting albumin, but there is one exception: they are for some curious reason not precipitated by picric acid, although the other alkaloidal reagents give positive results.

This whole class of bodies gives a well-marked biuret-reaction, and also Millon's reaction, except in the case of the analogue of peptone B, which also does not give it. Negative results are obtained with the lead sulphide- and xantho-proteic reactions, and also with the tests of Millon and Adamkiewicz.

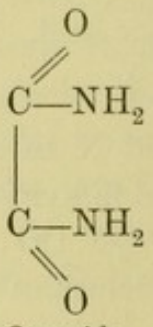
In addition to these albuminous substances are also found simple disintegration-products, which is not remarkable if one considers how susceptible albumins are to the action of all alkalies and how oxidation by means of potassium permanganate may produce a rise of temperature of 12–16°.

With Ehrmann[1] we may assume that oxidation will produce in the albumin-molecule, if it is built up according to Hofmeister's theory (see p. 139), the following changes:—

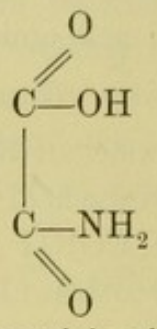
$\begin{matrix}\text{NH . CH . CO} \\ \quad\vert \\ \quad\text{R}\end{matrix}$ — $\begin{matrix}\text{NH . CH . CO} \\ \quad\vert \\ \quad\text{R}\end{matrix}$	when oxidised gives rise to
$\begin{matrix}\text{NH . CH . CO} \\ \quad\vert \\ \text{COOH}\end{matrix}$ — $\begin{matrix}\text{NH . CH . CO} \\ \quad\vert \\ \text{COOH}\end{matrix}$	by splitting off of CO_2 forms
$NH . CH_2 . CO$—$NH . CH_2 . CO$	by further oxidation
NH . CO . CO — NH . CO . CO	results.

[1] Ehrmann, *Über die Peroxyprotsäuren*, Inaugural Dissertation, Strassburg, 1903.

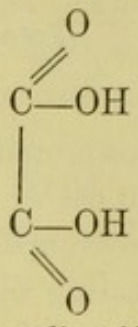
From this last complex, hydrolysis would give rise to oxamide, oxaminic acid, oxalic acid, and ammonia.



Oxamide,

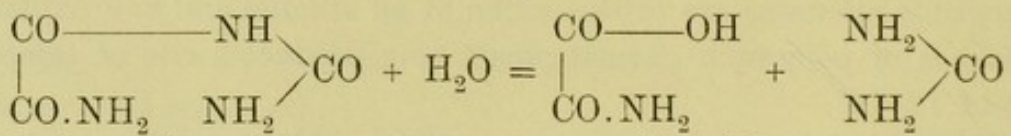


oxaminic acid,



oxalic acid.

Oxamide was first obtained by Löw[1] on oxidising egg-albumin with $KMnO_4$. He believed the oxamide to be formed secondarily out of the HCN and the NH_3 set free by the oxidation of the albumin, and this view is also shared by Hofmeister,[2] by Halsey,[3] and by Ehrmann, as explained above, but Kutscher and Schenck[4] incline to the view that oxamide occurs as such in gelatine, and state that the biuret-reaction (see p. 141) may be partly due to it. They obtained oxamide to the extent of 1·5 per cent on oxidising gelatine at 100° with 5 parts of calcium-permanganate and decomposing the slightly soluble lime-salts with hot ammonium carbonate. Ammonium oxaminate in considerable quantities and guanidin-picrate amounting to 8 per cent of the gelatine were also found. The ammonium oxaminate Schenck[5] derives from glycocoll, which gives rise to a substance which Seemann[6] identified as oxalur-amide. The latter under the influence of ammonia then breaks up into oxaminic acid and urea.



Oxalur-amide or oxalan, oxaminic acid, + urea.

From the oxaminic acid, oxalic acid $[CO.OH]_2$ is formed secondarily, and this explains the oxaluria which Lommel[7] produced by feeding animals on gelatine, for the latter is very rich in glycocoll or the mother-substance of oxalic acid. Casein, which is poor in glycocoll, yielded only traces of ammonium oxaminate when oxidised by Kutscher and Schenck.

That it is possible to synthetise urea or oxaminic acid from such

[1] O. Löw, *Journ. f. prakt. Chem.* [2] **31**. 129 (1885).

[2] F. Hofmeister, *Arch. f. experim. Pathol. u. Therapie*, **37**. 426.

[3] Halsey, *Zeit. f. physiol. Chem.* **25**. 325 (1898).

[4] Fr. Kutscher and M. Schenck, *Ber. d. deutsch. chem. Ges.* **38**. 455 (1905).

[5] M. Schenck, *ibid.* p. 459.

[6] J. Seemann, *Zentralbl. f. Physiologie*, **18**. 285 (1904), and *Zeitschr. f. physiol. Chem.* **44**. 229 (1905).

[7] Lommel, *Deutsch. Arch. f. klin. Med.* 1899.

non-nitrogenous substances as lactic acid and tartaric acid by means of strongly ammoniacal permanganate solutions, Hofmeister [1] and Halsey [2] have shown, but on the other hand there cannot be any doubt [3] that urea is formed directly from albumin, especially in the light of Kutscher and Zickgraf's discovery of guanidin amongst the products of oxidation, and Kossel and Dakin's urea-forming arginase (see index), and this 'direct' urea, although it only amounts to 10 per cent of the total urea excreted,[4] must be accounted for, and therefore Kutscher, helped by Schenck, Zickgraf, and especially Seeman, has made a number of investigations into the structure of the albumin-molecule by means of graduated oxidation.

If we assume with Kossel that an albumin-molecule possesses a central region or protamin-nucleus, round which the other amino-acids are arranged according to Hofmeister's conception (see p. 139), and if the biuret-reaction depends on the special way in which the arginin-radicals of the protamin are linked together,[5] then the disappearance of the biuret-reaction should coincide with the maximal yield of guanidin, which latter is derived from arginin. Zickgraf [6] has now actually found by treating the same amounts of boiling gelatine-solution with increasing amounts of calcium-permanganate solution that the maximal amount of guanidin-picrate is obtained at the time when the biuret-reaction gives negative results. Seemann [7] derives the larger amount of oxalic, succinic, and formic acids found amongst the oxidation-products, from radicals lying in proximity to the protamin-nucleus, because these readily oxidisable acids occurred in relatively large amounts, notwithstanding that the oxidation of gelatine had been carried very far, though not to the disappearance of the biuret-reaction. Seemann derives the oxalic acid from amino-chains according to the following plan : [8]—

$$CH_3—(CH_2)_x—\underset{\displaystyle NH_2}{\underset{|}{CH}} . CO . NH—R \longrightarrow$$

$$COOH—(CH_2)_x—H + NH_3 + \mathbf{COOH . CO . NH—R}$$

or $$\longrightarrow CO_2 + (CO_2)_x + NH_3 + COOH . CO . NH—R$$

[1] F. Hofmeister, *Arch. f. experim. Pathol. u. Pharm.* **37**. 436.

[2] Halsey, *Zeitschr. f. physiol. Chem.* **25**. 329 (1898).

[3] J. Seemann, *ibid.* **44**. 238 (1905).

[4] Drechsel, *Ber. d. deutsch. chem. Ges.* **33**. 3101 ; Gulewitsch, *Zeitschr. f. physiol. Chem.* **30**. 526 and 532 (1900).

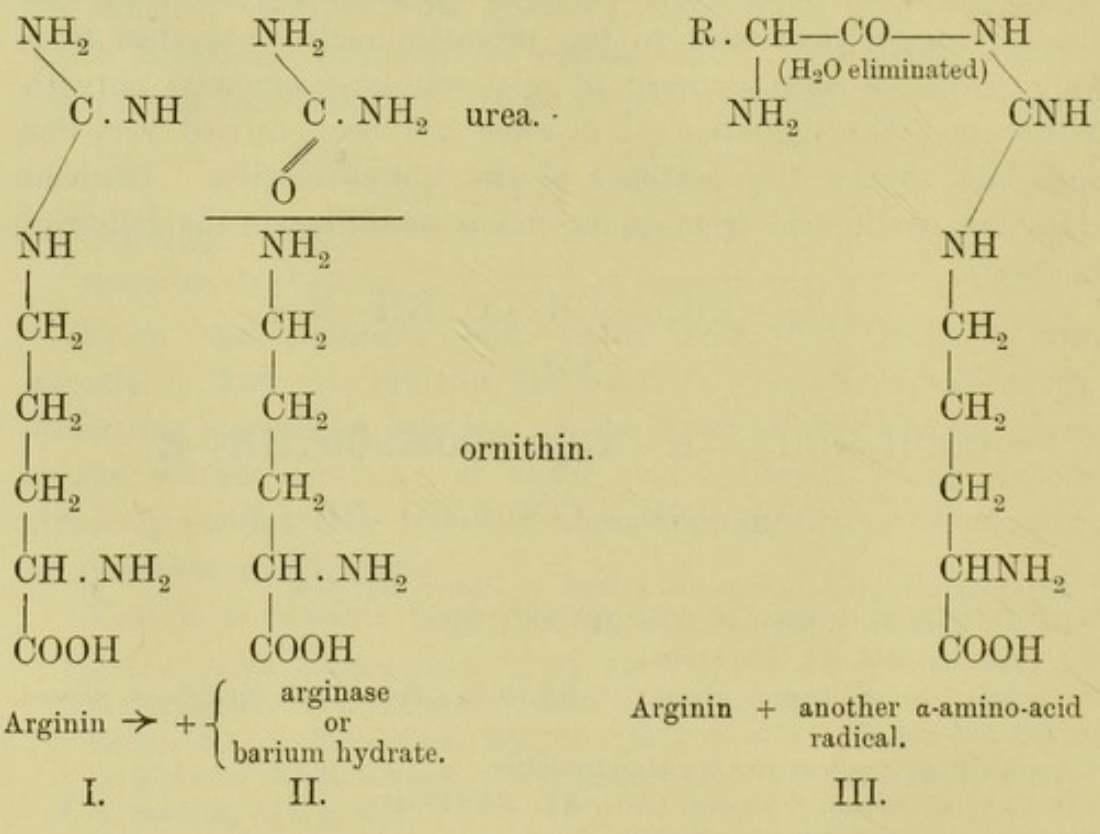
[5] Arginin itself does not give the biuret-reaction.

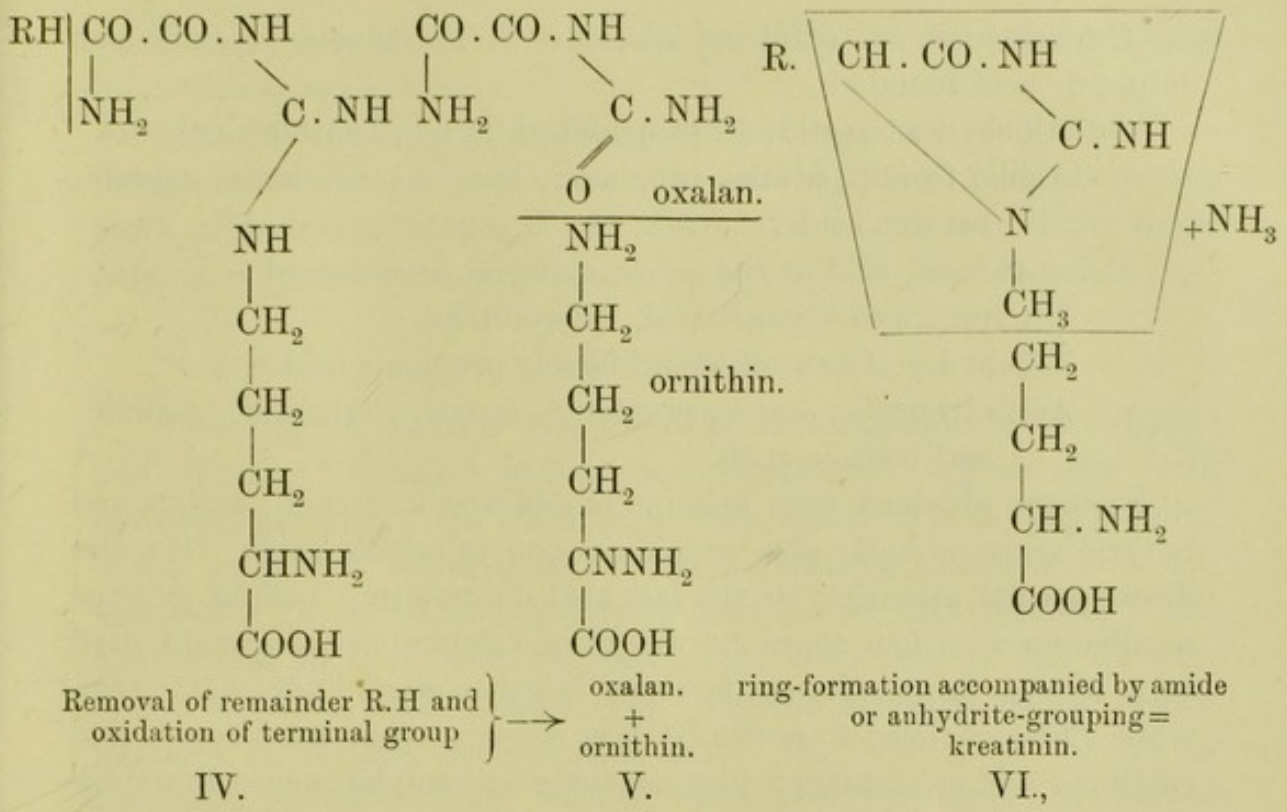
[6] G. Zickgraf, *Zeitsch. f. physiol. Chem.* **41**. 259 (1904).

[7] J. Seemann, *ibid.* **44**. 229 (1905).

[8] Compare with Ehrmann's view on p. 243.

The latter by hydrolysis will give rise to HO . OC—CO . OH or oxalic acid. Succinic acid arises, according to Seemann, probably from arginin or from a radical which, when albumin is hydrolysed, gives rise to asparagin or aspartic acid. Glutaric acid was not found by Seemann although Zickgraf had previously found it amongst the oxidation products of lysin, and he accounts for its absence by assuming that it is either still in the 'protamin-nucleus,' or that the complex which on hydrolysis gives rise to lysin becomes broken up at once during oxidation in such a manner as to be no longer recognisable. Tyrosin, from the ease with which it splits up, is placed also into the periphery of the albumin molecule. Oxaluramide or oxalan and oxaluric acid, which were first recognised as such by Seemann, the latter derives from arginin, and states that their presence accounts for the deficit in the amount of guanidin which should be there theoretically, judged by the arginin-content of the gelatine molecule. As direct oxidation of arginin does not yield oxaluric acid, but guanidin-butyric acid or guanidin + succinic acid (Kutscher), or urea and ornithin, when treated with barium hydrate (Schulze) or arginase (Kossel and Dakin), Seemann reasons that the arginin group must be attached at its guanidin-end to other amino-acids, from which the oxalic acid compound of the oxaluric acid arises, and he expresses his views in the following figures I. to VI. :—





which show the formation of urea and ornithin (II.); the union of arginin with any other amino-acid, such as glycocoll or tyrosin, or even di-amino-acid; for example lysin and arginin (III.), the formation of oxalan or oxalur-amide and ornithin (IV.) and (V.). This way of deriving oxalan, as Seemann points out, is a further support of Hofmeister's theory as to the union of amino-acids, for, apart from this arginin compound, the only other complex from which oxalan could be derived is leucin imide, see p. 33. Should even arginin not be the mother substance of the free guanidin found by Kutscher and Otori [1] in auto-digested pancreas, and by Otori [2] amongst the hydrolytic products of pseudo-mucin, and should guanidin even be derived from an as yet unknown complex, the derivation of oxaluric acid from a guanidin-compound would still hold good.[3] The volatile products obtained by means of oxidation were first studied by Liebig. On oxidising gelatine, by means of chromic acid, Schlieper [4] obtained:

Nitrites: hydrocyanic acid, valero-nitrite and (?) valero-aceto-nitrite.

Acids: acetic-, valerianic-, and benzoic acids.

Oil: smelling of cinnamon.

[1] Kutscher and Otori, *Zentralbl. f. Physiol.* **18**. 248 (1904).

[2] Otori, *Zeitschr. f. physiol. Chem.* **42**. 453.

[3] Apart from any question of oxidation, Seemann points out the possibility of kreatinin being derived directly from arginin. See formula VI.

[4] A. Schlieper, *Liebig's Annalen*, **59**. 1 (1846).

Guckelberger on oxidising casein with manganese peroxide and sulphuric acid found :[1]

Aldehydes : acetic-, (?) propionic-, butyric-, benzoic aldehydes.

Acids: formic-, acetic-, propionic-, butyric-, valerianic-, caproic-, benzoic acids.

Using chromic acid as the oxidising agent he obtained :

Nitrites : hydrocyanic acid, valeronitrite.

Aldehydes of benzoic and probably propionic acid.

Acids : formic-, acetic-, propionic-, butyric-, valerianic-, caproic-, and benzoic acids.

Seemann obtained from gelatine in addition to formic-, acetic-, and butyric acids probably also propionic- and valerianic acids. He also draws special attention to the fact that on oxidising boiling gelatine or albumin with four times its weight of calcium permanganate, used as a ten per cent solution, he never failed to smell hydrocyanic acid, while Plimmer failed to notice HCN on using manganese peroxide and sulphuric acid or potassium permanganate and sulphuric acid as oxidising media. (See index under hydrocyanic acid.)

Bernert has drawn special attention to both oxidation and hydrolysing forces being at work during the oxidation of albumins, and Cohnheim sums up by saying: "The dissociation and oxidation of albumins with caustic potash and permanganate follows therefore the same course as does the ordinary dissociation of albumins. A portion of the albumin is dissociated in the usual manner, and the dissociation-products are then partly converted into final products and partly preserved as intermediate substances. That portion of the albumin which does not dissociate also undergoes a change, for though it contains as much sulphur as does the mother substance, it is not possible to split off more than a small portion of the sulphur by means of lead acetate and sodium hydrate; it still contains an aromatic nucleus, but not the oxyphenyl group. This last fact may be explained on the assumption that in the benzene-nucleus a change has occurred, analogous to that taking place during iodisation. The most probable explanation seems to be that the hemi-group, which is always readily detached, is removed altogether by oxidation, while the more resisting anti-group and a radical containing the carbohydrate is left over. Of the two aromatic complexes the hydroxylated complex of the hemi-group disappears, while the other one persists. The percentage composition of the products of oxidation seems also to support the suggestion offered, for Pick has shown that the anti- and the hemi-groups (see index) hardly differ from one another as far as their general

[1] G. Guckelberger, *Liebig's Annalen*, **64**. 39 (1848).

percentage composition is concerned, and that their sulphur values in particular agree closely.

"Whether the higher percentage value of oxygen is really due to an oxidation, or whether it is due to the carbohydrate radical becoming more pronounced because of the diminution in the size of the molecule, is as yet an open question."

Oxyprotein

Following up the older and neglected investigations of Chandelon [1] and Wurster,[2] Schulz [3] has treated crystallised egg-albumin with hydrogen-peroxide. He obtained a body which he calls 'oxyprotein.' When proteid is oxidised in acid or in alkaline solutions it is dissociated. On p. 93 it has already been stated that Neuberg found amino-acids to have been converted into acetaldehyde and isovaleraldehyde. No such change was induced, however, when Schulz oxidised in strictly neutral solutions. On adding an excess of hydrogen-peroxide, along with a little platinum black, to neutral solutions of albumins, he noticed, after the lapse of some weeks if he worked at the room temperature, or more quickly on warming the mixture, that the whole of the albumin was deposited. This precipitate he called 'oxyprotein.'

Apart from a higher percentage of oxygen, oxyprotein does not differ from ordinary albumin; it gives all the albumin-reactions, inclusive of Millon's reaction and the sulphur test. This substance is an acid which is insoluble in acids, water, or salt solutions, while it is readily soluble in alkalies and alkali-carbonates. It is precipitated from its solutions by acids, but only at first, for after having been kept for some time in alkaline solutions it is only partly precipitable. It differs from acid-albumin and many acid-proteids in being only re-dissolved by a large excess of acid. The alkali-salts of oxyprotein are further precipitated by small amounts of sodium chloride and other neutral salts. It is non-coagulable. The alkaloidal reagents precipitate it, but not so the salts of the heavy metals—copper and silver—which may perhaps be due to an absence of neutral salts. Alcohol does not precipitate the alkali-salt.

Schulz believes therefore peroxide of hydrogen to produce really only an oxidation of the albumin without any other changes, the oxygen entering some indifferent group and rendering it acid.

[1] Chandelon, *Ber. d. deutsch. chem. Ges.* **17**. (2143) (1884).

[2] C. Wurster, *ibid.* **20**. 263 (1887).

[3] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **29**. 86 (1899). (Here also the older literature.)

Formaldehyde Compounds

The action of formaldehyde on albumin was first studied by Trikat [1] in 1892. Blum [2] noticed that the addition of formaldehyde makes albumin non-coagulable, and called the new compound 'methylene-albumin.' Benedicenti,[3] Bach,[4] Weigle, and Merkel,[4] Alsberg and Goldschmith,[5] and Lepierre [5] have investigated these methylene albumins, the fullest account published being that of Schwarz,[5] who worked in Hofmeister's laboratory. He also studied the action of acet-, benz-, and other aldehydes.

A few drops of formalin (*i.e.* 40 per cent formaldehyde) added to several cubic centimetres of a solution of crystallised serum-albumin will prevent its coagulation by heat. Part of the formaldehyde disappears at once, while another part combines only gradually; the maximum amount is absorbed after two months, when 43 molecules of aldehyde are taken up for 100 molecules of the albumin-nitrogen. The methylene-albumin does not coagulate on heating; it is not precipitated by alcohol in the absence of salts, but is precipitated from salt solutions; it gives most of the albumin-reactions. The ethylene-albumin resulting from acetaldehyde is very soluble in acids and alkalies, but insoluble if the reaction be neutral. It behaves like denaturalised albumin. Methylene-albumin is not precipitated, however, by salts. The higher aldehydes precipitate albumins and have but little action. Concentrated solutions of albumins are converted by formaldehyde into jellies.

According to Schiff [6] and Schwarz, the aldehyde radical probably combines with the NH_2-groups, thereby converting the proteid into an acid and simultaneously denaturalising it. Schwarz points out, however, that there are other ways in which aldehyde may act. Special attention is drawn to the observations of Schwarz that halogen-albumins do not unite with formaldehyde, that no albumin-radicals are given off by bringing formaldehyde and albumins together, and that methylene-albumins are digested by pepsin, but not by trypsin—'perhaps,' because the trypsin becomes destroyed.

According to Spiro,[7] albumins combine also with esters, ketones,

[1] Trikat, *Compt. Rend.* **114**. 1278 (1892).
[2] F. Blum, *ibid.* **22**. 127 (1896).
[3] A. Benedicenti, *Arch. f.* (*Anat. u.*) *Physiol.* 1897, p. 217.
[4] According to Schwarz.
[5] L. Schwarz, *Zeitschr. f. physiol. Chem.* **31**. 460 (1900). (Here the older literature.)
[6] H. Schiff, *Liebig's Annalen*, **319**. 287 (1901).
[7] K. Spiro, *Hofmeister's Beiträge*, **4**. 300 (1903).

plurivalent alcohols such as sugars, and with aromatic alcohols such as recorsin.

Bechhold [1] has described a phosphoric acid ester of albumins.

Iron Compounds of Albumins

In addition to the salts which albumins form with different metals in their ionic state, and also with ionic iron, there exist also compounds in which the iron does not play the part of an ion, and in which, for this reason, it cannot be directly demonstrated by the ordinary reagents. How this 'masked' iron can be best revealed is described in the author's *Physiological Histology*, pp. 290-293. Ascoli [2] is of the opinion that the iron does not fix on to the albumin at all, but to the nucleic acid or to the para- or pseudo-nuclein of the nucleo-albumin. Before the differences between salts and non-electrolytes were understood, much was written about these compounds, and the expression was used that iron, iodine, etc., were in 'organic union,' and such iron-compounds as Bunge's hæmatogen [3] and Schmiedeberg's ferratin [4] were supposed to be of great value to the animal organism, for the latter was supposed to absorb iron only as 'organic iron' albumin, and not as an albuminate of iron or as an ordinary iron-salt. The experiments of Gottlieb,[5] Voit,[6] Kunkel,[7] Abderhalden,[8] and others have shown this conception to be wrong, for the body is able to absorb iron, as it does halogens, in the ionic state, and then subsequently to de-ionise it. That, on the other hand, the body may get rid of iron in the non-ionic state in the form of hæmoglobin or other iron-containing cell-constituents is also beyond doubt. Further information as to how iron is bound up in the body is given in the chapter dealing with the nucleo-proteids and with plasminic acid, see p. 447. For the iron of hæmoglobin see index.

The compounds which albumins form with silver and with osmium are mentioned on pp. 342, 343.

[1] H. Bechhold, *Zeitschr. f. physiol. Chem.* **34**. 122 (1901).

[2] A. Ascoli, *ibid.* **28**. 246 (1899).

[3] G. Bunge, *ibid.* **9**. 49 (1884).

[4] O. Schmiedeberg, *Schmiedeberg's Arch. f. experiment. Patholog. und Pharmak.* **33**. 101 (1893).

[5] R. Gottlieb, *Zeitschr. f. physiol. Chem.* **15**. 371 (1891).

[6] F. Voit, *Zeitschr. f. Biol.* **29**. 325 (1892).

[7] A. Kunkel, *Pflüger's Arch. f. die ges. Physiol.* **61**. 595 (1895).

[8] E. Abderhalden, *Zeitschr. f. Biologie*, **39**. 113 (1899).

CHAPTER VIII

THE GENERAL PHYSICAL PROPERTIES OF ALBUMINS (ACCORDING TO COHNHEIM)

ALBUMINS, when in a dry state, appear as white or nearly colourless, loose, voluminous, non-hygroscopic powders. Some albumins are known to form crystals, but most are amorphous. Some are soluble in water, while others dissolve only in salt solutions, in dilute acids, or alkalies; all are insoluble, however, in pure alcohol, ether, chloroform, benzene, and all the other solvents in general use. In stronger solutions of alkalies and acids and in glacial acetic acid they dissolve and undergo dissociation. On being burned they leave behind the characteristic smell of burnt hair; they give rise to a voluminous slightly combustible coal, and when burned completely, leave behind an ash in which are found sulphuric acid derived from the sulphur of the albumin, and usually several other inorganic elements such as calcium, and occasionally phosphoric acid.

Solutions of genuine albumins do not diffuse through animal membranes or vegetable parchment, and belong therefore to Graham's[1] class of colloidal bodies. What we have to understand under the expression 'colloidal' has not yet been definitely settled. [The author's views are given on p. 254.] Most people do not regard the colloids as being in real solution, while Zsigmondy[2] has undoubtedly proved this to be the case. For albumins the question has been definitely settled, because Sjöqvist[3] and Bugarszky and Liebermann[4] have shown that albumin solutions conduct the electrical current, and that they may act both as kations and anions—in other words that they without doubt form solutions which obey the laws formulated by van 't Hoff. The albumins are indeed

[1] Th. Graham, *Philosophical Transactions*, **151**. I. p. 183 (1861).

[2] R. Zsigmondy, *Zeitschr. f. physik. Chem.* **33**. 63 (1900).

[3] J. Sjöqvist, *Skandinavisches Arch. f. Physiol.* **5**. 277 (1894).

[4] St. Bugarszky and L. Liebermann, *Pflüger's Arch. f. d. ges. Physiol.* **72**. 51 (1898).

especially suited for demonstrating the transition from colloids into crystalloids, for some of them exhibit all the properties of colloids and yet crystallise; crystalline egg-albumin, judging by the 'gold-number' of Schulz and Zsigmondy,[1] occupies a position intermediate between colloids and crystalloids. Casein and the mucins cannot be coagulated like the ordinary albumins, but they change their physical characteristics on being 'denaturalised,' and amongst albumoses are found all transitions between hetero-albumose, on the one hand, which does not diffuse in neutral and only very slowly in acid and alkaline solutions, and between peptones, which diffuse readily, on the other hand. What factors we are dealing with in colloidal solutions we do not know. Hofmeister[2] believes the two chief characteristics of colloids to be their great tendency (1) to become insoluble under the slightest non-chemical provocations, as *e.g.* on slight evaporation of the water in which they are dissolved, or on coming in contact with porous substances, and (2) to form exceedingly thin membranes or particles, which have the power of swelling up on being moistened, provided they have been dried previously. He attributes to this behaviour of colloids the great difficulties we experience in working with albumins, particularly when we endeavour to prepare them in a pure state. Owing to these very same properties, albumin possesses in a higher degree than does any other substance the power of forming tissues, and protoplasm with its peculiar semi-fluid structure.

As in the case of other colloids, so in the case of albuminous substances it is easy to rob them of their peculiarities, and once albumins have changed it becomes impossible to restore them; the process is irreversible, and the changed albumin is said to be denaturalised or coagulated. The dissociation-products and derivatives of albumins, namely, the albumoses, peptones, halogen-albuminates, methylene-albumins, etc., are no longer colloids, and can therefore not be denaturalised.[3] It is characteristic of the natural or real albuminous bodies, or the albumins proper, that they become permanently denaturalised, and that they cannot be rendered soluble again without extensive dissociation or other change of their original state, if once they have been coagulated by heat or some other process.

[1] F. Schulz and R. Zsigmondy, *Hofmeister's Beitr.* **3.** 137 (1902).

[2] F. Hofmeister, *Zeitschr. f. physiol. Chem.* **14.** 165 (1889).

[3] That the author does not agree with these views will become apparent later.

THE GENERAL PHYSICAL PROPERTIES OF ALBUMINS (ACCORDING TO THE AUTHOR)

The question of the physical state of albumins is so important from the biological and chemical points of view that the author has thought it best to give his own views in a connected manner, although he has dealt with them more fully (up to the year (1901-2) in his *Physiological Histology*. In the following account cognisance is, however, taken of papers bearing on the colloidal nature of substances which have been published in the last three years.

For purposes of discussion it is necessary to have a clear understanding as to the meaning of 'solution,' 'electrolyte,' 'hydrolyte,' and 'colloid,' and therefore these terms are defined in the first instance.

SOLUTION.—A substance, on coming into contact with a fluid, is said to pass into solution when its molecules separate from one another and, diffusing into the fluid, mix with the molecules of the latter. The resulting mixture, consisting of the molecules of the solvent and the solute,[1] may form so homogeneous a system as not to interfere in any way with the transmission of light, or, to use a technical term, the mixture may be 'optically void,' *i.e.* contain no visible particles. On the other hand, the solute may consist of particles of such size as to interfere more or less with the transmission of light, when we speak of 'colloidal' solutions (see below) or of suspensions. All solutions are therefore mixtures.

A substance in solution, as van 't Hoff has shown, is in every way comparable to a gas. There is, however, one difference, for in the case of an ordinary gas the amount contained in the fluid is proportional to the amount of the same gas outside the fluid, or, in other words, the gaseous tension in the fluid is proportional to the partial pressure exerted by the gas outside the fluid. In the case of dissolved solids, however, the solid cannot leave the fluid, because the very fact of a substance dissolving at all depends on definite electro-chemical interactions between the solvent and the substance dissolving, as has been shown by Brühl.[2] According to this observer, the power of acting as a solvent depends on the latter possessing some atom which is potentially plurivalent; for example, oxygen in water is divalent, but capable of becoming tetravalent; the nitrogen of ammonia is trivalent, but with a tendency to become pentavalent, and so on. To this must be added the conception that the body passing into solution

[1] A solute is any substance which has passed into solution.

[2] J. W. Brühl, *Zeitsch. f. physik. Chem.* 10. 1 (1899).

may undergo an analogous change. In the light of Brühl's conception, and taking also into consideration that even pure water is partially dissociated, and possesses a high dielectric constant,[1] the following possibilities suggest themselves :—

1. The substance and the solvent, by mutually diffusing into one another, form mixtures without the solute undergoing electrical dissociation. This happens, for example, if sugar or mercuric cyanide dissolve in water, and also happens as the preliminary step in all cases where electrolytes dissolve; but in the case of electrolytes the primary 'passing into solution' is followed by a secondary chemical dissociation as described below.

The author believes, when diffusion takes place, that the solvent has one electrical charge, while the solute has the opposite charge. The mixture being a binary system, it is impossible for an electrical current to pass through it, as this would mean moving both the solvent and the solute.[2]

2. The substance undergoes in the solvent electrolytic dissociation, as in the case of electrolytes or salts containing radicals capable of giving rise to strong positive ions or kations, and to strong negative ions or anions. Thus in the case of common salt, sodium becomes +, while chlorine becomes −:

$$NaCl + xH_2O \rightleftarrows Na^{\circ} + Cl' + H_2O.$$

In this case an electrical current passes through the mixture of solvent and solute, because we are dealing with a ternary system consisting of a medium, the solvent, in which both negative and positive ions, derived from the solute, are freely movable.

3. The substance, being composed of a potential, strong kation and a potential, feeble anion, or *vice versa*, undergoes hydrolysis, which means that the weaker ion of the salt is replaced by a stronger ion derived from the solvent. If the solvent is water, and if the weaker ion of the solute is electro-positive, its place is taken by the acid

[1] A dielectricon is a substance without any electrical charge of its own, but capable of having an electrical charge induced in it. According to Coehn droplets having the smaller dielectrical constant become electro-negative towards media with higher dielectrical constants, in a mixture consisting of two non-miscible fluids. According to Drude (Drude, *Zeit. f. physik. Chem.* **23.** 308 (1897)) the dielectrical constants for the following substances are : Water 80·9, glycerine 56·2, methyl-alcohol 32·6, ethyl-alcohol 25·8, prophyl-alcohol 22·8, acetone 21·8, amyl-alcohol 16·0, aldehyde 18·6, acetic acid 9·7. These substances are all electro-positive towards glass, while the following bodies are electro-negative : Chloroform, ethyl-ether, valerianic acid, carbon disulphide, xylol, toluol, benzol, oil of turpentine.

[2] Mann, *Physiological Histology*, 1902, p. 45. See also in this book, pp. 268 and 279, under Billitzer.

hydrogen-ion of water; if the weaker ion of the solute is electro-negative, then it is replaced by the alkaline hydroxyl-ion of water. Thus corrosive sublimate and water, or sodium carbonate and water, behave as follows:—

$$HgCl_2 + xH_2O \rightleftarrows [HgOH] + H^\circ + Cl' + Cl + H_2O$$
$$Na_2CO_3 + xH_2O \rightleftarrows [H_2CO_3] + [Na^\circ + OH']_2 + H_2O.$$

4. The substance, being composed of two feeble radicals, forms with the solvent a hydrate which is only capable of undergoing complete dissociation if along with this substance another salt is present, by the dissociation of which either acid hydrogen- or alkaline hydroxyl-ions are liberated. This view is fully discussed in Chapter VI. under the heading of 'Theoretical Considerations.' See also footnote on p. 258.

ELECTROLYTE. — An electrolyte is defined by Arrhenius[1] as a substance which imparts to water, which itself is a non-conductor, the power of allowing an electric current to pass through it, in virtue of the substance being in a state of electrical dissociation or ionisation, there being formed, while no current is passing, two sets of ions, the one having an electro-negative, the other an electro-positive charge.

HYDROLYTE.—If only one of the components of a salt becomes an ion, while the other component transfers its positive charge to a hydrogen atom of the water, and thereby converts the latter into the acid hydrogen-ion, H°, or its negative charge to the hydroxyl group, OH, of water, and thereby changes the latter into the alkaline hydroxyl-ion, OH′, then the salt is said to undergo hydrolytic dissociation, and substances behaving in this manner may be termed hydrolytes. Examples of electrolytes and hydrolytes have been given under Nos. 2 and 3 in the previous paragraph on 'solution.'

COLLOID.—This term was introduced by Thomas Graham[2] in 1861 for certain substances which differ from 'crystalloids' in diffusing very slowly in water, in being unable to pass through animal bladders and vegetable parchment, and in not crystallising readily. Graham states: Crystalloids and colloids "are like different worlds of matter." According to Graham, a colloid may occur in one or more of these three states:—

1. As a fluid mixture or 'sol'; a watery mixture, for example, being called a 'hydrosol.'

2. As a firm mixture or 'gel'; thus ordinary gelatine-jelly is a 'hydrogel' of gelatine.

[1] S. Arrhenius, *Zeit. f. physik. Chem.* **1.** 631 (1889).

[2] Thomas Graham, *Phil. Trans.* **151.** 183 and 373 (1861).

3. As a solid; for example, as dry silicic acid or glass.

Graham first noticed that colloidal substances are held in solution by a singularly feeble force, being "precipitated by the addition to their solution of any substance from the other class" (*i.e.* crystalloids), and that they are also altered by heat. He called the solid constituent of gels, which contracts on heating, the clot, and the exuding liquid the serum. Colloids, which, by a reversal of the causes producing their precipitation, may be rendered soluble again, Hardy calls 'reversible.' If a colloid cannot be brought back to its original soluble state it is irreversible.

The author distinguishes between insoluble, semi-soluble, and soluble colloids. A soluble colloid is one in which all the component particles carry definite electro-positive or electro-negative charges, as will be shown later, while an insoluble 'colloid' is iso-electric, *i.e.* carries no electrical charges, and as long as a colloid remains in this insoluble state it exhibits none of the characteristics usually attributed to colloids and enumerated below. According to the nature of the particular colloid we are working with, the conversion of the insoluble into the soluble state is either comparatively easy or very difficult, and the more a colloid is rendered truly iso-electric, the more difficult is it, other things being equal, to reconvert it into the soluble form. This reconversion in the case of albumins is often quite impossible, because when the iso-electric point is approached, the different groups of amino-acids in the albumin-molecule rearrange themselves intramolecularly to compensate for the removal of the electrically charged ions by means of which they were kept in solution. In addition to this change, amino-acids may also be converted from real acids and bases into pseudo-acids and into pseudo-bases (see pp. 218, 219).

For a historical account of investigations into the nature of colloids up to the year 1902, see the author's *Physiological Histology*, Clarendon Press, 1902, pp. 28-70.

Summing up our present knowledge, colloids, when 'in solution,' have the following characteristics:—

1. They polarise transmitted light.

2. Possessing a low osmotic pressure, they raise the boiling-point or affect the freezing-point of water only very slightly.

3. They are not coagulated irreversibly by a rise of temperature, provided electrolytes are absent and provided their chemical constitution does not become permanently altered.

4. They move either with or against an electrical stream which is being passed through them, and they are therefore either electro-positive or electro-negative, but they offer a great resistance to the

flow of the electrical current, owing to their increased bulk and diminished surface.

5. They undergo hydrolytic dissociation, as in the case of arsenic trisulphide (see below).

6. They are rendered more colloidal and are readily made insoluble by electrolytes the potent ion of which[1] has an electrical sign the opposite to that carried by themselves, and they are made less colloidal by the addition of ions of the same sign.

7. Colloids of opposite electrical sign precipitate one another if they are in equivalent amounts, but if either of the two colloids is added in excess, then the colloidal precipitate, which was formed in the first instance, may redissolve.

8. One colloid in solution does not penetrate another colloid which forms a rigid system, or, in other words, colloids do not pass through animal or vegetable membranes.

9. As a rule they do not crystallise readily.

POLARISATION-PHENOMENA.—To make polarisation of light the criterion as to whether a substance is or is not a 'colloid' is not permissible for these reasons: when in Tyndall's experiment a beam of light is passed through a solution, its track may either become very evident owing to the partial reflection of the light, or the beam is hardly visible. In the former case light appears polarised because the mean wave-length of light visible to our eye has its straight course interfered with by the presence of particles, each of which is at least one-half, and may be many times the diameter of the mean wave-length.[2] If we put the scale of light visible to our eyes as lying between the Fraunhofer lines A and K, *i.e.* between λ7606 and λ3934 (see p. 479), then it must be admitted that wave-lengths below and above these limits may also be polarised by particles of an appropriate size, although these wave-lengths are not visible to us directly. It follows, therefore, that when we call a substance a colloid, because it shows a beam of transmitted light, we are interpreting physico-chemical phenomena from a narrow point of view. Because we can see particles of a certain size interfering with light visible to our eyes,

[1] The potency of an ion is determined by the degree to which its electro-affinity is satisfied by the other ion with which it is linked together. If both ions have strong electro-affinities, as in the case of potassium chloride, then neither ion can exert its influence readily; but if one of the ions is weak, as, for example, the CO_3 radical in potassium carbonate, Na_2CO_3, and the Hg-radical in corrosive sublimate, $HgCl_2$, then the stronger ion causes the hydrolysis of water, or may act on other substances of the opposite electrical sign which are dissolved in the water along with itself.

[2] See Stokes (*Phil. Trans.* 1852, p. 463), Strutt [Lord Rayleigh] (*ibid.* **41.** 107, 274, 447), and Lommel (*Pogg. Ann.* **131.** 105).

it does not mean that a fundamentally new state of matter is reached whenever we pass from invisible to visible particles. There is no transition between electrolytes and colloids, as far as Tyndall's test is concerned.

Picton in 1892[1] divided arsenic-sulphide, As_2S_3, solutions, according to their physical state, into four classes, which he called α, β, γ, and δ. The α-solution is termed a pseudo-solution, because under a magnification of 1000 diameters the fluid is seen to contain crowds of minute suspended particles in rapid Brownian movement. The β-solution, forming the transition to the γ-variety, is composed of particles so small as to be microscopically invisible. The γ-solution differs from the α and β ones in diffusing and exerting osmotic pressure, but it cannot be filtered through a porcelain filter without the solid separating out, while the δ-solution contains sulphide particles of so small a size as to pass readily through the filter.

Now Picton's α-solution is comparable to what is ordinarily called a colloid, and his δ-solution to what is usually termed an electrolyte. The difference between a colloid and an electrolyte is, therefore, in one respect, purely one of size, or a quantitative one; the difference becomes qualitative only in respect to the unit of electrical charge carried by each individual particle.

Other Phenomena.—If polarisation does not allow us to distinguish between electrolytes and colloids, then all the other characteristics mentioned on p. 257, under Nos. 2 to 9, point directly to colloids being electrolytes as long as they are in 'solution.'

This view, first advanced in 1902 by the author in his *Physiological Histology*, p. 45, accounts in general for the movement of colloids 'in solution,' when they are subjected to an electrical current, and also explains the special case of the behaviour of heat-coagulated albumin. Hardy's observation that iso-electric heat-coagulated albumin moves neither towards the anode nor towards the kathode, while after the addition of a trace of acid it moves towards the kathode, and after the addition of an alkali towards the anode, the author explained thus:—"As the proteid acquires the charge of the positive hydrogen-ion of acids, and the negative charge of the hydroxyl-ions of alkalies, we may assume the hydrogen- or hydroxyl-ions to unite with aggregates of proteid-molecules, and thus to form new ions consisting of the $(\text{colloid} + H)^{\circ}$ or $(\text{colloid} + OH)'$. The anion of the acid which was added (for example, the negative chlorine- or acet-ions) or the kation of the alkali (for example, the positive sodium-ions) become the companion-ions to the $(\text{colloid} + H)^{\circ}$ or the $(\text{colloid} + OH)'$-ions."

[1] Harold Picton, *Journ. Chem. Soc.* **61.** 137 (1892).

This view, expressed by the author in 1902, he believes still to hold good for a colloid such as that of gold, which moves towards the positive electrode or anode when its watery solution is subjected to an electrical current. When Bredig makes his colloidal gold solutions [1] by passing a current of 10 to 12 amperes through gold electrodes which are immersed in acid-free water and which are kept 1 to 2 mm. apart, he finds the negative electrode or kathode to break up into colloidal particles of gold. According to the author the + hydrogen-ions of the water transfer their electrical load at the kathode to the gold, which now passes into solution as + gold-ions. These gold-ions, having a very low electro-affinity, then unite with the − OH-ions of the water, which have a stronger electro-affinity, and there are formed new electro-negative ions, namely, $[AuOH]'$, which travel towards the positive anode. The negative colloidal gold-ion has as its partner a positive hydrogen-ion of the water. This explanation helps us to understand why the addition of minute traces of alkali, *i.e.* OH-ions, to the water in which the colloidal gold solution is being made, greatly facilitates the formation of the colloidal gold, and why, on the other hand, any free acid will at once precipitate the colloidal gold for $[AuOH]' + H^\circ = Au + H_2O$.

In the case of gold-solution the problem is comparatively simple, but it becomes much more complex if we are dealing with a substance such as arsenic sulphide, As_2S_3. This compound is formed by passing a stream of sulphuretted hydrogen gas, H_2S, through a solution of arsenic trioxide, As_2O_3, and then removing the excess of H_2S by means of an inert gas. Arsenic trioxide when in solution gives rise to arsenious acid, $As(OH)_3$, which by dissociating into $[As(OH)_2O]' + H^\circ$ liberates the acid H°-ion, and hence gives rise to the acid reaction of an arsenic trioxide solution. Sulphuretted hydrogen in watery solutions also dissociates to a slight extent into $H^\circ + SH'$, and hence again the solution gives an acid reaction.

We are dealing, therefore, with the interaction of two substances which are potential acids, and which for this reason have the tendency of mutually precipitating one another.

Now, $As_2O_3 + 3H_2S$ give rise to $As_2S_3 + 3H_2O$. The arsenic trisulphide formed in this way undergoes in its turn a hydrolytic dissociation into $As_2O_3 + 3H_2S$. The possibility of such a dissociation was pointed out to Freundlich [2] by Dr. Böttger, and Freundlich actually found that H_2S, being a gas, can leave a colloidal arsenic sulphide solution, and thereby lead to a disintegration of the colloidal

[1] Georg Bredig, *Zeit. f. angew. Chem.* 1898, p. 951.

[2] Herbert Freundlich, *Zeit. f. physik. Chem.* **44.** 129 (1903).

solution which is more rapid in an open vessel than if the solution be preserved in a closed bottle.[1]

On the assumption that As_2S_3 really dissociates into $As_2O_3^{\circ\circ\circ}$ and $3H_2S'$, and these in their turn into $As(OH)_2O' + H^\circ$ and $H^\circ + HS'$, the complexity of the colloidal solution must be very great, and therefore its equilibrium must be very unstable. By the addition of strong acids the dissociation of $As(OH)_2O' + H^\circ$ and that of $H^\circ + HS'$ will be prevented, and therefore $As_2O_3 + 3H_2S$ are formed first, and ultimately the completely undissociated As_2S_3. By the addition of alkalies, on the other hand, either ortho-arsenites (*e.g.* $Ca_3(AsO_3)_2$) or meta-arsenites (*e.g.* $Ca_2As_2O_5$ or $KAsO_2$) result, which keep the arsenic in solution because both potassium and calcium possess much greater electro-affinities[2] than does arsenic. The author has thought it necessary to enter into this question because other writers always speak of spontaneous changes taking place in colloidal solutions without having taken proper precautions for excluding the possibility of chemical action. The author has found that using paraffined vessels to exclude the chemical action of glass, and keeping bottles well filled with colloidal solutions to diminish the air space, and using paraffin stoppers, that colloidal solutions keep with very little alteration after a preliminary change, due to mechanical disturbance, has taken place (see p. 274).

When a colloidal solution becomes semi-soluble, or, in other words, more colloidal, when, for example, Picton's δ-arsenic sulphide solution is changed into γ-, then β-, and ultimately into the α-variety, the following changes occur:—In a freshly prepared non-colloidal arsenic sulphide solution, As_2S_3 is dissociated into $[As_2O_3]^{\circ\circ\circ}$ and $3[H_2S]'$, and this dissociation is also met with in colloidal solutions, as has been shown by Freundlich.[3] When the colloidal solution becomes less colloidal there occurs, according to the theory of the author, a diminution in the amount of electrical dissociation; and this diminution is accompanied by a gradual increase in the size of colloidal particles. Picton and Linder[4] were the first to notice that the size of the colloidal particles increases when the point of coagulation is neared, and that there is a reaction other than mechanical between solvent and solid, even in these cases of colloidal solution.

[1] Freundlich has omitted to analyse the remaining solution, and the possibility of the arsenic solution which was kept in an open vessel having absorbed CO_2 from the air, and having thereby become precipitated, must also be taken into account, for by acids arsenic sulphide is precipitated completely.

[2] Mann, *Physiological Histology*, 1902, p. 14.

[3] Herbert Freundlich, *Zeit. f. physik. Chem.* **44.** 129 (1903).

[4] S. E. Linder and H. Picton, *Chem. Journ.* **61.** 137 (1892).

A non-colloidal arsenic sulphide solution, *i.e.* one in which the component particles are smaller than half a wave-length (see p. 258), is only capable of existence in the presence of free hydroxyl-ions, and if these be removed there occurs at once a change which expresses itself optically by light being polarised, and this depends, as has already been shown, on particles being formed in the solution which are larger than half a mean wave-length.

The removal of the hydroxyl-ions from the arsenic sulphide solution may produce one of two changes. If it required one hydroxyl-ion, OH′, for each arsenic sulphide molecule, As_2S_3 (or an arsenite molecule), to remain in solution, then with the removal of each hydroxyl-ion one arsenic sulphide molecule will cease to exist as an ion, and form a sediment as quickly as the viscosity of the water allows it to do so, provided it cannot form a binary system with water (see under solution, p. 255). If all the hydrogen-ions, each in custody of one arsenic sulphide molecule, were neutralised, then the whole of the sulphide would settle as an insoluble mass. The other possibility, and the one which the author believes to be actually at work, is that the As_2S_3 molecule itself undergoes a dissociation as soon as the hydroxyl radicals are removed, and that in this way are formed $[As_2O_3]^{\circ\circ\circ} + 3[H_2S]'''$.[1] If all the As_2S_3 molecules underwent this hydrolytic dissociation the arsenic sulphide solution would still be non-colloidal, as it is improbable that molecules having such low molecular weights as $As_4S_6 = 492$,[2] or $As_2O_3 = 198$, or $H_2S = 34$, are capable of polarising light. We must therefore assume that the particles in a colloidal solution of arsenic sulphide are composed of at least several molecules, and that these aggregates are kept in solution by an electrical charge. It is immaterial whether we assume that the colloidal particles are formed by a mechanical conglutination (see p. 274) of molecules or by such chemical action as anhydride-formation, as seen, for example, in the case of dextrose when it is converted into colloidal starch.

The essential point is that each colloidal aggregate must carry a definite electrical load, because it can move with or against an electrical stream, and that there must be other ions in the solvent, possessing an electrical sign the opposite from that carried by the colloid. As such a colloidal solution is precipitated by certain agencies, we must next inquire as to how a colloidal solution consisting of smaller particles is transformed into one containing larger units.

[1] The dissociation may be such that the positive load is carried originally by the H_2S, and the negative load by the As_2O_3 or $[As_2O_3]'' + 3[H_2S]^{\circ\circ}$.

[2] The correct formula for arsenic sulphide is As_4S_6, and is not As_2S_3.

When arsenic sulphide, As_2S_3, is in solution, it partly dissociates into $[As_2O_3]^{\circ\circ\circ}$ and $3[H_2S]'''$. The latter then dissociates still further into $H^\circ + HS'$, and thereby renders the whole solution acid. Comparing various salts which undergo hydrolysis and in doing so form acid solutions, we find, on the one hand, solutions which are almost colloidal, namely, $HgCl_2 = HgOH + 2HCl$; next, colloidal compounds such as arsenic trisulphide, $As_2S_3 = As_2O_3 + 3H_2S$; and finally, salts which are thrown down as insoluble basic compounds if their saturated solutions be diluted, as happens in the case of the nitrate of bismuth $[Bi(NO_3) + 5H_2O] = Bi(OH)_2NO_3 + 2HNO_3$. Whether a salt undergoing hydrolysis does or does not form insoluble basic salts depends on the strength of the base, on the power of dissociation possessed by the acid radical to which the base is joined, and as to whether the acid is an oxy-acid, for while, as just stated, mercuric chloride forms clear solutions, mercuric sulphate and mercuric nitrate form insoluble basic salts if they are diluted sufficiently.

In the case of arsenic sulphide, As_2S_3, we are dealing with both a feeble kation $[As]^\circ$ and a feeble anion $[S]'$, but the S of the anion is stronger than is the As of the kation, in consequence of which the compound As_2S_3, on coming into contact with water hydrolyses, which means the stronger anionic S′ links on to two kations, H°, of the water to form SH_2 or sulphuretted hydrogen, while the kationic As° links on to three anions, OH′, of the water to form $As(OH)_3$. Both SH_2 and $As(OH)_3$ have the tendency to dissociate electrolytically in such a way as to liberate free, acid, hydrogen kations, but the SH_2, possessing greater electrolytic power, only allows of a very partial electrolytic dissociation of the $As(OH)_3$ radical, the dissociation of the $As(OH)_3$ being just sufficient to keep it in solution in the form of particles of such size as to be capable of polarising light, *i.e.* to produce a colloidal solution. Another way of looking at this question is to say that the water + H_2S forms one phase, and that the $As(OH)_3$ forms a second phase, and that $As(OH)_3$ is soluble in the H_2S + water phase because a certain amount of difference of potential can be set up at the interfaces, in consequence of which the two phases tend to mix with one another, *i.e.* tend to pass into solution. (See the author's definition of 'solution' on p. 254.)

The colloidal nature of arsenic sulphide depends thus on the partial solution of the $As(OH)_3$ complex. As arsenic sulphide in pure water tends to split up into the two acid solutions $[SH]' + H^\circ$ and $[As(OH)_2O]' + H^\circ$, it will be readily understood that the formation of these two acids will be prevented by the presence of any stronger acid, *i.e.* of any other acid which, if present in equivalent amount,

will give rise to a greater number of free acid H° ions in the same bulk of fluid.

The precipitation of the colloidal arsenic sulphide solution is therefore in every respect comparable to the precipitation of any non-colloidal acid by the addition to its saturated solution of a second stronger acid.

The next questions are difficult, namely, whether the basic radical As_2O_3 is appreciably dissociated into $As(OH)_3 \rightleftarrows [As(OH)_2O]' + H^\circ$, and whether As_2S_3 may be considered capable of giving rise to such complex-ions as $[As_2O_2S]''$ and $[As_2OS_2]''''$ or to more complex arsenic-sulph-hydroxyl-ions. As in an arsenic sulphide solution the colloidal particles are repelled more from the negative than from the positive pole, according to Linder and Picton[1] they must carry negative loads. The author again supposes that the colloidal particles are hydroxyl compounds, comparable to colloidal gold, mentioned above on p. 260, and that each particle is composed of a sufficient number of arsenic-sulph-hydroxyl radicals to have assumed dimensions larger than one-half a mean wave-length (see p. 258).

Provided the author's hypothesis is correct, "we may consider the solution of an ordinary dissociated electrolyte as representing a double 'colloidal' solution, and the formation of insoluble hydrates during hydrolysis as the precipitation of one of the two colloidal solutions."[2] "If the aggregated particles in a colloidal solution are completely broken up into the composing units by acquiring definite charges, then the colloidal solution loses its colloidal character and becomes a solution of a completely dissociated electrolyte, as happens, for example, in the case of silicic acid, which in the presence of HCl gives negative results with Tyndall's experiment, but which in the absence of hydrochloric acid soon undergoes a change by means of which an aggregation into particles is brought about, and now Tyndall's experiment gives positive results."

The change from a so-called 'electrolytic' into a so-called 'colloidal' solution the author explained as follows:—"If to a solution containing a definite number of electro-positive (colloid + H)°-ions there is added an alkali containing the same number of electro-negative hydroxyl-ions, then the H° of the colloid and the OH' of the alkali unite to form electrically neutral water, and the colloid, having lost its electrical charge, is precipitated; if, however, not a sufficient number of OH'-ions were added to bind all the hydrogen-atoms, then the colloid-aggregates re-arrange themselves into larger aggregates, which

[1] S. E. Linder and H. Picton, *Chem. Journ.* **61.** 137 (1892).

[2] Mann, *Physiological Histology*, 1902, p. 46.

will remain in 'solution' as long as the H-ions, joined to the colloid, enable its aggregates to maintain a definite charge. As the colloidal particles become larger and larger, and the number of electrical charges for a given volume of solution fewer and fewer, complete precipitation will probably be induced at a time short of perfect neutralisation of the charges, because of the specific gravity of the colloid-aggregates overcoming the viscosity of the fluid."[1]

When a colloid becomes iso-electric it ceases to be a colloid; and provided that the framework which is formed during the process of rendering a colloid iso-electric is broken down mechanically, the newly formed iso-electrical or non-ionised compound will fall to the bottom of the vessel as an insoluble precipitate.

Why the colloid does not unite in every case with the radical having the stronger electro-affinity,—why, for example, after the addition of an acid the colloid unites with H°, which has less electro-affinity than K°, while after the addition of an alkali it does not unite with K° but the OH', which latter has also less electro-affinity than K°,—seemed, in 1902 to the author, "to be determined by the fact that H° and OH' are those very radicals which by their union form water, and which therefore may have special chemical affinities for the colloid, inasmuch as the latter owes its existence to having been formed in water." Now the author is of the opinion that colloids link on to H° or OH' for the very reason that these radicals have less electro-affinity, on the principle that a compound becomes the more insoluble the greater the agreement is in the amounts of the electro-affinities carried by the kat-ion and by the an-ion. According to this view, silver has the same amount of + electro-affinity as chlorine has − electro-affinity, and hence silver chloride is insoluble. The same reasoning would apply to other insoluble salts, and seems to be supported by the fact that colloids carrying a negative charge are most readily precipitated by colloidal hydrates carrying a positive charge. See later, p. 270, under Spring and Biltz.

In this connection the precipitation of colloidal solutions by the addition of 'neutral' salts must be mentioned. Even if we assume that the added neutral salt does not interfere in any chemical manner with the colloid we are experimenting with, it is evident, if the author's view is correct—namely, that electro-affinities in equivalent intensities but of opposite sign attached to two radicals lead to these two radicals forming insoluble compounds—that salts which are soluble and capable of electrical dissociation must for this very reason be composed of ions which differ from one another as regards their electro-affinities. If,

[1] Mann, *Physiological Histology*, p. 46.

however, either the kation or the anion is stronger, then the unsatisfied balance of electro-affinity represents available energy which, when brought into contact with colloids, will lead to the precipitation of the latter, provided that the colloid has an electrical sign which is the opposite to that carried by the stronger ion of the 'neutral' salt. This explanation offered by the author has further in its favour the fact that the electro-affinities of colloids are very feeble.

On Acclimatisation of Colloids, and on their 'Spontaneous' Change

Before giving further evidence in support of the view that colloids are electrolytes, it is necessary to discuss the so-called 'acclimatisation' of colloids and their 'spontaneous change.' (See p. 323.)

Acclimatisation.—Freundlich [1] has stated that generally the precipitation of a colloid varies according to the quickness with which a given quantity of salt is added. Suppose it requires the addition of 10 ccm. of a given salt solution to produce complete coagulation of a colloid, provided the salt solution be added quickly, then after the gradual addition of 5 ccm. of this salt solution it will take still 10 ccm. to complete the coagulation. Freundlich comes therefore to the conclusion that in precipitating a colloid it is not a mere question of adding a certain number of ions, and that we are not dealing with chemical equilibria, but that the agency bringing about coagulation is a time-factor, dependent on the rate of diffusion. If all the salt solution is added at once, then a marked diffusion of ions is called forth, owing to great differences in the concentration of the solution, and correspondingly great differences of potential will be established in the biphasic colloid + water system at the limiting surfaces of the two phases, in consequence of which rapid 'coagulation' will ensue. For further information on the views of Freundlich, see p. 268.

The author cannot accept this explanation, for the conversion of an electro-positive or an electro-negative colloid into an iso-electric, *i.e.* non-electric, non-colloidal, or insoluble substance, depends directly on the abolishment of hydrolytic dissociation, while the separation of the non-colloidal substance is dependent on entirely different causes. If we imagine a colloidal solution containing a sufficient number of colloidal particles to allow these to be in actual contact with one another when they are distributed throughout the solution, it follows that these particles must aggregate whenever the existing difference of potential between the colloidal particles and the solvent is done away with,

[1] Herbert Freundlich, *Zeit. f. physik. Chem.* **44**. 129 (1903).

because the surface tensions and consequently the surface areas of two substances in 'solution' are inversely proportional to the amount of the existing difference of potential. Therefore to remove the difference of potential is equivalent to making the particles approach one another and collectively offer a smaller surface, for the surface increases as the square, while the cubic capacity increases as the cube.

If the solute and the solvent in their free state are both fluids, they will completely separate from one another if in solution, as soon as the difference of potential which keeps them in solution is abolished; thus water and oil in the iso-electric state separate into two layers. It is quite different, however, if the solute in its free state is, for example, such a solid as arsenic sulphide. By the gradual addition of acids or acid salts the arsenic sulphide will aggregate into larger and larger particles, till ultimately an exceedingly delicate framework is formed in the meshes of which is the solvent. This framework will gradually become more and more resistant owing to the increase in surface tension, and therefore the small trabeculæ formed in this way will offer greater mechanical resistance to a diminution of their surface, and hence we require to add, 'after having acclimatised' colloid, still about the same amount of a coagulating electrolyte as would have been sufficient in the first instance if we had added it quickly.[1]

The real cause of the rapid coagulation of a colloidal solution on the quick addition of an electrolyte sufficient to bring about complete coagulation seems to be the following:—The strong diffusion currents lead to the formation of irregular mechanical aggregates, or to a conglutination of the particles as described on p. 274.

Spontaneous Change.—For a colloid to change 'spontaneously' is a matter of impossibility, still the expression is used constantly, and therefore an inquiry into the causes leading to spontaneous coagulation is needed. Hantzsch has shown that certain colour bases are especially liable to become converted into pseudo-bases, and, as pointed out on p. 219, this conversion is accompanied by a loss of basic capacity; if, further, albumins are mixtures of pseudo-bases and pseudo-acids, it is quite conceivable that owing to the disappearance of a predominating acid or basic function the albumin molecule may become iso-electric, and thereby be rendered insoluble.

Not taking into consideration the possibility of chemical action

[1] The question as to whether a colloid, by forming a resisting framework, is capable of resisting the electrical stress of an electrolyte by the development of torsional electricity cannot be entered into here.

such as that due to the liberation of alkalies by the decomposition of glass vessels or due to the absorption of gases, the following conception seems to the author worthy of being considered:—The transformation of insoluble arsenic sulphide into the colloidal electrolytic variety requires a strong force to overcome the initial inertia of the iso-electric sulphide, which is held together by a high surface tension; but once having been set in motion a comparatively small force will be sufficient to keep the sulphide in the colloidal state, and therefore it is possible to dialyse away a great deal of the acid which was required in making the colloidal solution without leading to a precipitation of the colloid. If, however, a feeble force in one direction is sufficient to maintain a colloid in solution, then also will it require only a feeble force in the opposite direction to precipitate a colloid from its solution, and this accounts for the ease with which colloidal solutions may be thrown down. As in the case of a body once set in motion, its momentum will carry it a certain distance, so also in the case of colloids. A 'solution' made by mechanical means will remain for a certain time in solution, but gradually as the component particles lose their momentum it will become insoluble.

As pointed out on pp. 266 and 323, true 'colloidal solutions' depending for their solubility on the presence of electrolytes are permanent as long as all chemical change is prevented.

Further Evidence that Colloids are Electrolytes

To the same conclusion as the author, namely, that colloids are electrolytes, have subsequently come Billitzer, working under Nernst, and Freundlich, working in Ostwald's laboratory. The author's views were expressed in 1902 in his *Physiological Histology*. See this book, pp. 259, 262, 264.

Billitzer[1] arrived in 1903 at the conclusion that colloids may be regarded as ions, for he found it impossible to explain the movement of colloidal particles in an electrical field on v. Helmholtz's hypothesis[2] that a separation of the positive and the negative charge in electrolytes is brought about by the formation of a double electrical layer, which was so constituted that on two sides of a plane immeasurably thin[3] there were developed equivalent but opposite amounts of electricity.

Freundlich[4] explains the behaviour of colloids on the assumption

[1] Jean Billitzer, *Ann. d. Physik*, **316**. 902 and 937 (1903).
[2] H. v. Helmholtz, *Pogg. Ann.* **165**. 228 (1853).
[3] See Lord Kelvin, *Nature*, 31st March and 19th May 1870.
[4] Herbert Freundlich, *Zeit. f. physik. Chem.* **44**. 129 (1903).

that the surfaces of colloidal particles are semi-permeable, which means that they allow of the ready passage of ions of the opposite electrical sign to that carried by themselves, *i.e.* of either kations or of the anions, while the other ions which cannot enter the colloidal particles remain in the solvent. This explanation amounts to the same as that given by the author, namely, that the [colloid + the entered ion] is an ion. Very interesting in this connection is an observation made by A. Fischer,[1] who noticed that "the basic dyes are absorbed at once by the acid nucleoproteids, while with acid stains there is a delay, in about the proportion that methyl green will have stained already intensely, when acid fuchsin only just shows the faintest indication of staining. In the course of ten minutes, however, this difference disappears in material which was fixed in indifferent reagents." Reversely "the acid dyes diffusing through the sections stain at first only the cytoplasm, and several seconds later the nuclei, which ultimately are also stained as intensely as the cytoplasm." Fischer failed to understand the importance of his own observations, for he uses his facts to prove the absence of any real difference in the absorptive powers of nucleins with regard to acid and basic dyes; while to the author,[2] Fischer's observations have this significance: each particle, either kat-ion or an-ion, has an aversion for ions of its own kind or those of the same electrical sign; thus the positive kat-ion H° will not only repel other H° ions, but also, for example, those of potassium, K°. On the other hand, positive kat-ions will readily unite with negative an-ions.

The view that colloids are electrolytes is further supported by the fact that colloids of opposite electrical sign precipitate one another, as has long been known to histologists. Romanowsky[3] in 1891 combined equi-molecular proportions of the basic methylene-blue and the acid eosin, and thus obtained the water-insoluble eosinate of methylene-blue.[4] Quite recently the same phenomenon has been studied by Biltz,[5] who calls these unions 'adsorption-compounds' (see p. 270).

After giving various further examples of the fact that hydrosols of opposite electrical sign mutually precipitate one another if they are mixed in equivalent amounts, he shows that mixtures of hydrosols possessing the same electrical sign—such as the purple of

[1] A. Fischer, *Fixirung, Färbung und Bau des Protoplasmas*, 1899, p. 94

[2] Mann, *Physiological Histology*, 1902, p. 339.

[3] Romanowsky, *Zur Frage d. Parasitol. u. d. Therap. d. Malaria*, St. Petersburg, 1891.

[4] Other instances are given in the editor's *Physiological Histology*, pp. 441-444.

[5] W. Biltz, "Mutual Interactions of Colloidal Substances," *Ber. d. deutsch. chem. Gesell.* **37**. 1095 (1904).

Cassius, which is a hydrosol of stannic acid and gold—are thrown down together by electrolytes having the opposite electrical load. He also found that the precipitating action of mixtures of electrolytes and colloids is an additive effect, and that in many cases an action which seems to be brought about by an electrolyte is caused at least partly by the presence of colloids. In this connection he draws attention to the work of Spring,[1] who showed that solutions of the salts of plurivalent metals (such as aluminium chloride or ferric chloride) are not optically void, and therefore must contain colloidal hydroxide; and also to the work of Mylius,[2] who accounts for the fact that metaphosphoric does, while orthophosphoric does not, coagulate albumin by showing that metaphosphoric acid contains polymolecular particles, *i.e.* that it is in fact a 'colloidal' solution. Mylius further shows that all acids which precipitate ordinary white of egg, after it has been diluted, contain complex molecules.

Biltz objects to a chemical explanation of colloidal solutions, and considers Bredig's view to be correct, namely, that the cause of the relatively great stability of pure colloidal solutions is the electrical difference of potential between the colloid and the solvent. The author wishes to point out that according to all laws of physical chemistry the first essential for chemical interaction is the establishment of an electrical load, or, in other words, that only those substances which carry an electrical load are capable of acting upon one another 'chemically.' To say, as does Biltz, that we are dealing with adsorption-phenomena when a colloid is precipitated, is not giving an explanation at all, but amounts simply to stating the premise over again in a roundabout manner. If the cause of the adsorption is the ionic difference of potential between two substances, then adsorption means simply chemical union.

The inter-relation of suspensions and colloids in viscid media; the behaviour of colloids upon one another, and how under certain circumstances one colloid may prevent the precipitation of a second colloid,—are fully discussed by Arthur Müller.[3]

Whitney and Ober[4] were able to show that different metals combine in equivalent amounts with colloids in forming precipitates, thus:—

[1] Spring, *Bull. de l'Acad. Roy. de Belg.* 1900, p. 483.

[2] F. Mylius, *Ber. d. deutsch. chem. Ges.* **36**. 775 (1903). (The reader's attention is especially directed to this important paper.)

[3] Arthur Müller, *ibid. Ges.* **37**. 11 (1904).

[4] Whitney and Ober, *Zeit. f. physik. Chem.* **39**. 630 (1902).

1·9 per cent As_2S_3.	Chloride Solutions 25 ccm.	Amount of Salt added expressed in Grammes.	Free Chlorine remaining in Solution calculated as Acid.
100 ccm.	K	2·00	0·0038
100 ccm.	Ba	0·1394	0·0039
100 ccm.	Sr	0·1071	0·0042
100 ccm.	Ca	0·0706	0·0040

This table is exceedingly interesting, because it shows not only a marked difference between the monovalent potassium and the divalent barium, strontium and calcium, confirming Hans Schultze's [1] observation that the relative coagulative power of mono-, di-, and trivalent metals varies greatly, but also shows, according to the author's opinion, that within the divalent metals the power of precipitating colloids increases with the diminishing electro-affinity of the metals.[2] This fact is a further support of the author's views on electrical dissociation and solubility of salts, see pp. 255, 265, and 289.

The fact that colloids may decompose such a 'neutral' salt as barium chloride and induce its hydrolysis shows that colloids must be chemically active, *i.e.* that they must be electrolytes or hydrolytes (see pp. 254-256). The first observer to point out that 'inert' substances may decompose neutral salts in the presence of water, and that they may join either with the acid or basic radical set free, was v. Bemmelen,[3] who investigated such porous substances as animal charcoal, silicic acid, and coagulated colloids.

Changes induced in Colloids by Physical and Chemical Means

The author's view that colloids are electrolytes and capable of reacting with H° and OH' ions was expressed by him in 1902 [4] in the following way:

"(1) Direct union between an acid and the colloid:

$$\text{Colloid} + HCl + \text{water} = (\text{colloid} + H)^{+} + Cl^{-} + H_2O.$$

[In this case the ion $(\text{colloid} + H)^\circ$ is a complex ion, comparable to the ion $(PtCl_6)''$ of the so-called platinum chloride H_2PtCl_6.]

[1] Hans Schultze, "Schwefelarsen in wässeriger Lösung," *Journ. f. prakt. Chem.* **25**. 431 (1882), and **26**. 320 (1883).

[2] Mann, *Physiological Histology*, p. 14. A table of electro-affinities copied from Abegg and Herz is given on p. 313 of this book.

[3] v. Bemmelen, *Zeit. f. anorg. Chem.* **23**. 321 (1900).

[4] Mann, *Physiological Histology*, p. 48.

"(2) Indirect action of a kat-ion radical on the colloid:

$$\text{Colloid} + \text{water} + \text{HCl} = (\overset{+}{\text{colloid}})\ (\overset{-}{\text{water}})\ \overset{+}{\text{H}} + \overset{-}{\text{Cl}}.$$

[In this case the colloid acts as a dielectricon or a body which has no electrical charge of its own, but in which an electrical charge may be induced owing to the colloid being a hydrate. The author holds further that the development of a charge in a 'dielectricon' is accompanied by some kind of definite chemical change in the dielectricon.]

"(3) Direct action of an an-ion radical on the colloid:

$$\text{Colloid} + \text{HCl} + \text{water} = (\overset{+}{\text{colloid}})\ \overset{-}{\text{Cl}}\ \overset{+}{\text{H}} + \text{water}."$$

"In this last case the chlorine-ion, which has greater negative electro-affinity than the hydrogen-ion has a positive-affinity, may be assumed to satisfy its full negative-affinity by evoking a positive response of the colloid, in which case the positive H° and the positive colloid° will together completely balance the negative Cl′." [See also the author's definition of a neutral salt, p. 265 of this book.]

The changes which colloids—defined in the manner just given—may undergo, were then classified in this way:[1]

"(A) Changes in temperature leading to 'setting':
1. By lowering the temperature.
2. By raising the temperature.

"(B) Mechanical shaking producing 'conglutination':

"(C) Physical factors inducing 'coagulation':
1. By alterations in the electrical tension between the colloid and its solvent.
2. By dehydration (or 'salting out').

"(D) Chemico-physical factors causing 'precipitation,' owing to—
1. A withdrawal of the H° or OH′ radical of the colloid.
2. A removal of salts.
3. The formation of insoluble or irreversible salts.
4. Heat action.

"(E) Chemical action accompanied by physical change, owing to the formation of additive compounds between colloids and non-electrolytes by the process of oxidation or reduction."

This classification has again been adopted in the following pages, with the addition of a chapter on so-called spontaneous coagulation on p. 323. (See also p. 266.)

[1] Mann, *Physiol. Histol.* p. 48.

1. Setting of Colloidal Solutions.

This question has been investigated by Hardy,[1] who calls jellies which on heating become fluid, and on cooling revert to the solid state, 'reversible jellies.' When colloidal matter occurs in the state of a gel, which means a mixture of a fluid and a solid, then "it may consist of a solid mass containing spherical fluid droplets, or of solid droplets, which by hanging one to the other form a framework, in the spaces of which fluid is held. These terotypes present important mechanical peculiarities. The former is firm and elastic, and it maintains its structural integrity even under high pressure. The latter is much more brittle and manifests a tendency to spontaneous shrinking, which is due to a continuous increase in the surface of contact, or possibly union between droplet and droplet. These gels with the open solid framework therefore specially manifest that property of spontaneous shrinkage to which Graham applied the term 'synaeresis.'"[2]

The author believes that the setting of gelatine which occurs on cooling is in every respect comparable to the firming which molten alloys of metals undergo when they are cooled. What attracts molecules[3] of the same kind to one another when the temperature begins to fall is the circumstance that they possess the same surface-tension; or with other words that they cannot develop amongst themselves a difference of potential, which according to the author's view is essential for the diffusion of one substance into another one, as explained on p. 255. As, further, a solution containing an electrolyte becomes a better conductor when it is heated, and as this increase in conductivity is not due to an additional electrolytic dissociation of salt-molecules, it must be due to an increase in the mobility of the already existing ions. We find, therefore, in a cooling salt solution an increasing rigidity of the ions analogous to the increasing viscosity of a cooling gelatine solution.

When a gelatine solution cools, we have, therefore, firstly an aggregation of the gelatine molecule on the one hand, and of the water molecule on the other hand, because each species of molecule possesses one surface tension, and secondly a 'setting' owing to the firming of each individual molecule.

As examples of a partial setting when the temperature is raised

[1] Hardy, *Journ. of Physiol.* **24.** 172 (1899).

[2] For further information see author, *Physiological Histology*, p. 49.

[3] The expression molecule is meant to include every kind of particle from an electron to the largest aggregate of atoms capable of forming a definite compound.

may be mentioned, the coagulation of the caseinogen salts of calcium, magnesium, barium, strontium, caffeine, and strychnine, which, according to Osborne,[1] occurs between 35° and 45°.

2. Conglutination or Aggregation by Mechanical Means

Berthold[2] observed in 1886 that 'mechanical coagula' may be produced by shaking white of egg with distilled water, and that this coagulum has the fibrillar appearance described by Flemming as occurring normally in protoplasm. Ramsden[3] then found, quite independently, that solid molecular aggregates of albumin may be obtained by mere agitation of various albuminous solutions. Filtered, clear solutions of white of egg, for example, on being shaken in a test-tube soon form masses in the shape of long strands and flocculi. The results of Ramsden have been criticised by Starke,[4] who believes that these mechanical coagula are due to a drying of the proteid solution whenever it comes into contact with air. To prove his point he fills a bottle completely with proteid solution, drops in pieces of glass rods, corks the bottle to exclude all air, and then shakes vigorously for some weeks and obtains no coagula. Such an experiment excludes, however, those very conditions as to surface tension which are necessary for Ramsden's results (see below).

The author[5] has explained the formation of mechanical aggregates by assuming that the particles, which give rise to visible masses, adhere to one another as would pieces of butter or any similar substance, on being thrown together. The author introduced, for the purpose of making a sharp distinction between chemical coagulation and the formation of mechanical aggregates for the latter, the term 'conglutination,' and explained the formation of mechanical aggregates on shaking, for example, a solution of egg-white in the following way: "The molecules of white of egg in a dilute watery solution may be supposed to be evenly distributed and spheroidal in shape. If in sufficient number to touch one another, regular geometrical figures will result, as are seen, for example, in foams, with this difference, that each space in the foam must be imagined to be filled by one molecule of proteid. Such solutions of 'proteid' appear clear, because all the molecules, being symmetrically arranged, will form a homogeneous mass."

"Where the solution is in contact with the air, as on its free

[1] W. H. Osborne, *Journ. of Physiol.* **27**. 398 (1901).
[2] Berthold, *Studien über Protoplasmamechanik* (1886).
[3] Ramsden, *Arch. f. (Anat. u.) Physiol.* 1894, p. 517.
[4] Starke, *Zeit. f. Biol.* **40**. 419 (1901).
[5] Mann, *Physiological Histology*, 1902, p. 51.

surface, the proteid-molecules are subjected along with the water-molecules to the effect of surface tension, and may be supposed to form collectively an elastic proteid membrane. The latter appears clear, because the component molecules are all similarly arranged, forming a homogeneous layer. By shaking, the surface particles are, suddenly, partly released from tension and partly subjected to still greater stress, and being unable to resume at once the original shape, owing to their viscosity, masses of distorted and compressed molecules will be produced. On continuing the process of shaking, the already-formed aggregates act as nuclei to which other surface molecules attach themselves, and thus strands and fibres are formed of sufficient size to become visible to the naked eye."

In further support of this view it was pointed out by the author[1] that "the tendency of weak alcohols to prevent the formation of mechanical coagula is very interesting, and is probably due to a double action, namely, partial dehydration of the proteid molecules, and also to an alteration in the surface tension of the fluid."

More recently Ramsden,[2] by ingenious experimentation, has succeeded in showing that true membranes are actually formed, and that an essential condition for the formation of these aggregates is the presence of a free (*i.e.* gas or air) surface, and that aggregates similar to those formed by egg-white may be obtained with all proteid solutions, including even the simplest peptones, and with a very large number of other colloidal substances (soap, quinine, ferric hydrate, saponin, aniline dyes, etc.).

At any appropriate surface particles of the previously dissolved colloid pass spontaneously out of solution and form a delicate surface pellicle which exhibits many of the characteristic properties of solid matter. This rearrangement of the system :

$$\text{Water} \rightleftarrows \text{dissolved colloid} \rightleftarrows \text{gas},$$

is due to the fact that the total energy of surface tension is thereby diminished. If such a surface with its coating of solid particles be forced to diminish rapidly in area, these particles are heaped up and become visible as 'mechanical surface aggregates,' which in some cases (serum-albumin, quinine, peptone, aniline dyes, etc.) are completely resoluble in the mother liquid ; in other cases (egg-albumin, ferric hydrate, acid- and alkali-albumin) portions of the aggregates are found to have been rendered permanently insoluble, *i.e.* to have been 'coagulated.' In a paper not yet published, Ramsden

[1] *Physiological Histology*, 1902, p. 104.

[2] W. Ramsden, *Proc. Roy. Soc.* **72.** 156 (1903).

shows that with egg-albumin only the lines of maximum shear (the wrinkles) persist as coagulated proteid, while in the intervals between these lines the coating particles retain their solubility.

Mechanically coagulated proteid thus obtained bears a strong general resemblance to fibrin, and, like fibrin, can be 'coagulated' still further by heat. Ramsden maintains that mechanical surface coagula must be regarded as consisting of chemically unaltered colloid particles which by mere mechanical inpaction have been driven into closer physical union. This union is held by Ramsden to be physical rather than chemical, in the same sense that the union of silicic acid particles must be regarded as physical, when a colloid solution of silica is coagulated by the addition of an electrolyte.[1] In all such cases there is no limit to the size of the molecule which can be built up, and the chemical term 'polymerisation' is undesirable, since it would then be necessary to regard the continuous solid framework of a gel as composing one large molecule.

Ramsden therefore advances the view that some forms of coagulation, including mechanical surface coagulation, and fibrin formation (see p. 382), are essentially physical processes, due entirely to the close physical union of previously distinct colloid particles and involving no demonstrable chemical change.

Incidentally may be mentioned that all bubble-forming liquids have been shown by Ramsden to yield mechanical surface aggregates, and conversely that the power of forming thin films, bubbles, or froth, in any limpid liquid, is due to the presence of solid surface coatings. Similarly the permanence of any good emulsion is due to the formation of a coating of solid particles, or veritable haptogen-membrane, at the interfaces between the emulsified globules and the menstruum in which they are suspended.

The haptogen-membrane which forms when milk is boiled is fully discussed by Jamison and Hertz.[2]

3. Coagulation due to Alterations in the Electrical Tension between the Colloid and its Solvent.

An electrolyte in dissociating into its ions always gives rise to an amount of positive electricity which equals the amount of negative electricity, and the electrical charges are carried by the ions. Each kind of ion travels, further, with its own velocity, the two fastest ions being the acid hydrogen- and the alkaline hydroxyl-ions.

If now, for example, hydrochloric acid is poured into water, the

[1] The author does not hold this view; see pp. 268-271.

[2] R. Jamison and A. F. Hertz, *Journ. of Physiol.* **27.** 26 (1901).

positive hydrogen-ions, travelling much faster than the negative chlorine-ions,[1] will diffuse more quickly into the surrounding water, and, carrying their positive charge with them, render the region of the water where they arrive more positive than that part containing the slowly wandering chlorine ions. If one electrode is placed where the hydrogen-ions are abundant and another one where the chlorine-ions were left behind, an electrical current can be readily demonstrated.[2] Similarly, if we start with a liquid chain composed, for example, of 5 molecular per cent sodium chloride solutions at the ends and 10 molecular per cent hydrochloric and 10 molecular per cent caustic soda in the middle,

5 'per cent' NaCl	10 'per cent' NaOH	10 'per cent' HCl	5 'per cent' NaCl

then by the interaction of the alkali and the acid a 5 molecular per cent salt solution will be formed in the middle:

A	B	C	D	E
5 'per cent' NaCl	10 'per cent' NaOH	5 'per cent' NaCl	10 'per cent' HCl	5 'per cent' NaCl

On diluting A and E a current will pass from B to D, that is, from the alkali to the acid, because the hydrogen-ions, H°, will pass from D into E more quickly than the chlorine-ions, Cl', and similarly the hydroxyl-ions, OH', enter A more freely than do the slower ions; therefore an excess of negative chlorine-ions being left in D and an excess of positive sodium-ions in B, the latter becomes electro-positive to D and the current in the solution passes from B to D.

The same result will be obtained by strengthening the salt solution in C, and for the same reason.

On strengthening the salt solutions in A and E, or by diluting the salt solution in C, a current will be set up from the acid to the alkali, from D to B, because the fast hydrogen-ions will carry their positive charges from D to C at a quicker rate than do the slower hydroxyl-ions carry their charge from B to C. When the hydrogen-ions meet the hydroxyl-ions, neutral water is formed, but there being, owing to the rate of migration, more hydrogen-ions than hydroxyl-ions, a positive stream is set up from D to B.[3]

Should precipitates be formed which do not act as conductors, such

[1] Hydrogen-ion = 320; OH-ion = 174; chlorine-ion = 65; K-ion = 65; Na-ion = 44.

[2] The idea that differences of potential are due to differences in the rate of migration of ions was first suggested by Nernst, 1888.

[3] Worm-Müller, *Poggendorff's Annalen*, 104, 114 (1870). See also Max Oker Blom, *Pflüger's Arch.* **84.** 191 (1901), **85.** 543 (1901).

as are obtained on coagulating egg-white, there is set up but little interference with the strength of the current.

In the liquid chain represented above by A to E, we have salt solutions at the end, while in the middle are equivalent solutions of alkalies and acids in equal volumes, and of such strength as to form by their union a salt solution of the same concentration as seen in the end-links A and E. Given such a chain, no current is set up, though a chemical union takes place.

If two solutions of an acid varying in their concentration are interpolated between the same salt solution, a current passes from the stronger to the feebler solution, the electro-motive force increasing with the difference in concentration. If the dilute acid is replaced by water, the current passes from the acid to the water, and with the dilution of the acid diminishes in electro-motive force. Here again the current is caused by the hydrogen-ions migrating rapidly from a place of greater to that of lesser osmotic pressure, although the hydrogen -ion is not subjected to a greater pressure than is its fellow ion.

The greatest difference of potential will be set up in solutions to which is added an electrolyte the radicals of which differ greatly in their rate of migration, as is the case with hydrochloric acid, for example, $H^{\circ} = 320$ and $Cl' = 65$, while no differences of potential can be obtained, for example, from potassium chloride [$K^{\circ} = 65$ and $Cl' = 65$]. The facts just stated will allow us to understand, if colloids owe their existence to carrying definite charges, how such charges will be influenced by electrolytes; how in some places the colloidal charge will be diminished while in others it will be increased, or what comes to the same, how in the former case bigger aggregates are formed, while in the latter instance the original aggregates will become smaller or even pass into solution. Thus by the diffusion of electrolytes alone the original state of the colloid becomes greatly altered, and if in addition new insoluble compounds are formed between the radicals of the electrolyte and the colloid, then the temporary structural changes brought about by the disturbance of the electrical equilibrium may become 'fixed.'

The author has explained on p. 259 that, according to his view, a colloid remains a colloid only as long as it carries a definite electro-positive or electro-negative load, and that a non-electric condition is induced by ions of the opposite electrical charge (Picton and Linder, Spring)—a view which is also supported by Hardy's experiment on heat-coagulated albumin. The latter in an alkaline solution behaves as an anion, while in an acid medium it behaves like a kation. The author assumed that H°-ions or OH'-ions combined with the colloid to form

complex-ions, or, as he put it in 1902, in his *Physiological Histology*, p. 46, "If the aggregates in a colloidal solution are completely broken up into the composing units by acquiring definite charges, then the colloidal solution loses its colloidal character and becomes a solution of a completely dissociated electrolyte. Reversely, if to a solution containing a definite number of electro-positive (colloid + H°) -ions there is added an alkali containing the same number of electro-negative hydroxyl-ions, then the H° of the colloid and OH′ of the alkali unite to form electrically neutral water, and the colloid having lost its electrical charge is precipitated; if, however, not a sufficient number of OH′-ions were added to bind all the hydrogen-ions, then the colloid aggregates rearrange themselves into larger aggregates, which will remain 'in solution' as long as the H-atoms, joined to the colloid, enable its aggregates to maintain a definite .charge. As the colloid particles become larger and larger and the number of electrical charges for a given volume of solution fewer and fewer, complete precipitation will probably be induced at a time short of perfect neutralisation of the charges, because of the specific gravity of the colloid-aggregates overcoming the viscosity of the fluid." The same principle has recently been put forward by Billitzer,[1] who, without accounting for the reason why colloids possess their original charge, simply states that the precipitating ion is carried down by colloids in such quantities as have sufficed to electrically neutralise the original charge on the colloidal particles: "Because an ion carries a load, which is considerably in excess of that carried by the suspended particles of the colloid, an electrical neutralisation of colloidal particles can only occur, if so many colloidal particles aggregate round an ion as to neutralise its charge. Hereby complexes of large diameter are formed which are thrown down along with the precipitating ion whenever the critical point has been reached at which the forces of gravitation make themselves felt. The ions form condensation-nuclei round which the suspended particles aggregate." Billitzer also speaks of true and false colloids, the former he believes to be precipitated by whatever electrolyte one adds, while false colloids are characterised by being only precipitated by one set of electrolytes, while others instead of causing a precipitation may lead to greater subdivision of the particles. As examples of false colloids are mentioned especially gelatine and egg-white.

The division into true and false colloids is quite untenable, Billitzer has not taken into account that the coagulation of his 'true' electrolytes is brought about by the difference in the rate of diffusion of the ions of the electrolyte which is added, nor has he in the 'false'

[1] J. Billitzer, *Zeitschr. f. physik. Chem.* **51**. 133 (1905).

colloids taken into account the amphoteric character of amino-acids and therefore of albumins.

Billitzer objects, however, to having the term of false colloids restricted to those which possess a molecular weight of less than 30,000, as Sabanajew [1] proposes to do. Billitzer in 1905 has, however, arrived at the same conclusion as the author did in 1902, namely, that colloids are feeble electrolytes, see p. 279.

4. The Salting-out of Albuminous Substances

Albuminous substances may be precipitated from their solutions without undergoing any alteration, by various inorganic salts.

Virchow in 1854 first stated [2] that magnesium sulphate (which Claud Bernhard had introduced), sodium chloride, and sodium sulphate, by abstracting water from albuminous substances in solution, rendered them insoluble.

Hofmeister [3] in 1886 also suggested quite independently that such neutral salts as sodium or potassium chloride and sodium nitrate coagulate albuminous matter by withdrawing water from the albumin-molecules, as these salts have a greater affinity for the water than has the albumin.

Nasse, in 1887,[4] was of opinion that coagulation did not depend merely on the albumin molecules in their struggle for water becoming vanquished by the salt molecules, because the quotients of the concentrations of certain given salts, necessary for producing coagulation, are not the same for different albumins; thus in the case of ammonium and magnesium sulphates, the quotients were for:

Gelatine	Egg-white	Serum-albumin	Hemi-albumose	Peptone
0·84	1·03	0·94	0·85	1

Although Nasse believes that in gelatine we are dealing practically with dehydration, there is no doubt that other factors have to be considered as well, in salting out with neutral salt-solutions.

In 1888 Hofmeister [5] pointed out that whatever the nature of a colloid, the salts always came in the same order, if the concentration at which they commenced to precipitate was made the standard. But on studying his tables the agreement is not a complete one. That the essential factor, when neutral salts are used, is one of salting out the author firmly believes, and in his *Physiological Histology* he has

[1] Sabanajew, *Journ. d. russ. chem. Ges.* 1891, p 80.—Abstracted in *Chem. Centralbl.* 1891, p. 10.

[2] Virchow, *Virchow's Arch.* **6.** 572 (1854).

[3] Franz Hofmeister, *Arch. f. experiment. Pathol. u. Pharmak.* (1886).

[4] Nasse, *Pflüger's Arch.* **41.** 506 (1887).

[5] Hofmeister, *Arch. f. experiment. Pathol. u. Pharmak.* **24.** 247 (1888).

compared the precipitation of an albumin to the crystallisation of a supersaturated solution. The idea, that salts act as 'dehydrating agents,' has been followed up by several of Hofmeister's pupils, and in particular by Kauder[1] and Lewith,[2] and in a modified form by Spiro,[3] Posternak,[4] and Pauli.[5]

Kauder introduced the principle of fractional precipitation as described more fully below, while Lewith determined experimentally a number of salts that do (+) or do not (−) coagulate albumin. His results, arranged in tabular form by the author,[6] are as follows :—

	Potassium.	Sodium.	Ammonium.	Magnesium.	Calcium.	Barium.
Acetate . .	+	+	−	−	−	−
Chloride . .	+	+	−	−	+	−
Nitrate . .	−	+	−	−	+	−
Phosphate . .		+				
Sulphate . .	−	+	+	+		
Sulphocyanate .			−			
Iodide . .	−	−	−	−	−	−
Bromide . .	−	−	−	−	−	−
Chromate . .			−			
Bicarbonate .		−				

Lime salts occupy amongst the alkalies and alkaline earths a peculiar position, as they lead to the formation of insoluble precipitates. Sodium phosphate and chlorate coagulate only slightly, while potassium chlorate does not coagulate.

The effect of saturated salt solutions on albumin is as follows :—

Salt.	Solubility at 18°.	Effect on Albumin.
Potassium acetate .	97·844	Complete precipitation of all proteids except peptones.
Ammonium sulphate .	76·8	Complete precipitation of all proteids except peptones.
Zinc sulphate . .	135	Complete precipitation of all proteids except peptones.
Sodium chloride . .	35	Incomplete globulin precipitation.
Magnesium sulphate .	25·8	Complete ,, ,,
Potassium sulphate .	12·5	No precipitation.
Sodium sulphate . .	5	Incomplete globulin precipitation at ordinary temperature but complete precipitation, resembling that produced by ammonium sulphate, at 37°.

[1] Kauder, *Arch. f. experiment. Pathol. u. Pharmak.* **20**. 411 (1886).

[2] Lewith, *ibid.* **24**. 1 (1888).

[3] K. Spiro, *Physik. u. physiol. Selektion* : Habilitationsschrift, Strassburg, 1897 ; and in *Hofmeister's Beiträge*, **4**. 300 (1903).

[4] Swigel Posternak, *Ann. de l'Inst. Pasteur*, **15**. pp. 85, 169, 451, 570 (1901).

[5] W. Pauli, *Hofmeister's Beiträge*, **3**. 225 (1903).

[6] Mann, *Physiological Histology*, 1902, p. 31. See also p. 52.

Spiro[1] regards the salting-out process in the light of the laws regulating the coefficiency of distribution. This is fully discussed by the author in his *Physiological Histology*,[2] who there points out that to say a substance distributes itself between two other substances, as does, for example, an organic acid between ether and water, is not to give an explanation at all, but amounts simply to a recapitulation of the fact. For an explanation we must know 'why' any given substance distributes itself by preference in one of two substances, and such an explanation must have a chemical basis.

From the table on p. 281 showing the effect of saturated salt solutions on albuminous substances, it is evident that solutions behave quite differently according to the nature of the salt. If we multiply the solubility by the molecular weight we find ammonium sulphate, potassium acetate, and magnesium sulphate agree in giving high figures as compared with sodium chloride, but potassium sulphate does not coagulate although it gives a higher figure than does sodium sulphate.[3]

Posternak published in 1901[4] an extensive series of experiments on coagulation, but this work escaped the author in the summary of colloids in his *Physiological Histology*. Posternak believes colloids to be characterised by being in the form of micellæ, a term which was first used by v. Nägeli.[5] A micella is defined by Posternak as the smallest quantity of a colloid, possessing the physical properties of the colloid taken as a whole, and formed by the association of molecules of large size. A micella is, however, not of invariable size, for it may be diminished more or less according to the number and the mobility of ions present in the solution. The ions diminish the size of the micellæ by imparting to them an electrical charge, the diminution in the size of the micellar magnitude being proportional to the charge or to the electrifying power of the ions, and therefore an insoluble colloid cannot dissolve in distilled water. While according to Posternak's view ions have the tendency to produce a solution of the colloidal micellæ, non-dissociated salt molecules are believed to have the opposite effect, *i.e.* to produce precipitation. A struggle is believed to go on continuously between the ions and the non-dissociated molecules, and the greater the mobility of the ions the less protected will a micella be. Thus if sodium chloride and potassium chloride

[1] K. Spiro, *Hofmeister's Beiträge*, **4.** 300 (1903).

[2] Mann, *Physiological Histology*, 1902, pp. 328, 331, 311.

[3] Mann, *Physiological Histology*, 1902, p. 31.

[4] Swigel Posternak, *Ann. de l'Inst. Pasteur*, **15.** pp. 85, 169, 451, 570 (1901).

[5] C. v. Nägeli, *Mechanisch-physiologische Theorie d. Abstammungslehre*, München and Leipzig, 1884.

be added in equivalent amounts to two portions of a 1 : 1000 HCl-solution of the reserve material from the seeds of *Picea excelsa*, the sodium salt will cause a precipitate long before the potash salt does so, because the mobilities of the sodium ion are much less than that of the potassium ions, namely, as 44·4 : 65·2. Similarly equivalent solutions of the chlorides of magnesium, strontium, and barium stand in the proportions of 0·311 : 0·366 : 0·414, while the mobilities of these elements are as 49 : 54 : 57·3. Posternak was, however, not the first to point out the importance of the mobilibity of ions; this was first done by Spring [1] who compared solutions having the same conductivity (chlorides of K, Na, Rb. Li, Ca, NH_3) and found that they produced flocculation in the order of the mobility of their ions, except in the case of lithium chloride, which takes much less time to coagulate than does the potash salt, because lithium chloride undergoes hydrolysis and thereby gives rise to the formation of hydrogen ions, which possess the greatest mobility.

In addition to the changes which micellæ undergo under the influence of ions and of non-dissociated salt molecules, they are said to possess 'adhesiveness,' a term based on Duclaux's 'molecular adhesion,' which is the same as what others call 'surface attraction' or 'adsorption.' As the adhesive energy is proportional to the extent of the surface, and as different albumins differ from one another in the degree of their adhesive affinity, it follows that albumins of different origin must possess micellæ of different sizes. As an increase of temperature augments the micellar 'adhesiveness' the micellæ must possess the power of increasing their surface with a rise of temperature. This increase in size is termed micellar dilatability or elasticity.

Posternak does not believe in the Virchow-Hofmeister explanation of the action of neutral salts in coagulating albumins by the withdrawal of water, as the reserve material of *Picea excelsa* seeds dissolved in 1 : 1000 HCl is readily precipitated by traces of salt, while it is not rendered even cloudy on being saturated with glucose (see also Spring, p. 298). He believes the micellæ of albumins to be provided with Ehrlich's haptophore-groups, by means of which they attract the non-dissociated salt molecules, which then form a protective envelope, and so protect the micellæ from being dissolved by the ions, as explained above.

The weaker a solution is in proteid the greater must be the amount of salt we require to add to produce flocculation:

[1] W. Spring, *Bull. des science, Acad. Roy. de Belg.* 1900, p. 483.

Concentration of Picea-albumin dissolved in 1 : 1000 HCl.	Amount of ammonium chloride required for flocculation, in molecular concentration.
1 per cent	0·385
0·5	0·419
0·25	0·493
0·125	0·537
0·0625	

Other interesting points discovered by Posternak were, that the same acid radical produces in different salts different effects :

HCl	NH_4Cl	K Cl	NaCl
0·388	0·385	0·380	0·325,

and that the same acid which in dilute strengths favours solution causes precipitation when it is concentrated. This fact is attributed to a change in the electrical conductivity, thus :

Strength of HCl.		Molecular concentration.		Conductivity.
1 : 1000	=	0·0273	=	0·95
1,415 : 1000	=	0·388	=	0·86

In the first case the quotient $\frac{\text{dissociated molcules}}{\text{non-dissociated molecules}} = 19$, while in the second case it is 6.

Ordinary abumins being electro-negative are coagulated, according to Hofmeister and Pauli, see p. 288, by kations in the following order :—

$$Li > Na > K > NH_4 > Mg,$$

while the electro-negative anions tend to prevent coagulation in this order :

$$Fl > SO_4 > P_2O_5 > \text{citrate} > \text{acetate} > Cl > NO_3 > Br > I > CNS.$$

Posternak has now observed the very interesting fact that if the reserve material of Picea dissolved in 1 : 1000 HCl be taken, that the order of the above salts is inverted, the electro-positive albumin is now precipitated by anions in this order :

$$CNS > I > Br > NO_3 > Cl > \text{acetate},$$

while the coagulation-inhibiting kations follow in this order :

$$Mg > NH_4 > K > Na.$$

This phenomenon is interesting in connection with Hardy's experiment on heat-coagulated albumin, which may behave either as a kation or as an anion (see p. 278). Pauli has also drawn attention to the fact

that the above order, in which salts precipitate electro-negative albumins, is the same as that in which they prevent the imbibition of water by gelatine-plates and in which they increase the melting point of gelatine.

Pauli [1] has carefully investigated the effect which is produced on eggwhite by the addition of the neutral salts of the alkalies and of magnesium. In the first instance he confirms Schäfer's observation (see p. 290) that two salts in combination will do what one salt by itself is unable to do, for if potassium or sodium chloride and sodium acetate be used in such strengths as not to cause coagulation, they will on being mixed give rise to coagulation, and $KCl + NaC_2H_3O_2$ will produce a greater effect than if $NaCl + NaC_2H_3O_2$ are used. In the former case the kation is different in the two salts, while in the latter case they are the same.

The dibasic magnesium sulphate + the monobasic sodium chloride also augment mutually their coagulating efficiency, as is proved by the following table given by Pauli :—

To 2 ccm. of eggwhite were added 8 ccm. of a 4·5 normal $MgSO_4$ solution in the quantities stated, and then weighed amounts of crystalline NaCl.

Salts.	Change Produced.		Salts.	Change Produced.	
	Immediately	After 24 hours.		Immediately.	After 24 hours.
1. $MgSO_4$ 2·5	Clear.	Slight turbidity greater than in No. 4.	6. 2·00 $MgSo_4$ + 3·5 NaCl.	Turbidity as in No. 9.	Milky turbidity.
2. $MgSO_4$ 3·00	Very slight turbidity.	Milky turbidity.	7. 2·5 $MgSO_4$ + 1·00 NaCl	Slight turbidity.	Slight milk turbidity.
3. NaCl 3·5	Clear.	Slight opalescence.	8. 2·5 $MgSO_4$ + 1·5 NaCl.	Turbidity more marked than in No. 7.	Milky turbidity as in No. 2.
4. NaCl 4·00	Slight opalescence.	Very slight turbidity.			
5. 1·00 $MgSO_4$ + 3·5 NaCl	Very slight turbidity.	Slight turbidity.	9. 2·5 $MgSO_4$ + 2·00 NaCl	Turbidity more marked than in No. 8.	Opaque milky turbidity.

[1] W. Pauli, *Hofmeister's Beiträge*, **3.** 225 (1903).

If, to a solution of ammonium sulphate, of sufficient strength to produce coagulation, be added equivalent amounts of tartrates, sulphates, and acetates it will be seen that, in the order given, these salts show a diminishing power of assisting the ammonium sulphate in bringing about coagulation.

Tribasic-, in combination with mono- and di-basic salts, also exhibit a summation of effect as is shown by the behaviour of potassium citrate $K_3C_6H_5O_7$ + NaCl.

As many neutral salts do not possess the power of causing egg-white to coagulate, Pauli studied their influence on salts which do coagulate, and found that magnesium and ammonium chloride, sodium and potassium bromide produce a distinct increase in the coagulating power of NaCl and KCl, while ammonium bromide, sodium, potassium, and especially ammonium iodide markedly inhibit the coagulating power of NaCl.

Potassium nitrate increases, proportionately to the amount in which it is used, the coagulating power of potassium and sodium chloride, while magnesium nitrate in a normal solution and ammonium nitrate are indifferent. Potassium and ammonium thiocyanates inhibit the action of sodium chloride. Fluorides are especially powerful in producing reversible coagulates. Arranged in the order of their coagulating power, expressed in normal strengths, they are NaFl (1·00); KFl (1·25); NH_4Fl (2·00). Potassium and ammonium fluoride have their action increased by ammonium bromide, iodide and thiocyanate. Ammonium chloride and nitrate do not influence potassium fluoride, while they slightly increase the coagulating power of ammonium fluoride, as the latter contains the same kation.

Ammonium sulphate has its power increased by increasing amounts of ammonium chloride, while increasing strengths of ammonium iodide and bromide diminish the amount of coagulation.

Potassium tartrate has its action increased by ammonium chloride, sodium, and potassium bromide; no effect is produced by ammonium nitrate, while the coagulating power is diminished by ammonium bromide; sodium-, potassium-, ammonium-iodides; magnesium chloride, nitrate and bromide; and sodium-, potassium-, ammonium-, and magnesium-thiocyanates.

Potassium citrate is augmented by ammonium chloride and sodium bromide, is left indifferent by potassium bromide, and is inhibited by all other salts.

Hofmeister was the first to state definitely that both the basic and the acid radicals of salts play a part in coagulating albumin, which in modern terminology means that coagulation is dependant on

the additive effect of the two ions of a salt, and if this be so, Pauli points out that the total effect produced by a mixture of several salts in solution must be determined by the sum of all the ionic actions. Pauli's modified view is given below. Acetates of the alkalies being somewhat better coagulants than are the chlorides, one obtains a greater effect in coagulating eggwhite by a mixture of sodium acetate + potassium chloride than by an equivalent mixture of sodium acetate + sodium chloride, as the dissociation of the sodium chloride by saturating the solution partially with sodium-ions interferes with the dissociation of the sodium acetate molecules.

The fact that sodium sulphate does, while potassium sulphate does not coagulate albumin, Hofmeister explained by assuming that in the case of potash salts the solubility of these salts was not sufficient to allow the solutions of requisite concentration being obtained. To test this hypothesis Pauli employed two methods: He made a 20 per cent potassium sulphate solution at 100° in a test tube, poured some warm oil on the solution, and allowed it to cool in a big beaker till the temperature had fallen to 50°, then added, by means of a pipette, some albumin solution, which, forming a layer between the oil and the 20 per cent potassium sulphate solution, produced a distinct turbidity which was reversible on dilution.

Potassium nitrate employed similarly could not be made to yield a coagulum, and therefore recourse was had to the following method: To a solution of a salt, capable of coagulating albumin, but of such strength as to be just inefficient, potassium nitrate was added in the crystalline form, when the mixture was found to coagulate.

This experiment does not seem to the author to be at all conclusive, for if the potash salt dissolves at all it can only do so at the expense of the other salt in solution; by attracting water molecules to itself it must make the other salt-solution more concentrated, and the latter being more concentrated may cause the coagulation of the albumin quite irrespective of the potash salt.

Do both ions of a salt cause coagulation, or is only one of the ions concerned? and if so, what does the other ion do? This question suggested itself to Pauli because some electrolytes, such as the acetates, nitrates, and chlorides of ammonium and magnesium, cause no coagulation, notwithstanding the fact that they are very soluble; and again the fact that NH_4 coagulates in ammonium sulphate, but does not do so in ammonium acetate, while at the same time the acet-ion coagulates in sodium acetate, while it does not coagulate in ammonium acetate, makes it impossible to believe that both ions of a salt act in the same sense, and therefore Pauli has come to the conclusion that in electro-

lytes which cause coagulation we are dealing with the algebraic sum of the antagonistic properties of ions having opposite electrical charges. He assumes that kations have a coagulating and anions a dissolving influence on egg-white, because acids, which have the kation $H^{\cdot}$ in common, cause coagulation, while bases, having the anion OH' in common, cause albumins to dissolve.

If the coagulating values of a series of kations are indicated by $f, f', f'' \ldots\ldots$ and the inhibiting values of a number of anions by h, h', h'', then by combining electrolytes the three following states are possible:

$$\sum (f, f', f'' \ldots\ldots) \gtreqless \sum (h, h', h'' \ldots\ldots),$$

which means that it is possible to add to a solution of a coagulating electrolyte, other electrolytes which either increase or diminish or leave unaltered the coagulating power of the first electrolyte.

In the following table Pauli has arranged the kations in ascending order from left to right, magnesium being the feeblest and lithium the strongest kation, while the anions are so arranged that the one with the fullest inhibiting power, namely, fluorine, comes first, while the strongest inhibitor, namely, thiocyanate, comes last.

Kations → / Anions ↓	Mg	NH_4	K	Na	Li
Fluoride		+	+	+	
Sulphate	+	+	+	+	+
Phosphate		+	+	+	
Citrate		+	+	+	
Tartrate		+	+	+	
Acetate	−	−	+	+	
Chloride	−	−	+	+	+
Nitrate	−	−	−	+	+
Chlorate		−	−	+	
Bromide	−	−	−	−	+
Iodide		−	−	−	
Thiocyanate	−	−	−	−	

It will be seen from the table that the feeble precipitating power of magnesium and ammonium is already interfered with by the acetates and chlorides, while potassium is not affected by nitrates, and so on.

The criticism which the author has to make to Pauli's very important investigation, apart from the objection already raised on p. 287, is shortly this: It has been assumed throughout by Pauli that the salts only act upon one another, and not also on the albumin. If we consider what effects, especially the halogen salts, have in preventing, for example, the setting of gelatine, we must bear in mind

that an analogous change may very well be produced in egg-white, and that for this reason in the above table the iodides and thiocyanates have apparently so strong an inhibiting action on all kat-ions. The formation of double salts has also not been taken into account, nor has sufficient attention been paid to the amphoteric character of the albumin. Posternak's researches (see p. 282) seem to have escaped Pauli also.

That, however, so-called 'neutral' salts are in reality not neutral, but are composed of ions in which either the negative or the positive electro-affinity preponderates, has been explained on p. 265, and Pauli's observations seem to bear out the author's views, and there cannot be any doubt that the preponderating positive or negative electro-affinity is the factor which makes the dehydration of the albumin molecule possible, and thus leads to its coagulation. The author is therefore of the opinion that salting-out with neutral salts is in the last instance simply a question of dehydration according to the old view of Virchow and Hofmeister, and that the albumin molecules, when they become dehydrated, behave as would other salts, by losing all power of dissociating either electrolytically or hydrolytically.

The salting-out process has become of the very greatest importance in studying the chemistry of albuminous substances, as it does not lead to the denaturalisation of albumins. Another great advantage of salting-out is that the individual albumins may be separated from one another to a much greater extent than by any other precipitation-method, and salting-out has therefore rendered the greatest service in the preparation of pure albumins.

Salting-out with sodium chloride and magnesium sulphate has been investigated by Tolmatscheff,[1] Hammarsten,[2] Burkhardt,[3] and Halliburton.[4] Ammonium sulphate, the most efficient of all the salts, was introduced by Heynsius[5] and extensively used by Kühne,[6] who also

[1] Tolmatscheff, 'The Analysis of Milk,' *Hoppe-Seyler's Medizin.-chem. Untersuchungen*, p. 272 (1867).

[2] O. Hammarsten, 'Paraglobulin,' *Pflüger's Arch. f. d. ges. Physiol.* **17**. 413 (1878); K. V. Starke, 'Abstract of the Swedish Original by Hammarsten' in *Maly's Jahresber. f. Tierchemie*, **11**. 17 (1881).

[3] Burkhardt, *Arch. f. exp. Path. u. Pharm.* **16**. 322 (1882).

[4] W. D. Halliburton, 'Muscle-Plasma,' *Journ. of Physiol.* **8**. 133 (1887); and 'Proteids of Milk,' *ibid.* **11**. 448 (1890).

[5] A. Heynsius, *Pflüger's Archiv f. d. ges. Physiol.* **34**. 330 (1884).

[6] W. Kühne, *Verh. des Heidelberger naturh.-mediz. Vereins*, N.F. **3**. 286 (1885); W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **20**. 11 (1884); **22**. 423 (1886); S. Wenz, **22**. 1 (1886).

showed[1] that two factors have to be considered in salting-out, namely, not only the concentration but also the absolute quantity of the salt used and the absolute quantity of the albumin to be salted out. This fact—the ignorance of which has repeatedly led to errors—is readily explained on the supposition that salting-out is equivalent to a distribution of the solvent between the albumins and the salt. An exact investigation of a large number of salts, as far as salting-out is concerned, has been undertaken by Hofmeister[2] and his pupil Lewith[3] (see above, p. 281), and also by Posternak and Pauli.[4] (See below.)

For the chemistry of proteids the question as to the extent to which albumins in solution may be precipitated completely by different salts is an especially important one. In this respect Cohnheim distinguishes four groups of salts :—

1. Sodium : -chloride, -sulphate, -acetate, and -nitrate. They salt out certain albumins, such as fibrinogen and casein, even if their solutions are not quite saturated.

   The author points out, however, that according to Pinkus sodium sulphate in saturated solutions at 37° is in every respect comparable to a saturated ammonium sulphate solution at the ordinary temperature.[5]

2. Magnesium sulphate, which allows us to draw a sharp line between the more readily precipitable albumins, *e.g.* globulin, and the less readily precipitable ones, *e.g.* albumins proper.

   Magnesium + sodium sulphate, used in combination, precipitate, according to Schäfer,[6] also those albumins which are not readily precipitable.

3. Potassium acetate, calcium chloride, and calcium nitrate. They precipitate all native albumins from their solutions, but the lime salts render the albumin-precipitates very quickly insoluble.

4. Ammonium sulphate and zinc sulphate[7] are the most energetic precipitants. They precipitate also all the dissociation-products of albumin, except the peptones, and in this respect they may be considered to be complete (Kühne).

This order has been established empirically.

[1] W. Kühne, 'Experiences with Albumoses and Peptones,' *Zeitschr. f. Biol.* **29**. 1 (1892).

[2] F. Hofmeister, *Arch. f. experiment. Pathol. u. Pharmakol.* **24**. 247 (1887); **25**. 1 (1888).

[3] J. Lewith, *ibid.* **24**. 1 (1887).

[4] W. Pauli, *Hofmeister's Beiträge*, **3**. p. 225 (1902).

[5] Pinkus, *Journ. of Physiol.* **27**. 57 (1901). See also Haslam, *ibid.* **32**. 267 (1905).

[6] E. A. Schäfer, *Journ. of Physiol.* **3**. 181 (1880).

[7] A. Bömer, *Zeitschr. f. anal. Chem.* **34**. 562 (1895); E. Zunz, *Zeitschr. f. physiol. Chem.* **27**. 219 (1899).

Why salts follow one another in this order we do not know at present, but below an attempt will be made to account theoretically for some of the facts.

A number of salts which are good precipitants, such as sodium chloride and magnesium sulphate, possess the property of precipitating certain albuminous compounds not only in saturated but also in partially saturated solutions. This property was first discovered by Hammarsten[1] and made use of by Hofmeister's pupil Lewith,[2] and by Halliburton.[3] The systematic employment of fractional precipitation by means of ammonium sulphate and zinc sulphate led in the hands of Hofmeister and his pupils[4] to the crystallisation of albumins; the preparation of many albumins in a pure form, and to much light being shed on the constitution of albumoses.

It has been found that each albumin begins to be precipitated whenever the concentration of the salt has reached a certain point, and that at a definite higher concentration the precipitation comes to an end because practically the whole of the albumin has been thrown down. Hofmeister states that the precipitation-limits for ammonium sulphate "are quite as characteristic for an albumin as is the solubility of a crystalline substance." The special precautions which are necessary for completely separating different albuminous fractions from one another are given on p. 184.

Kühne has observed that the concentration of an albumin must not be altered to any extent when making salting-out experiments, and this observation has been amply confirmed by Hofmeister, Kauder, Pick, and Zunz. It is therefore best to proceed as follows:—Certain definite amounts of an albumin solution are taken; to these amounts

[1] O. Hammarsten, 'Fibrinogen,' *Pflüger's Arch. f. d. ges. Physiol.* **19**. 563 (1879); **22**. 431 (1880). [2] J. Lewith, *Arch. f. experim. Pathol. u. Pharmak.* **24**. 1 (1887).

[3] W. D. Halliburton, 'Proteids of Kidney and Liver-Cells,' *Journ. of Physiol.* **13**. 806 (1892).

[4] G. Kauder, 'Albumins of the Blood-Serum,' *Schmiedberg's Arch. f. experiment. Pathol. u. Pharmakol.* **20**. 411 (1886); J. Pohl, *ibid.* **20**. 426 (1886); F. Hofmeister, 'Crystalline Egg-Albumin,' *Zeitschr. f. physiol. Chem.* **14**. 165 (1889); E. P. Pick, *ibid.* **24**. 246 (1897); F. Umber, *ibid.* **25**. 258 (1898); Fr. Alexander, 'Caseine-Albumoses,' *ibid.* **25**. 411 (1898); R. Bernert, 'Oxidation with Potassium Permanganate,' *ibid.* **26**. 272 (1898); E. Zunz, 'Zinc Sulphate,' *ibid.* **27**. 219 (1899); E. Zunz, 'Peptic Dissociation of Albumin,' *ibid.* **28**. 132 (1899); E. P. Pick, *ibid.* **28**. 219 (1899); W. Reye, *Fibrinogen*, Dissertation, Strassburg, 1898; H. Krieger, *Crystalline Albumins*, Dissertation, Strassburg, 1899; F. Goldschmidt, *Albumin and Acids*, Dissertation, Strassburg, 1898; A. Magnus-Levy, 'Bence-Jones' Albumin,' *Zeitschr. f. physiol. Chem.* **30**. 200 (1900); O. Maas, 'Dissociation with Alkalies,' *ibid.* **30**. 61 (1900); L. Langstein, 'Ova-Albumin,' *ibid.* **31**. 49 (1900); E. Fuld and K. Spiro, 'Serum-Globulin,' *ibid.* **31**. 132 (1900); E. P. Pick and K. Spiro, 'Coagulation,' *ibid.* **31**. 251 (1900); E. P. Pick, 'Deutero-Albumoses,' *Hofmeister's Beiträge*, **2**. 481 (1902); O. Porges and K. Spiro, *ibid.* **3**. 277 (1902).

are then added different quantities of saturated ammonium-sulphate solutions; and, finally, by the addition of distilled water all the different mixtures are brought to the same volume. If it is stated that the precipitation-limits for globulin are 2·9 and 4·6, then is meant that if in 10 ccm. of fluid (globulin + ammonium sulphate + water) there are present 2·9 ccm. of cold saturated ammonium sulphate, that globulin begins to be precipitated, while if 4·6 ccm. of saturated ammonium sulphate be present, that the precipitation of globulin has come to an end, because the whole of the globulin is precipitated. As the solubility of ammonium sulphate is 76·8° at room-temperature, it is easy to calculate what percentage of ammonium sulphate is required for bringing about incipient and complete precipitation of any one albumin, as soon as we know what amounts of saturated ammonium sulphate have to be added for any given quantity of fluid.

The boundary lines drawn between individual albumins, by means of fractional precipitation with ammonium sulphate, agree fairly well with those obtained by means of other salts, and the groups of albumins separated from one another by these means show also in other respects great similarity.

The complex albumins, namely, fibrinogen, casein, and the nucleo-albumins of the cell-plasm, are partially precipitated by even incompletely saturated solutions of magnesium sulphate and sodium chloride. The upper precipitation-limits of these substances for ammonium sulphate are 3·0.

The albumins of the second group, including the different globulins, the mucins, and also the primary albumoses, are only precipitated by completely saturated solutions of magnesium sulphate. The precipitation-limits of this group of albumins lie for ammonium sulphate between 2·7 and 4·6.

The albumins of the third group, including the albumins proper and hæmoglobin, are not precipitated by the above salts, except they be used in the combination: magnesium- + sodium-sulphate; while they are precipitated by almost completely saturated solutions of ammonium sulphate or zinc sulphate. The deutero-albumoses resemble the true albumins, but it is possible to subdivide them still further by means of fractional precipitation, as has already been pointed out on p. 179, where the researches of Pick and Haslam have been described.

Fuld and Spiro,[1] Porges and Spiro,[2] Joachim,[3] and Freund and

[1] E. Fuld and K. Spiro, *Zeitschr. f. physiol. Chem.* **31**. 132 (1900).
[2] O. Porges and K. Spiro, *Hofmeister's Beiträge*, **3**. 277 (1902).
[3] J. Joachim, *Wiener klinische Wochenschrift*, 1902, Nr. 21.

Joachim,[1] have also fractionated globulin by means of potassium acetate and ammonium sulphate. Oppenheimer,[2] working with serum-albumin, and Cohnheim with the nucleo-proteid of natural gastric juice, have further observed that these apparently uniform substances may be broken up by means of ammonium sulphate into several fractions with definite precipitation-limits, which latter remain constant even after repeated reprecipitation, and that the fractions obtained in this way do not differ from one another in any other respect. The question naturally arises whether one is justified in regarding these fractions really as distinct chemical individuals. Cohnheim believes that admixtures of any kind, that salt formation and similar factors, may well produce these differences, for Fuld and Spiro have found that in one of the globulin fractions, namely, in euglobulin, ferments, lime salts, etc., are present. The author holds that the various fractions must be considered to be definite individual substances, but as to whether they occur naturally is quite a different question. If a sufficient number of subfractions could be taken, then we ought to pass uninterruptedly from the mono- and di-amino-acids through the di- and polypeptids to peptones, and so upwards. That, however, such individual complexes as the hemi- and anti-, the thio-, and glyco-groups exist seems to be beyond doubt.

The salts which albumins form with acids and with bases may also be salted out, as has been shown by Paal,[3] Cohnheim,[4] and Spiro and Pemsel,[5] and this property may be used for determining the acid and the basic capacity of albumins. The salts of albumin with acids are indeed even more readily precipitated than are the albumins themselves, for Pick and Zunz have found, if the reaction of an albuminous solution be acid, that the precipitation-limits for ammonium sulphate and zinc sulphate are always lowered, *i.e.* that precipitation commences and terminates with a smaller percentage of these salts. Salkowski[6] found similarly that all native albumins may be precipitated from their solutions by means of sodium chloride if the reaction be acid, while if the reaction be neutral the albumins are not precipitated at all and the globulins only partially. Kühne[7] has also shown that sodium chloride precipitates albumoses much more thoroughly if the reaction be acid. On the other hand, salts of

[1] E. Freund and J. Joachim, *Zeitschr. f. physiol. Chem.* **36**. 407 (1902).
[2] C. Oppenheimer, *Arch. f.* (*Anat. u.*) *Physiol.* 1903, 201.
[3] C. Paal, *Ber. d. deutsch. chem. Ges.* **25**. 1202 (1892); **27**. 1827 (1894).
[4] O. Cohnheim, *Zeitschr. f. Biolog.* **33**. 489 (1896).
[5] K. Spiro and W. Pemsel, *Zeitschr. f. physiol. Chem.* **26**. 231 (1898).
[6] E. Salkowski, *Zentralbl. f. d. medizin. Wissenschaften*, 1880.
[7] W. Kühne, *Zeitschr. f. Biol.* **20**. 11 (1884); **29**. 1 (1892).

albumins with bases are much less readily precipitable than are the natural albumins; urea, according to Spiro,[1] acts also as a base, and prevents thereby precipitation.

According to the unanimous statements of Kühne, Umber, and Siegfried,[2] it is quite impossible to precipitate one or several of the deutero-albumoses without having previously converted them into sulphates or into ammonia salts. The observation of Hopkins[3] and Krieger,[4] that albumins crystallise more readily from half-saturated ammonium-sulphate solutions if the solution be acid instead of neutral, seems also to come under this heading, for the crystalline albumins are apparently not free albumin, but the sulphate or some analogous salt (see p. 326).

5. Precipitation of the Colloid due to the withdrawal of the Hydrogen or Hydroxyl Radical[5]

For descriptive purposes, the hydrogen and hydroxyl compounds of colloids are discussed by themselves, but it must be remembered that they differ from other compounds only in degree and not in kind.

Pure albumins and globulins Starke[6] regards as acid or alkali albumins or globulins. They are insoluble in water and neutral salt solutions, but are soluble in very dilute acid or alkalies, because they unite with the H° or the OH'-ions to form new compounds, as can be demonstrated by using tropæolin as an indicator.

Alkali and acid albumins and globulins occur naturally. Thus globulins are found both in the acid extracts of lentils made with 5 per cent salt solution and also in the blood. They occur in combination with either the acid H°- or the alkaline OH'-ions. The relationship between the colloid and the acid H° or the alkaline OH' has been expressed as follows:

1. The acid, or alkali, and the proteid form a salt which is capable of dissociation, and therefore of remaining in solution (Starke).

2. The proteid is partly in solution and partly in the colloidal state. The colloid as a whole is kept in suspension, because the acid or alkali which was added establishes a difference of potential between the solid and the fluid phases of the colloid, giving rise to a double electric layer round each solid particle (Hardy).

[1] K. Spiro, *Hofmeister's Beiträge*, IV. p. 300 (1903).
[2] M. Siegfried, *Zeitschr. f. physiol. Chem.* **27**. 335 (1899).
[3] F. G. Hopkins and S. N. Pinkus, *Journ. of Physiol.* **23**. 130 (1898).
[4] H. Krieger, Dissertation, Strassburg, 1899.
[5] Reprinted from the author's *Physiological Histology*, p. 55.
[6] Starke, *Zeitschr. f. Biol.* **42**. 187 (1901).

3. A neutral pseudo-basic proteid, by addition of the acid H°, is converted into a real base, and the pseudo-acid colloid by the basic OH′ radical is changed into a real acid (the Author).

4. The proteid forms with the H° a kat-ion, or with the hydroxyl-ion, an an-ion, the an-ion in the former case being the an-ion of the acid which was added, while in the second case the kat-ion is the kat-ion of the alkali we added (the Author).

Whatever view we adopt, if we are dealing with a (colloid + H°), then, by the addition of the OH′ group neutral water is formed, $H^{\circ} + OH' = H_2O$, and the charge on the proteid also disappears, immaterial in what way it was induced. For reasons given on p. 264 the colloid then aggregates into larger masses, and is ultimately completely precipitated.

The precipitation in this case depends therefore on the chemical union between the hydroxyl- and the hydrogen-ions producing electrically neutral water, and thereby diminishing or completely destroying the electrical charges on the colloid. Another method of rendering the hydrogen-ion inert is to convert it into the non-ionic state by removal of the an-ion to which it was linked, as in the following case: Given faintly acid solutions—for example, an acid albumin—coagulation may be induced by the addition of neutral salts of the alkalies or of alkaline earths, because this addition drives out the carbon dioxide contained in the water and thereby renders the solution less acid. The less acid the albumin solution is, to begin with, the less neutral salt will be required for complete coagulation.

Alkali-albumins in an alkaline solution are coagulated by the addition of CO_2, or other dilute acids; by small quantities of alkaline earths, probably because of the formation of insoluble hydrates; further, by dilution with water or dialysis against pure water; and lastly, by saturated solutions of sodium chloride or magnesium sulphate. All these reactions, with the exception of the last, which is caused by salting-out, see p. 280, depend directly on the alkalinity or the acidity of the proteid compound being interfered with. When Starke states that the acidity of a dilute acid is diminished by neutral salt, it must be remembered that this only holds good if the acid and the salt have a common ion. Starke also observed that if the same amount of alkali is added to two test-tubes, one of which contains pure distilled water while the other contains an equal amount of pure sodium chloride solution made up with the same distilled water, litmus-paper is turned much bluer by the salt solution. The explanation he offered was that alkalies in the presence of neutral salts dissociate to a greater extent, and therefore give a more pronounced alkaline reaction. This

view cannot be upheld, because by the addition of neutral salts the CO_2 normally present in water is discharged, and therefore the alkali which is added will not be partly bound by the acid, and in consequence will produce a stronger effect on the litmus. The author arrived at this conclusion by adding neutral litmus solution to the distilled water, and then boiling it to get rid of the CO_2, and his conclusions are supported by the criticism of Starke's paper in Wolff and Smits' article.[1]

6. Precipitation due to a Removal of Salts

Proteids, which require the presence of neutral salts to remain in solution, are widely distributed amongst both plants and animals, and are represented by the globulins proper and the closely allied muscle-proteids (paramyosinogen and myosinogen). These substances are precipitated from their solutions either if the salts normally present are removed by dialysis, or if the concentration of the inorganic salts is greatly diminished by the addition of water. Various causes have been assigned for this precipitation.

W. Pauli[2] has studied the effect of both non-electrolytes and of electrolytes on globulin solutions. He found pure water containing grape-sugar in quantities varying from mere traces up to 3·25 normal = 68·5 per cent, or containing pure urea up to three or four times normal strength, always precipitated globulin as if neither sugar nor urea had been present, and the globulin which separated out in these solutions could always be readily dissolved by the addition of neutral salts. He drew the conclusion that globulin requires for its solution the presence of a neutral salt which has dissociated into its ions, and that the non-dissociated molecules of a salt play no part. The ion-action depends on the number of ions present and on the quantity of the globulin, for only that amount of globulin passes into solution for which sufficient salt has been added, and the subsequent clearing of the globulin solution is proportional to the amount of salt added.

The fact that free ions render globulin soluble leads Pauli to suppose that the negative and positive ions unite with the proteid-molecules in a loose, chemical manner, the kat-ions attaching themselves to certain radicals in the proteid, while the an-ions join on to other groups. This supposition is supported by the following analogous case, which was pointed out to Pauli by Hofmeister:—Amino-acids unite simultaneously with both alcohol and hydrochloric acid to form beautifully crystalline,

[1] K. Wolff u. A. Smits, *Zeitschr. f. Biol.* **41**. 437 (1901).

[2] W. Pauli, *Pflüger's Arch.* **78**. 315 (1899); compare also with W. Huiskamp, *Zeitsch. f. physiol. Chem.* **34**. 32 (1901).

very permanent, water-soluble compounds, for example, $HCl \cdot NH_2 \cdot COO(C_2H_5)$. After removing the HCl of this body with Ag_2O, the remaining radical soon decomposes by giving off the alcohol radical. See also Chapter VI.

This view is supported by the observation of Stewart[1] that the globulins on being precipitated carry salts down with them; but against Pauli's view, according to Cohnheim, is the fact that the addition of albumin does not influence the electrical conductivity of sodium-chloride solutions (according to Bugarszky and Libermann).[2]

On this principle globulin and sodium chloride would form the compound (HCl) Globulin (NaOH)—H_2O, or more probably according to the formula, Globulin + NaCl = Cl – Globulin – Na. In support of this last view Pauli refers to the investigations of Spiro and Pemsel,[3] who showed that albumins can bind both acids and bases.

Cohnheim has further pointed out that Pauli's view does not account for the precipitation of the globulin which results from diluting its solution, as in this case no ions are removed, and he suggests that globulins are rendered insoluble owing to their strong hydrolytic dissociation. Osborne[4] has also stated that the globulin precipitation is caused by the hydrogen-ions of the water, which again only means that hydrolysis occurs.

This hydrolysis we may assume[5] to be induced on the same principle as is the hydrolytic decomposition of many salts of the heavy metals which form clear solutions as long as they are concentrated, but which on dilution at once undergo hydrolysis, forming insoluble hydrates.

Starke[6] in 1900 found that he could obtain a substance giving all the characteristic reactions of ordinary globulins by diluting white of egg with ten times its bulk of water and then dialysing the solution at a temperature of 75-85° C.

There is formed by this process a substance which is quite insoluble in pure water, and also in neutral salt solutions, but which, when treated with very dilute alkalies, becomes soluble. Starke found after adding the same amount of alkali to two identical quantities of globulin that more globulin passed into solution if neutral salts were present, and explained his results on the assumption that alkalies undergo greater dissociation in the presence of neutral salts, and that for this reason they produced a greater effect. As shown on p. 295

[1] G. N. Stewart, *Journ. of Physiol.* **24**. 460 (1899).

[2] St. Bugarszky and L. Libermann, *Pflüger's Arch. f. d. ges. Physiol.* **72**. 51 (1898).

[3] Spiro and Pemsel, *Zeitschr. f. physiol. Chem.* **33**. 401 (1901).

[4] T. B. Osborne, *ibid.* **33**. 225 (1905).

[5] Mann, *Physiological Histology*, 1902, p. 57.

[6] J. Starke, *Zeitschr. f. Biol.* **40**. 419, 494.

Starke's explanation is impossible, but apart from the fact that neutral salts do drive out carbon dioxide from solutions, we must remember Pauli's work (see p. 285), and therefore arrive at the conclusion that globulins will pass into solution only in the presence of free hydrogen or by hydroxyl-ions, and that, after the union of these ions with the albumin has taken place, the 'neutral' electrolytes will greatly facilitate the passing into solution of globulins, by establishing differences of potential which will diminish the surface tension of the globulin particles.

Finally, the conception developed by the author in Chapter VI. should not be lost sight of, namely, that amino-acids develop the tendency of forming internal salts, and of becoming insoluble owing to the loss of their electrical charge, whenever they are removed from the influence of electrolytes or when they are in the presence of such mixtures of electrolytes that the sum of the electro-affinities of the amino-acids and the other salt-ions of one sign (negative or positive) are balanced by the electro affinities of the ions of the opposite sign. In either case the ionic dissociation of the solvent, for example, water, must also be taken into account.

7. The Formation of Irreversible Salts

That salts of the alkalies and of magnesium give rise to reversible salts has been explained on p. 280, while now the effect of the salts of the alkaline earths—calcium, barium, and strontium, and of the heavy metals—zinc, iron, copper, silver, mercury, and lead—will have to be studied.

Spring, in a number of very important papers, has studied the behaviour of finely suspended particles, particularly in relation to various electrolytes.[1] He objects to the view, first put forward by Barns and Schneider,[2] that colloidal particles surround themselves by either physical or chemical means with a definite water-jacket, because there is no definite proportion between the amount of colloid which is precipitated and the amount of electrolyte which has been added; he points out at the same time that the addition of electrolytes stops the Brownian movement, shown by suspensions whenever flocculi are formed. Spring also discovered that all plurivalent salts which undergo hydrolysis give rise to colloidal solutions, as tested by

[1] W. Spring and de Boek, 'Colloidal Coppersulphide,' *Bull. de la Soc. Chim. de Paris*, **48**. 165 (1887); 'A Water-Soluble Manganese Oxide,' *ibid.* p. 170. Spring, 'The Influence of Electricity on the Sedimentation of Turbid Fluids,' *Bull. de l'Acad. Roy. de Belg.* [3] **35**. 780 (1898); *ibid.* 1899, p. 300; *ibid.* 1900, p. 483.

[2] See Mann's *Physiological Histology*, p. 33.

Tyndall's experiment (see p. 258), and that their precipitating power is directly proportional to the degree of their colloidal nature, and also pointed out that ox serum, which always shows a strong Tyndall reaction, readily precipitates ferric hydrate.

He further showed if a colloid solution of gum-mastic was placed in a cylindrical vessel, and if a saturated solution of copper sulphate, or aluminium, or iron chloride, was introduced at the bottom of the cylindrical vessel, care being taken to avoid all air bubbles, that the mastic solution acted as a semi-permeable membrane. For example, in the case of copper sulphate the presence of free sulphuric acid could be demonstrated in the upper layers of the solution, while the copper hydrate, enveloped in particles of gum-mastic, had become precipitated. This conception of Spring, published in 1900, has been again brought forward as something new by several German investigators, namely, by Biltz,[1] Freundlich,[2] Landsteiner and Jagic,[3] Neisser and Friedmann.[4] The author was unable to procure the papers published in the Münchener periodical, and quotes from Pauli.[5]

While copper sulphate, if it be employed in the manner just indicated, separates freely into H_2SO_4 and $Cu(OH)_2$, which latter then unites with the mastic solution, quite a different result is obtained if the copper sulphate or some other salt and the mastic solution be mixed together, for in this case the flocculi of gum-mastic contain no hydrate of copper°.

It appears to the author that in this last case coagulation is brought about in the following way. The electropositive copper, $Cu^{\circ\circ}$, set free from the copper sulphate by electrolysis, combines with the neighbouring electronegative mastic-radicals in such a way as to form a compound $Cu^{\circ\circ} + Mastic''$; this compound is then broken up hydrolytically, and $Cu^{\circ\circ} + 2(OH)'$ is formed; the mastic having handed on its negative electrical charge to the hydroxyl radical, and thus having lost its electrical load, fuses with other mastic particles because the absence of a difference in potential leads to an increase in the surface tension (see p. 265).

7a. Compounds of Albumins with Alkaline Earths

The salts of the alkalies, alkaline earths, and heavy metals differ, according to Pauli,[6] essentially in this, that the importance of the an-ion, from the precipitation point of view, gradually becomes less

[1] Biltz, *Ber. d. deutsch. chem. Ges.* **37**. 1095.

[2] H. Freundlich, *Zeitschr. f. physik. Chem.* **49**. 129 (1903).

[3] Landsteiner and Jagic, *Münchener med. Wochenschrift*, 1903, No. 27.

[4] Neisser and Friedmann, *ibid.* 1904, Nos. 15 and 19.

[5] *Hofmeister's Beiträge*, **6**. 253, 1905.

[6] W. Pauli, *ibid.* **6**. 233 (1905).

and less. How important a part an-ions play in the salts of the alkalies has already been explained on p. 288.

The diminishing influence of the an-ion, the author thinks, may be accounted for by the plurivalent character of the kat-ion, which leads to the formation of colloidal solutions. This tendency of salts to form colloidal solutions, or, what means the same, to undergo hydrolysis, commencing amongst the alkaline earths, reaches its maximal development amongst the heavy metals. While, to give an example, the halogen salts of the alkalies are neutral and remain neutral on being added to colloids, the halogen salts of the alkaline earths are neutral in watery solutions, but become acid on being added to colloidal solutions, as was first shown by Whitney and Ober;[1] finally (according to the author's investigations, see p. 308) the alkaline salts of the heavy metals are acid in watery solutions, and remain acid in colloidal solutions. Expressed in tabular form we find therefore:—

Salt.	Reaction in a Watery Solution.	Reaction in a Colloidal Solution.
NaCl . .	Neutral	Neutral.
$CaCl_2$.	Neutral	Acid.
$HgCl_2$.	Acid	Acid.

It is evident that in the case of the alkaline earths the colloid assists the water in producing a hydrolysis of the calcium, barium, or strontium salts, and this is a farther proof that colloids must be chemically active, that they are indeed electrolytes, as first pointed out by the author (see p. 268), and it also follows, if it were not for the hydrolysis which the salts undergo, and which renders them colloidal, that they would not precipitate colloids. (See p. 270 for investigations of Spring.)

The compounds which the kat-ions of the alkaline earths and the kat-ions of the heavy metals form with colloids remain insoluble because the (kat-ion + colloid) compound possesses less electro affinity than does the H°-ion, which becomes linked on to whatever an-ion (*e.g.* Cl') originally accompanied the kat-ionic alkaline earth or heavy metal.

Calcium chloride, according to Pauli,[2] has in the crystalline form the precipitation limits of 8·8 normal, while the anhydrous preparation has the limits 9 to 9·2 normal. With 3 ccm. of $CaCl_2$ in these concentrations, and with 2 ccm. of egg-white, there is produced at once a bluish turbidity, which, after twenty-four hours, looks like 'thick milk.' Barium chloride has somewhat lower precipitation limits.

[1] W. R. Whitney and J. E. Ober, *Zeitschr. f. physik. Chem.* **39**. 630 (1901-1902).

[2] W. Pauli, *Hofmeister's Beiträge*, **5**. 27 (1904).

The chlorides and acetates of the alkaline earths generally require to be in much higher normal concentrations than do the corresponding alkali salts; reversely, the thiocyanite, iodide, and bromide of calcium, precipitate in relatively low concentrations, while the corresponding alkali salts do not precipitate at all.

As the sulphates, carbonates, and phosphates of the alkaline earths are insoluble, it was impossible to modify the precipitating power of the soluble salts of the alkaline earths by the addition of the alkali-sulphates, carbonates, and phosphates, but the effect of adding the alkali salt with monovalent an-ions was studied.

As shown on p. 288, certain salts may help in or may interfere with the precipitation of egg-white by other salts of the alkalies. Thus NaCl, $NaNO_3$, NaB augment, while NaI, NaCNS diminish precipitation. On the assumption that the kat-ions favour precipitation while an-ions do the opposite, it was further pointed out by Pauli, for the salts of alkalies that $Li > Na > K > NH_4 > Mg$, and that the an-ions came in this order: $CNS > I > Br > NO_3 > Cl > C_2H_3O_2$.

The alkaline earths differ from the alkalies, because in them the precipitating power of any kat-ion is increased by the an-ions in this order: $C_2H_3O_2 > Cl > NO_3 > Br > I > CNS$; in other words, the order which holds good for the alkalies becomes reversed for the alkaline earths, and this is attributed to the fact that the originally neutral reaction of the solutions of the alkaline earths are rendered acid by the albumin, which is quite analogous to the above-mentioned observation of Whitney and Ober, who worked with arsenic trisulphide. Whitney and Ober's articles have escaped apparently Pauli.

That, however, the albumin is not the only factor in altering the neutral reaction of the salts of the alkaline earths into an acid one is shown by the fact that the alkaline reaction of a radium carbonate solution may become neutral and even acid towards phenol-phthalein by the simple addition of neutral calcium chloride (Pauli). As sodium carbonate and sodium phosphate are always present in egg-white, one might expect the reaction of the albumins to be strongly alkaline, owing to the hydrolytic dissociation of the alkali salt into, for example, $NaOH + H_2CO_3$, of which the former, by electrolytic dissociation, gives rise to the strongly alkaline OH′-ion. The action of egg-white being neutral towards phenol phthalein, it follows that the OH radicals must somehow be satisfied by the albumin. On adding a salt of an alkaline earth to an albumin solution, Pauli believes the OH radicals, previously held by the albumin, to now attach themselves to the earthy kat-ions, $Ca(OH)_2$, $Ba(OH)_2$, $Sr(OH_2)$, and thereby to relatively increase the number of the acid H-ions, and so to produce the acid reaction.

Attempts to throw light on this question with ash-free albumin have as yet led to no definite results (Pauli).

There is, on the other hand, no difference in the order in which kat-ions follow one another in the alkalies and in the alkaline earths. Pauli states: "Albumin which has become changed by its firm union with the electropositive-ions of the alkaline earths[1] differs from native albumin in having its precipitation augmented by an-ions and inhibited by kat-ions." Pauli points out that his explanation agrees well with such other observations as those of Hardy (see p. 259), who pointed out that the electrical properties of coagulated albumin are reversed by changing the reaction of the medium in which it is placed; and of Posternak (see p. 282), who found the sequence of the precipitating ions in acid solutions to be the opposite from that which Hofmeister[2] and Pauli had determined for native albumin, etc.

The effect of adding alkali-salts to the salts of the alkaline earths Pauli has summed up thus: "The greater the precipitating power of the an-ion of the alkaline earth which we add to an albumin solution, the less will be the increase in the amount of the precipitate which we obtain by adding an alkaline salt with a feebler an-ion, and the inhibitory effect of the alkali-ions (*i.e.* kat-ions) may make itself felt beside the relatively small number of the earthy alkali kat-ions [as for example, in 1 normal $Ca(CNS)_2$]. The reverse holds good if the an-ion of the alkaline earth has a low precipitating value, while that of the added alkali has a high one, and if the number of the simultaneously acting kat-ions of the alkaline earth be great, as it is, for example, in 9 normal $CaCl_2$."

Pauli has also studied the effect of adding hydrochloric acid and caustic potash to the various salt solutions he used for coagulating and precipitating egg-white.[3] To exclude the formation of acid-precipitates, stronger solutions than 0·03 normal HCl were never used, especially as 0·01 normal HCl strongly reddens litmus. It was found that the mono-, di-, and tribasic neutral salts of the alkali metals were not appreciably influenced by 0·01 normal HCl.[4] With 0·02 normal HCl, the sulphates of Na, K, NH_4, and Mg produce a somewhat quicker, and with 0·02 normal NaOH a somewhat slower, coagula-

[1] The double electrical load is not sufficient to explain the behaviour of the ions of the alkaline earths towards albumin, for the divalent magnesium behaves like the alkalies, while the monovalent lithium in many respects approaches the alkaline earths, for its bromide precipitates, and the compounds formed thereby soon become irreversible.

[2] Hofmeister, *Arch. f. experim. Path. u. Pharm.* **25. 26. 27.**

[3] Coagulation and precipitation are terms used in the author's sense (see p. 272).

[4] In every case 2 ccm. of egg-white and 8 ccm. water were taken, and the salts then added in the solid form. It was necessary to at once mix the egg-white with the rest of the fluid to avoid alterations in the concentration of the electrolytes.

tion than with native albumin. After twenty-four hours no difference could be seen between the tubes which had HCl and KOH added and the control tubes. All precipitates were found to be reversible.

Using 6-normal solutions of non-coagulating electrolytes (Mg, NH_4, K, Na—acetates, chlorides, nitrates, bromides, iodides, and thiocyanates) acidification with 0·01 normal HCl converted only the thiocyanates and iodides into precipitating agents: $Mg > NH_4 > K > Na$. The precipitates were irreversible. On gradually increasing the amount of acid the chlorides, nitrates, and bromides also become precipitants, but ammonium salts do not precipitate till the acidity has reached 0·03 normal HCl.

Keeping the acidity 0·03 normal HCl constant, and increasing the concentration of salts, Pauli found the precipitating power of the salts, arranged according to their an-ions, to follow in this order: $CNS > Br > NO_3$, SO_4, while, arranged according to their kat-ions, he obtained the series $Na > K > NH_4 > Mg$.

Chlorides do not increase in the same ratio as do the other neutral salts, because the chlorides interfere to a certain extent with the electrical dissociation of the HCl, as both possess the Cl-ion in common. If the chlorides are only in strengths of 0·25 and 0·5 normal, the kat-ions form again the series $Na > K > NH_4 > Mg$, while in higher concentrations (1-normal) the order of the kat-ions is inversed and becomes the same as if only 0·01 normal HCl were present, showing that 1-normal chlorides greatly reduce the electrical dissociation of the HCL.

With 0·03 normal HCl the precipitation, on increasing the amounts of the salts, reaches a maximum, and then sinks on the further addition of salt. Using equivalent solutions the maximal precipitation of the egg-white was not always obtained with the same concentration of different salts. All precipitates on being diluted were found to be irreversible.

The acetates, fluorides, tartrates, and citrates of the alkalies, instead of producing an increased precipitation in the presence of HCl, were found to diminish the amount of the precipitate.

7b. Compounds of Albumins with the Heavy Metals

The following historical review is based on the papers by Vaubel,[1] Schulz,[2] and Galeotti.[3] The first metallic albuminate

[1] W. Vaubel, *Journ. f. prakt. Chem.* **60**. 55 (1899).

[2] Fr. N. Schulz, *Die Grösse des Eiweissmoleküls.* Jena (1903), Gastar Fischer.

[3] G. Galeotti, *Zeitschr. f. physiol. Chem.* **40**. 492 (1904).

formed synthetically seems to have been a copper compound, and, therefore, the copper albuminates are discussed in the first instance.

7c. Copper Albuminates

The copper compounds of albumin were first made by Orfila[1] because of their toxological importance. To him and to Christison belongs the credit of having clearly recognised that copper unites with albumin only in the form of its oxide, and not in the form of a salt. This view was also held by Rose,[2] the discoverer of the biuret-reaction. Mitscherlich's[3] statements to the opposite effect were disproved by Mulder[4] and Lieberkühn.[5] Other early workers were Lassaigne,[6] Bielicki,[7] Neebe,[8] Ritthausen,[9] Ritthausen and Pott,[10] and Mörner.[11] The recent investigations date from Harnack,[12] who preferred, by means of copper salts, 'ash-free' albumin, after Würtz, in 1844, had failed in preparing ash-free compounds by means of lead salts. Harnack's investigation called forth papers by Grübler,[13] Chittenden, and Whitehouse,[14]

[1] Orfila, *Toxic. Gén.* i. 535. See also Christison, *Uber d. Gifte*, Weimar, 1831, pp. 480 and 482.

[2] F. Rose, *Poggendorff's Annalen*, **28**. 132 (1833), Inaug. Dissert., Rostock (1833).

[3] C. G. Mitscherlich, *Arch. f. Anat. u. Physiol.* **4**. 91 (1837).

[4] Mulder, *Versuch einer allgem. physiol. Chem.* Braunschweig, 1851.

[5] N. Lieberkühn, *Poggendorff's Annalen*, **86**. 117 and 298 (1852).

[6] Lassaigne, *Journ. de chem. médic.* (2nd series), vol. 6 (according to Harnack).

[7] R. Bielicki, *Quædam de metallorum albuminatibus, eorumque effectu ad organismum animalium*, Dissertation, Dorpat, 1853.

[8] C. W. Neebe, *Versuche ü. d. Wirk. d. essigsäuren Kupferoxyds und einiger anderen organischsäuren Kupferoxyde*, Dissertation, Marburg, 1857.

[9] H. Ritthausen, *Die Eiweisskörper der Getreidearten*, etc. Bonn, 1871.

[10] H. Ritthausen and R. Pott, *Journ. f. prakt. Chem.* **7**. 361 (1873).

[11] K. A. H. Mörner. 'Alkali-albuminates in combination with alkaline earths and copper,' *Upsala Läkare förenings förhandl.* **13**. 24 (1877). See Maly's *Berichte*, **7**. 6 (1877).

[12] E. Harnack, (*a*) 'The Copper Compounds of Albumin,' *Zeitschr. f. physiol. Chem.* **5**. 198 (1881); (*b*) 'Method of Preparation and Properties of Ash-free Albumin,' *Ber. d. deutsch. chem. Ges.* **22**. 30, 46 (1889); (*c*) 'Sulphur Content of Ash-free Albumin,' *Ber. de deutsch. chem. Ges.* **23**. 40 (1890); (*d*) 'Studies of so-called Ash-free Albumin,' *Ber. de deutsch. chem. Ges.* **23**. 3745 (1890) (here is given a detailed account of how to prepare 'ash-free' albumin); (*e*) 'Further Studies of Ash-free Albumin,' *Ber. de deutsch. chem. Ges.* **25**. 204 (1892); (*f*) 'Discussion on Crystallised and Ash-free Albumins,' *Zeitschr. f. physiol. Chem.* **19**. 299 (1894); (*g*) 'The Sulphur of Ash-free Albumins as compared with that of Halogen Albuminates,' *Ber. d. deutsch. chem. Ges.* **31**. 1938 (1898).

[13] G. Grübler, *Journ. f. prakt. Chem.* **23**. 97 (1881).

[14] R. H. Chittenden and H. H. Whitehouse, 'On the Metallic Combinations of Albumin and Myosin,' *Studies from the Laborat. of Physiol. Chemist., Yale Univers.*, New Haven, **2**. 95 (1887).

Werigo,[1] Stohmann and Langbein,[2] Bülow,[3] Brunner,[4] Baal,[5] Schulz,[6] and Galeotti.[7]

The greatest amount of copper is bound by vegetable proteids, for Ritthausen gives these percentage figures :—

	CuO.	Ash.
Gluten-caseinogen from wheat . .	16·97	+
Legumin from peas	15·61	1·21
Spelt-gluten-caseinogen	15·23	0·57
Legumin from broad beans . . .	14·10	3·05
Legumin from oats	13·53	...
Conglutin from lupines	13·38–11·60	0·43–2·16

For animal albumins the following percentage figures are given :—

An albuminate from the sepia-case (Schulz) .	20 per cent CuO
Milk-casein (Ritthausen and Pott) . . .	16-17
Egg-white: F. Rose	1·6-1·69
Mulder	4·44
Mitscherlich	2·8-3·3
Lieberkühn	4·6
Bielitski	4·72-5·19
Lassaigne	4·95
Harnack—(*a*) preparation . .	1·35
(*b*) preparation . .	2·64

[1] B. Werigo, *Pflüger's Arch.* **48**. 127 (1890).

[2] F. Stohmann and H. Langbein, *Journ. f. prakt. Chem.* **44**. 336 (1891).

[3] K. Bülow, *Pflüger's Arch.* **58**. 207 (1894).

[4] A. Brunner, *Über Albuminfällung durch Schwermetalle*, Dissertation, Würzburg, 1897.

[5] C. Paal, (*a*) 'Action of fixed Alkalies on Egg-albumin,' *Ber. d. deutsch. chem. Ges.* **35**. 2195 (1902); (*b*) 'Colloidal Silver-oxide,' *ibid.* p. 2206; (*c*) 'Colloidal Mercuric Oxide,' *ibid.* p. 2219; (*d*) 'Colloidal Silver,' *ibid.* p. 2224; (*e*) 'Colloidal Gold,' *ibid.* p. 2236.

[6] Fr. N. Schulz, *Die Grösse des Eiweissmoleküls.* Jena, 1903 (Gustav Fischer).

[7] G. Galeotti, *Zeitschr. f. physiol. Chem.* **40**. 492 (1904).

[TABLE X

TABLE BY F. N. SCHULZ SHOWING THE PERCENTAGE IN WHICH VARIOUS HEAVY METALS HAVE BEEN FOUND IN COMBINATION WITH EGG-ALBUMIN.

Observers.	Cu.	Ag.	Pt.	Hg.	Pb.	Zn.	Fe.
Mulder . .	3·55	...	...	...	...	...	...
Mitscherlich .	2·24–2·64	...	...	...	...	...	...
Bielitzky . .	3·77–4·15	...	...	...	...	...	...
Lassaigne . .	3·96	...	...	...	...	...	...
Mörner . .	1·2	...	...	...	...	...	...
Harnack . .	1·35–2·64	...	...	...	...	...	...
Rose . . .	1·20–1·35	...	...	...	...	2·16	1·99
Lieberkühn .	3·68	6·1, 6·26	...	...	2*	3·7	...
Schwarzenbach [1]	...	..	5·7	...	...	...	...
Diakonow [2] .	...	...	0·8-6·3	...	...	...	...
Fuchs [3] . .	...	3·3	0·8-5·9 8·1	...	...	...	...
Commaille [4] .	...	...	9·10-8·02	...	...	...	...
Loew [5] . .	...	2·17, 2·28 4·31 4·39, 4·64	...	...	...	...	...
Chittenden and Whitehouse .	0·7, 1·21 1·71, 2·19	3·91, 4·09 4·86, 5·72	...	2·89 ...	2·2– 2·8† 2·4–32·1‡	0·91 ...	0·95 ...
Brunner . .	1·34	...	...	7· 5	...	...	1·06

* Values inconstant. † Precipitated with basic lead acetate.
‡ Precipitated with neutral lead acetate.

Galeotti,[6] by employing Gibb's method of representing geometrically the thermodynamic principles governing a pluriphasic system, has succeeded in solving the problem of the equilibrium between the different phases of egg-albumin or serum-albumin and $CuSo_4$ or $AgNO_3$ by a graphic method, for a temperature between 14° and 16°. If the centesimal composition of a given complex has been ascertained, it is possible to at once determine into how many phases such a complex may divide, and also what the composition of each phase must be.[7] Galeotti has arrived at the conclusion that the salts of the heavy metals with the albumins are not true compounds in constant proportions according to the theory of valency. The insoluble compounds which are formed on bringing together solutions of albumin with heavy metals, and which are usually called metallic albuminates, are loose compounds with varying constitutions.

As the combinations of the heavy metals with the albumin are

[1] Schwarzenbach, *Liebig's Annalen*, **133**. 125 (1865).

[2] Diakonow, *Hoppe-Seyler's med. chem. Unters.* 1867, p. 228.

[3] Fuchs, *Liebig's Annalen*, **151**. 372 (1869).

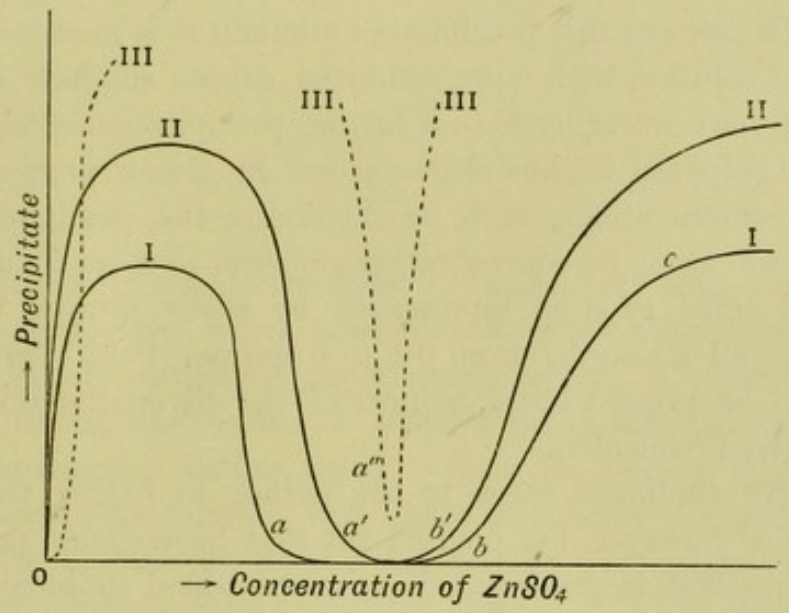
[4] Commaille, *Moniteur scientific*, Oct. 1866. Abstracted in *Jahresb. ü. Fortschr. d. Chemie*, v. Will, 1866 (Giessen, Ricker, publisher).

[5] Loew, *Pflüger's Arch.* **31**. 393 (1883).

[6] G. Galeotti, *Zeitschr. f. physiol. Chem.* **40**. 492 (1904).

[7] The reader will find a concise account of Gibb's principle as elaborated by Bakhuis-Roozeboom, Rijn van Alkemade and Schreinemakers in Galeotti's paper.

soluble in an excess of both the heavy metal and the albumin, Galeotti has spoken of such compounds as reversible ones, but Pauli points out rightly that the term 'reversible' should only be used for the compounds which, on dilution with water or on dialysis, are reconverted into the initial unaltered albumin. Reversible coagulates are thus only formed by the neutral salts of the alkalies and of magnesium. According to Pauli[1] the essential difference between the salts of the alkaline earths and those of the heavy metals is shortly this: In the alkaline earths both kat-ions and an-ions play a part in producing coagulation, although the effect produced by the an-ions is subsidiary. In the salts of the heavy metals the only coagulating factor is the kat-ion, the an-ions being negligible.



Pauli has experimented on egg-white with $ZnSO_4$, $CuSO_4$, and $AgNO_3$. He distinguishes the following three types of phenomena.

1. In the case of $ZnSO_4$, starting with very dilute concentrations of this salt 0·0008 to 0·001 normal in 1·10 egg-white, a coagulum is obtained, which on dilution is not reversible; in concentrations of 0·5 to 4 normal, $ZnSO_4$ does not cause coagulation; beyond 4 normal concentrations a precipitate is formed, which, on diluting the solution up to 4 and 0·5 normal, dissolves again, while, if the dilution be carried beyond 0·5 normal, an irreversible coagulum is formed. The greater the concentration of the albumin, the more does the indifferent zone become narrowed down, as is seen from the figure given by Pauli, in which I., II., and III. represent increasing strengths of albumin.

From this figure it also follows that in the region of the second precipitation the albumin will be precipitated by diminishing not only the albumin + salt-content, but also the salt percentage alone.

[1] W. Pauli, *Hofmeister's Beiträge*, **6**. 233 (1905).

2. The second type of coagulation is seen if one uses copper sulphate. It resembles $ZnSO_4$ in also giving a precipitate in very dilute solutions (0·0008 to 0·001 normal), and giving no precipitate in normal solutions,[1] but it differs from $ZnSO_4$ in giving with 1 : 10 egg-white a secondary precipitate even if used in strengths of six times normal. Galeotti has, however, shown that copper sulphate with very high concentrations of albumin behaves as follows: "On dissolving a large amount of copper albuminate in a supersaturated $CuSO_4$-solution and inspissating the fluid under a bell-jar with some H_2SO_4, one obtains at first only crystals and the solution remains clear, but gradually as the crystals increase in size, the fluid becomes turbid and a more or less abundant precipitate of albumin settles down." To dissolve this precipitated albumin it is necessary to dilute the original solution with more saturated copper sulphate solution, as addition of mere water leads to a further precipitation of albumin.

Copper sulphate is thus characterised by giving precipitates only in feeble concentrations, and by dissolving the precipitate, formed originally, whenever its concentration amounts to more than 1 normal.

3. The third type is represented by silver nitrate, which precipitates in all strengths from 0·1 to 6 normal (Pauli), but if excess of albumin be present large amounts of the silver-albumin-compound are redissolved (Galeotti).

Corrosive sublimate seems to the author to belong to the third type. Rose,[2] however, has pointed out that hæmoglobin prepared by Berzelius' method is precipitated by concentrated sublimate, but that on diluting the mixture it passes into solution, to be, however, reprecipitated by increasing the amount of sublimate. The author in his *Physiological Histology* was the first to make experiments to determine the antagonistic effects produced by sodium chloride on the precipitation of egg-white by corrosive sublimate, for this question is of great importance in connection with the fixing of tissues for histological purposes, the medicinal application of sublimate and the sterilisation of hands and wounds by sublimate. He based his explanation on the electro-affinity of metals (see footnote, p. 313).

The following experiments are taken from the author's *Physiological Histology*, pp. 109-113.

Experiment 8 a

In the first experiment 5 per cent watery solutions of sublimate and of common salt were used in the amounts stated in the table,

[1] The molecular weights of $ZnSO_4$ and $CuSO_4$ are 160·9 and 159·2.

[2] F. Rose, *Poggendorff's Annalen*, **28.** 132 (1833).

1 ccm. of normal egg-albumin being added to 10 ccm. of the sublimate-salt mixture. After the addition of the albumin the test-tubes were each shaken three times, a shake consisting of a single sharp movement.

	$HgCl_2$ 5 per cent.	NaCl 5 per cent.	Albumin.	Proportion in gram weights of $HgCl_2$: NaCl.
A .	10	...	1	10 : 0
B .	9	1	1	9 : 1
C .	8	2	1	4 : 1
D .	7	3	1	7 : 3
E .	6	4	1	3 : 2
F .	5	5	1	1 : 1
G .	4	6	1	2 : 3
H .	3	7	1	3 : 7
I .	2	8	1	1 : 4
K .	1	9	1	1 : 9
L .	0·5	9·5	1	0·5 : 9·5
M .	0·25	9·75	1	0·25 : 9·75

The immediate results are these :—In *A* a very coarse membranous precipitate is formed, which after five minutes commences to settle, leaving a faintly opalescent supernatant layer. *B* resembles *A*, but the precipitate is less coarse. *C* shows a finely floccular precipitate in a bluish opalescent mother liquor. Soon the flocculi aggregate into larger flocculi like cumuli, and these commence to settle quickly. *D* resembles *C*, but the flocculi are smaller and settle less quickly. The mother liquor is more opalescent than in *C*. The specific gravity of the egg-white lies between that of the solutions *D* and *E*, nearer *D*. The tube *D* shows a uniformly opalescent fluid with no flocculi. From *F* to *M* a gradually diminishing opalescence is seen, which in *M* is just perceptible.

After twenty-four hours in *A* a thick curdy precipitate occupies the lower quarter of the mixture, while the supernatant fluid is perfectly clear. *B* shows a thick curdy precipitate in the lower third, the middle third being distinctly opalescent and sharply marked off from the upper third, which exhibits the faintest trace of opalescence. *C* to *M* form a definite series in which the amount of the precipitate and the opalescence slowly decrease till only a few very light flocculi are seen in *H*, which thus forms the most homogeneous tube. In *I* to *M* the opalescence gradually diminishes till it is just visible in *M*, but there is no precipitate. Thus after twenty-four hours *H* shows the same condition which was seen in *E* immediately after mixing the egg-white with the sublimate-salt mixture.

General conclusions are given on p. 313.

Experiment 8 B

In Experiment 8 A the only constant was the egg-white, namely, 1 ccm., while both the sublimate and the salt solutions were variables. Following the suggestion of G. J. Burch the author repeated the experiment with two constants, namely, the sublimate and the egg-white, and two variables, the salt and the water, with Experiment 8 B, while in Experiment 8 C the albumin and the salt were constants, the sublimate and water being the variables. (The differences in the electrolytic dissociation of NaCl and the hydrolytic dissociation of HgCl were not taken into consideration.)

The molecular weight of sodium chloride is 58·4, and a 'normal' solution is made by dissolving 58·4 grms. of this salt in 1000 ccm. of water, sodium being monovalent. The molecular weight of mercuric chloride (sublimate) is 270·6, and, as mercury is divalent, to obtain a normal solution of sublimate equivalent to that of a monovalent substance such as sodium chloride, one-half the molecular weight in grams, namely, 135·3 grams, would have to be dissolved in 1000 ccm. of water. The solutions used in this experiment were a two-fifth normal sublimate solution, *i.e.* 54·1 grams in 1000 ccm. of water, and a double-normal salt-solution, *i.e.* 116·8 grams in 1000 ccm. of water. The reason for choosing a two-fifth sublimate solution was because the insolubility of sublimate prevents a 'normal' watery solution being made, its solubility being only 70 instead of 135·3 grams in 1000 ccm. of water. The sodium chloride was used in double its normal strength to bring the action of this salt quickly into play.

Care was taken that the total amount of fluid amounted in each case to 10 ccm., and that the sublimate, salt, and water were well mixed before adding the albumin.

25 ccm. of sublimate solution contain 1·3525 grams of $HgCl_2$, while 5 ccm. of salt solution contain 0·584 gram of NaCl. (Table on p. 311.)

The immediate results were:—*A* showed no change, while in *B* a dense flocculent coagulum appeared which commenced to settle after 10 minutes. The coagulum was so dense that ordinary letterpress could not be seen through the test-tube. *C* contained finer flocculi than *B*; letterpress at first was just visible, but the writing became more distinct after 10 minutes because of the settlement of the flocculi. *D* resembled *C*, but the flocculi were still finer, and hence the print plainer than in *C*; after 10 minutes individual letters could be recognised, the sediment was finer and less abundant than in *C*.

E to *L* exhibit a gradually descending series of opalescence. The

egg-white floated on the solutions from *A* to *G* and had to be mixed with the fixing solution by shaking. At *H* the specific gravity was approximately the same; the egg-white sent downwards finger-like processes resembling the tentacles of a medusa. *I* at first sight might be taken to be almost clear, and *L* compared with *A* showed the merest trace of opalescence. In *M*, within 2½ minutes after adding the albumin and shaking vigorously, a thick curdy precipitate settled down. In *N* the salt-solution did not quite prevent a slight opalescence, indicating that some albumin was still combined with sublimate. *O* showed a very faint opalescence due to incipient globulin precipitation.

	$HgCl_2$ in ccm.	NaCl in ccm.	Water in ccm.	Albumin in ccm.	Proportion in grams of $HgCl_2$: NaCl.
A .	...	2·5	7	·5	·0 : 2·92
B .	2·5	...	7	·5	1·3525 : 0
C .	2·5	0·5	6·5	·5	1·3525 : 0·584
D .	2·5	1	6	·5	1·3525 : 1·168
E .	2·5	2	5	·5	1·3525 : 2·336
F .	2·5	2·5	4·5	·5	1·3525 : 2·920
G .	2·5	3	3	·5	1·3525 : 3·504
H .	2·5	4	3	·5	1·3525 : 4·672
I .	2·5	5	2	·5	1·3525 : 5·840
K .	2·5	6	1	·5	1·3525 : 7·008
L .	2·5	7	...	·5	1·3525 : 8·176
M .	10	...	...	·5	5·41 : 0
N .	1	8·5	...	·5	0·641 : 9·625
O .	...	8·5	1	·5	0 : 9·625

After twenty-four hours:—In *A* the solution was absolutely clear. In *B* a heavy white curdy precipitate occupied the lower one-third, while the upper two-thirds showed a just perceptible opalescence. *C* to *G* showed a slight precipitate, most marked in *C* and gradually diminishing till just visible in *G*; the supernatant fluid showed a marked bluish opalescence, most marked in *C* and gradually diminishing till *G*. In *H* to *L* no precipitate was visible, and the solutions gradually become less opalescent, till in *L* the opalescence was just visible.

After forty-eight hours traces of a fine floccular precipitate were seen in all the tubes from *H* to *L*, being least marked in *L*.

M contained a dense precipitate in the lower one-fifth of the tube, the upper four-fifths being perfectly clear. With transmitted sunlight the flocculi in the precipitate were seen to be much coarser than in *B*. In *N* the opalescence was still visible, while *O* was perfectly clear, with the merest trace of a sediment.

Experiment 8 C

The sublimate and salt solutions were of the same strength as in Experiment 8 B, the constants in this case being the salt and the variables the sublimate and water.

	NaCl in ccm.	$HgCl_2$ in ccm.	H_2O in ccm.	Albumin in ccm.	Proportion in grams of $HgCl_2$: NaCl.
A .	10	1	84	5	0·0541 : 1·168
B .	10	2·5	82·5	5	0·13525 : 1·168
C .	10	5	80	5	0·2705 : 1·168
D .	10	10	75	5	0·5410 : 1·168
E .	10	20	65	5	1·082 : 1·168
F .	10	25	60	5	1·3526 : 1·168
G .	10	30	55	5	1·623 : 1·168
H .	10	40	45	5	2·164 : 1·168
I .	10	50	35	5	2·725 : 1·168
K .	10	60	25	5	3·25 : 1·168
L .	10	70	15	5	3·787 : 1·168
M .	10	80	5	5	4·328 : 1·168

The immediate results: All solutions from *A* to *M* are opalescent; *D* to *H* show a flocculent precipitate after the first three shakes; on continuing to shake this precipitate disappears, but re-forms after five minutes, though to a much lesser extent than at first. The specific gravity of egg-white lies between *I* and *K*, nearer to *I*. *I* after ten minutes shows traces of a flocculent precipitate, similar to the primary one seen in *D* and *H*. *K*, *L* and *M* are milky opalescent with no trace of flocculi ten minutes after the experiment.

After forty-eight hours all the tubes from *A* to *M* are markedly opalescent, the opalescence and the amount of precipitate in the bottom of the test-tube increasing gradually from *A* to *M*. In *M* the upper one-tenth of the solution is somewhat clearer than the subjacent portion, showing that a second crop of flocculi is about to settle down. *K* appears more milky than either *I* or *L*. *M* in general character strongly resembles *C* of Experiment 8 A.

General conclusions are given on the next page.

Experiment 9

A saturated solution of sublimate in 0·5 per cent sodium chloride (Gaule's solution), and this solution diluted with an equal bulk of water, were compared with a saturated solution of sublimate in distilled water and also with this solution diluted with an equal bulk of distilled water.

Result: The saturated sublimate-salt solution formed a sediment consisting of coarser flocculi than the saturated watery sublimate solution, and therefore did not settle down as firmly as the former. The sediments formed by the half-saturated solutions only settle down to one-half the extent of those formed by the saturated ones.

There was no difference noticeable in the sediments of the two half-saturated solutions, while the supernatant fluid in that test-tube containing the half-saturated salt-sublimate mixture was opalescent, because the salt leads to the formation of some very fine coagula, or partly dissolves the mercury precipitate. After shaking up the two half-saturated solutions, the one containing salt settles more quickly.

General conclusions: Sodium chloride, if present in even minute traces, has a distinct solving action on the sublimate precipitate, and if it be to the sublimate in the proportion of 3 : 7 gram weight (Experiment 8 A, *D*) the formation of a solid coagulum is prevented altogether, —the albumin-molecules being fixed separately give rise to a fine opalescent emulsion. Still further additions of salt, especially if the amount of gram weight of sodium chloride to the sublimate is as 5·84 : 1·35, prevent coagulation altogether (Experiment 8 B, *I*), because the sodium chloride, by its ready dissociation into sodium-ions and chlorine-ions, saturates the watery solution with the latter, and thereby prevents any more chlorine-ions being formed. Sublimate, which would normally break up hydrolytically according to the formula $HgOH + 2HCl$, and its hydrochloric acid into the hydrogen-ion with an acid reaction and the chlorine-ion, cannot do so now, as the formation of chlorine-ions in a solution already saturated by them is impossible.

When sublimate, therefore, is used in strong NaCl solutions it cannot dissociate hydrolytically, and no hydrogen-ions being formed the reaction of the sublimate and salt solution remains neutral, as has already been noticed by Lee and Mayer.[1]

When a proteid, coagulated by sublimate, is treated with a solution of sodium chloride it becomes soluble, because we are dealing with the following changes: From a table of electro-affinities[2] it will

[1] Lee and Mayer: *Grundzüge d. mikr. Technik*, 1901, p. 43.

[2] Abegg and Herz, *Chemisches Practicum*, Vandenhoek and Ruprecht, Göttingen, 1900 (English edition, Macmillan), gives the following table of electro-affinities :—

Kat-ions arranged in descending order of their electro-affinities—

K, Na, Li, Ba, Sr, Ca, Mg, Al, Mn, Zn, Cd, Fe, CO, Mi, Pb,
H, Cu, Ag, Hg, Pt, Au.

An-ions arranged in descending order of electro-affinities—

(F, NO_3, ClO_3), (Cl, So_4), Br, I, PO_4, CO_3, CrO_4, SiO_3, SH, H_2BO_3.
OH, CN, O, S.

be seen that sodium is a much stronger kat-ion than is mercury, and hence sodium will displace the mercury in the albuminate, a sodium instead of a mercury albuminate being formed; the mercury ions, turned out of their union with the albumin, link on to the chlorine an-ions to form sublimate, and as the solution is already saturated with an excess of chlorine-ions, the $HgCl_2$ cannot dissociate, and we arrive at the same result as if we had added a solution of sublimate in a strong salt solution to the proteid. The sodium albuminate does not coagulate, because all neutral salts, such as sodium chloride, fail to produce coagulation.

These experiments make it clear that it is by no means immaterial whether tissues are fixed in sublimate or in sublimate-sodium chloride solutions. Some years ago M. Heidenhain was good enough to inform me by letter that his object in using sublimate dissolved in salt solution was to increase the solubility of the former. At that time I had made these experiments, but refrained from arriving at any rash conclusion because I did not know then, nor do I now, to what extent the electrolytic dissociation of sublimate is prevented by the addition of salt, particularly as the new factor of increased solubility of the mercury salt, owing to the formation of double salts, comes in.[1]

When staining sections the instability of the mercury albuminate must also be borne in mind.

Experiment 10

To determine the coagulating power of the double salt $HgCl_2 + NaCl$ when used in different strengths. The double salt was made up as a 5 per cent solution.

	A	*B*	*C*	*D*	*E*	*F*
5 per cent double salt solution . .	10	8	6	4	2	1
Water	—	2	4	6	8	9
Albumin	1	1	1	1	1	1
Double salt by weight . . .	0·5	0·4	0·3	0·2	0·1	0·05

After twenty-four hours the amount of sediment diminished from *A* to *F*, showing that to get a complete precipitation, as far as this is possible in the presence of sodium chloride, the fixative must be to the object to be fixed in the proportion of 10 to 1. That precipitation was not complete was determined by adding some of the

[1] Experiments have been going on for some time to settle experimentally the precise effect produced on tissues by adding sodium chloride to sublimate solutions in different proportions.

5 per cent double salt solution to the tubes *D*, *E*, and *F*, when an increase in the amount of opalescence was observed.

Experiment 11

To determine the effect of a 10 per cent watery solution of the double salt $HgCl_2 + 2\ NaCl$.

	A	*B*	*C*	*D*	*E*
10 per cent double salt .	10	8	6	4	2
Water	—	2	4	6	8
Albumin	1	1	1	1	1

After twenty-four hours all test-tubes had a milky opalescent appearance, the tube *C* being the most milky because the specific gravity of this fixing solution was primarily equal to that of the albumin solution, and for this reason also no sediment was formed. In the remaining four tubes the sediment diminished slightly in this order, *E*, *D*, *B*, *A*.

That sublimate in the presence of sodium chloride loses its coagulating power and therefore also its toxicity is well shown by the investigations of Paul and Krönig,[1] who found that the number of colonies of bacteria increased with the amount of salt which was added to a sublimate solution. Subsequently Paul and Sarwey[2] showed that sublimate is less dissociated in high percentage ethyl alcohol than in methyl alcohol, and that alcohols greatly interfere with its power of dissociating and therefore with disinfection.

If we take all the factors into consideration it seems to the author that the union of the heavy metals with albumins is, in the case where metallic salts undergo hydrolysis, primarily an oxidative process, the metallic oxide, for example, HgO, uniting with some carbon-atom, analogous to the union of the unsatisfied oleic acid with osmium tetroxide.[3] While in those cases where metallic salts do not undergo hydrolysis, as in the case $AgNO_3$, the primary change is a union of the NO_3-ion with the amino-group of the albumin, while the union of the Ag-ion with the albumin is only made possible by the conversion of the Ag-ion into the oxide. (See p. 342 under Schadee van der Does.)

[1] Paull and Krönig, *Zeitschr. f. physikal. Chem.* **21.** (1896); *Münchener med. Wochensch.* 1897; *Zeitschr. f. Hygiene*, **25.** (1897).

[2] Theod. Paul and Otto Sarwey, *Münchener mediz. Wochensch.* 1901, No. 36, 37, 38.

[3] Mann, *Physiological Histology*, 1902, pp. 303-311.

8. Coagulation by Means of Heat

If watery solutions of albuminous substances are heated to a certain temperature, the albumin becomes coagulated. Little as we know as to the principles underlying this process, it is yet necessary to discuss it in full, as we constantly have to practise it.

Natural albumins are always changed chemically by heat action. They become more basic, for distinctly acid solutions become less acid and neutral solutions turn alkaline. On the other hand solutions of muscle-proteids, which are essentially globulins, are rendered acid by heat-coagulation, as was first shown for myosinogen and paramyosinogen by Halliburton, and then fully confirmed by G. N. Stewart.[1]

The two main factors influencing heat coagulation are, firstly, the reaction of the solution, and, secondly, the amount of salts present in the solution. Apart from the older investigations of Lieberkühn and Heynsius[2] and others, the chief advance in more recent times has been made by Frédéricq,[3] Halliburton[4] and his pupil Hewlett,[5] Neumeister,[6] Brunner,[7] and Starke.[8] They agree in emphasising that complete precipitation and coagulation of albumin is only possible if the reaction of the solution is slightly acid. Whenever the reaction is too strongly acid or too strongly alkaline, then a greater or smaller amount of albumin remains in solution and so escapes coagulation. The other fact, first noticed by Aronstein,[9] and then confirmed by Heynsius,[2] Harnack,[10] Bülow,[11] Starke,[12] Pauli,[13] and Erb,[14] is shortly this: If a solution of an albumin—for example, egg-white—has all its inorganic salts removed by prolonged dialysis, then heat may be applied without producing coagulation. But precipitation and

[1] G. N. Stewart, *Journ. of Physiol.* **24.** 450 (1899).

[2] A. Heynsius, *Pflüger's Arch. f. d. ges. Physiol.* **9.** 514 (1874).

[3] L. Frédéricq, a short summary is given in the *Zentralbl. f. Physiol.* **3.** Nr. 23, p. 601 (1890).

[4] W. D. Halliburton, *Journ. of Physiol.* **5.** 155 (1885).

[5] R. T. Hewlett, *ibid.* **13.** 493 (1892).

[6] R. Neumeister, 'Introduction of Albumoses and Peptones into the Organisms,' *Zeitschr. f. Biol.* **24.** 272 (1888).

[7] R. Brunner, Dissertation, Bern, 1894.

[8] J. Starke, 'Heat-Coagulation and Neutral Salts,' *Sitzungsber. d. Gesellsch. f. Morphol. und Physiol. in München*, 1897, p. 1.

[9] B. Aronstein, *Pflüger's Arch. f. d. ges. Physiol.* **8.** 75 (1874).

[10] E. Harnack, *Ber. d. deutsch. chem. Ges.* **22.** II. 3046 (1889); **23.** II. 3745 (1890).

[11] K. Bülow, *Pflüger's Arch. f. d. ges. Physiol.* **58.** 207 (1894).

[12] J. Starke, *Sitzgsber. d. Ges. f. Morph. u. Physiol. in München*, 1897, p. 1.

[13] W. Pauli, *Pflüger's Arch. f. d. ges. Physiol.* **78.** 315 (1899).

[14] W. Erb, *Zeitschr. f. Biol.* **41.** 309 (1901).

coagulation of the albumin are induced at once when salts are added to the heated solution.

Starke and Erb give the following explanation:—Albumins are always denaturalised by heat, whatever the reaction and whether salts are present or not, but the fate of the denaturalised albumin depends on various factors. Denaturalised albumin is insoluble in water and in neutral salt solutions—soluble, however, in acids and in alkalies. If therefore a feebly alkaline solution of albumin is heated, there is formed at once a soluble salt consisting of the denaturalised albumin and the metallic base; while if an acid solution of albumin is heated, there results analogously a soluble salt composed of the denaturalised albumin and of the acid we added. Only if the reaction is quite neutral does the denaturalised albumin, which in itself is insoluble, become completely precipitated.

The salts which denaturalised albumin forms with acids are called acid-albumins; those formed with bases are usually called alkali-albuminates, but Schmiedeberg[1] and Maas[2] employ the term 'albuminic acids.' Paal[3] calls the denaturalised albumin which is formed by the action of fixed alkalies—protalbinic and lysalbinic acids. Osborne[4] calls the denaturalised edestin—edestan. The salts of denaturalised albumin with hydrochloric, sulphuric, acetic acid, etc., are very soluble in water, but they are precipitated by even traces of salts. Alkali-albuminates are precipitated by larger amounts of neutral salts; of these alkali-albuminates the sodium-, potassium-, and ammonium-salts are readily soluble, while the calcium-, barium-, or strontium-salts are only slightly soluble. Therefore an alkali-albuminate is precipitated by a large amount of sodium chloride and by small amounts of a calcium salt.[5]

According to the view of Erb,[6] coagulation of serum-albumin takes place in the following manner:—An albumin solution containing no traces of salt is completely precipitated by heat, and not a trace remains in solution. The addition of a drop of dilute acid or dilute alkali prevents the coagulation apparently completely, as the solution remains clear on being heated; but the acid solution is completely precipitated if subsequently a trace of sodium chloride is

[1] O. Schmiedeberg, *Arch. f. experiment. Path. u. Pharmakol.* **39.** 1 (1897).

[2] O. Maas, *Zeitschr. f. physiol. Chem.* **30.** 61 (1900).

[3] C. Paal, *Ber. d. deutsch. chem. Ges.* **35.** II. 2195 (1902).

[4] T. B. Osborne, *Zeitschr. f. physiol. Chem.* **33.** 225 (1901).

[5] S. Ringer and H. Sainsbury, *Journ. of Physiol.* **12.** 170 (1891); S. Ringer, *ibid.* **12.** 378 (1891); also *ibid.* **13.** 300 (1892); F. W. Tunnicliffe, *Zentralbl. f. Physiol.* **8.** 387 (1894).

[6] W. Erb, *Zeitschr. f. Biol.* **41.** 309 (1901).

added. An acid solution of albumin which contains salts coagulates as soon as it is boiled. If the reaction be alkaline, precipitation is never so complete as with acid solutions, but an alkaline solution containing salts, especially a calcium salt, always becomes turbid, and is partially precipitated on being heated.

The following points are also of interest:—Any albumin solution, containing salts, acids, or bases, becomes more alkaline on being coagulated; therefore a solution which, to begin with, is neutral or even feebly acid becomes distinctly alkaline on boiling. Why this happens is not known definitely, but the author, who has discussed heat coagulation at greater length in his *Physiological Histology*, 1902, pp. 58-68, has arrived at the conclusion "that any factor which tends to prevent the formation of hydrogen-ions will also prevent coagulation." "By regarding proteids as hydrogen salts we may assume that the hydroxyl-radical of alkalies prevents coagulation either by preventing the intramolecular change" (*i.e.* conversion of pseudo-bases into real bases and pseudo-acids into real acids—see this book, p. 219) "which occurs normally when heating neutral or acid proteid solutions, or, if the intramolecular change does take place, by neutralising the acid hydrogen-ion which is liberated." The increase in alkalinity, which is produced by heating albumin solutions, is explained thus: "If the pseudo-acid radical[1] of the proteid is converted into a real acid, it will change the pseudo-basic radical into a real base, which latter, by splitting off ammonia, could produce the alkaline reaction." The view of the author is not only supported by Schadee van der Does'[2] observation that addition of silver oxide, Ag_2O, prevents heat coagulation (see index),—as does also the addition of osmium tetroxide, according to Mönckeberg and Bethe,[3]—but also by the recent investigations of Heffter,[4] who showed that a potential hydrogen-ion must exist in the albumin molecule, which, by uniting with sulphur, gives rise to sulphuretted hydrogen (see p. 97).

That albuminous compounds are plurivalent acids and bases is referred to on p. 218, and on the author's theory it is quite conceivable that, if not all, at least some of the amino-acid side-chains in the albumin-molecule, having become hydrolysed by heat, will then act on the remainder of the molecule as would any free acid. Heat coagulation is therefore brought about by one portion of the albumin molecule

[1] By an oversight the word pseudo-basic has been substituted for pseudo-acid in the original.

[2] Schadee van der Does, *Zeit. f. physiol. Chem.* **24.** 351 (1897).

[3] Mönckeberg and Bethe, *Arch. f. mikr. Anat.* **54.** 135 (1899).

[4] Heffter and Hausmann, *Hofmeister's Beiträge*, **5.** 214 (1904).

precipitating the remainder. Whether salts are needed or may be dispensed with will depend on the nature and the grouping of the amino-acid compounds in the albumin.

A chemical explanation as to why acid-albumins are precipitated by salts, is, according to Cohnheim, still outstanding, but it is definitely known that this phenomenon is not a salting-out process, for Panum,[1] Bülow,[2] Werigo,[3] Kieseritzky,[4] Rosenberg,[5] Goldschmidt,[6] v. Fürth,[7] Schulz,[8] Starke,[9] and Erb[10] have shown precipitation to be brought about by mere traces of salt.

The author is of the opinion that the precipitation of acid albumins by the addition of salts is in every respect analogous to the throwing down of colloidal solutions by 'neutral' salts, as explained on p. 289. The acid albumin + the strong an-ion radical of a 'neutral' salt possess collectively the same amount of electro-affinity as does the strong kat-ion of the 'neutral' salt. This view is further supported by the fact that there is a definite proportionality between the amount of acid present in the albumin and the amount of salt which is required to ensure precipitation. The smaller the excess of acid the less salt is needed for precipitating the albumin.

The author's theory explains also the observations of Goldschmidt and others, namely, that on neutralising a strongly acid solution of acid-albumin, a localised precipitate is formed in the acid solution by the addition of an alkali, and that this precipitate then dissolves, to be formed again by a further addition of alkali, to redissolve, and so on; for during the addition of the alkali a neutral salt is formed temporarily, and this neutral salt leads to the albumin being precipitated. A complete precipitation of all the acid-albumin is, however, exceedingly difficult, according to Werigo, Spiro, and Pemsel,[11] as the equivalent amounts of the inorganic constituents needed for the exact neutralisation of an albumin with its high molecular weight are so small as to bring them within the margins of experimental error.[12]

[1] P. Panum, *Virchow's Arch.* **4.** 419 (1851).

[2] K. Bülow, *Pflüger's Arch. f. d. ges. Physiol.* **58.** 207 (1894).

[3] B. Werigo, *ibid.* **48.** 127 (1891).

[4] W. Kieseritzky, *Die Gewinnung des Faserstoffs, Alkalialbuminats und Acidalbumins*, Dissertation, Dorpat, 1882.

[5] A. Rosenberg, Dissertation, Dorpat, 1883.

[6] F. Goldschmidt, *Säuren und Eiweiss*, Dissertation, Strassburg, 1898.

[7] O. v. Fürth, *Arch. f. experiment. Pathol. u. Pharmakol.* **36.** 231 (1895).

[8] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **24.** 449 (1898).

[9] J. Starke, *Sitzungsber. d. Münchener Gesellsch. f. Morphol. u. Physiol.* 1897, p. 1.

[10] W. Erb, *Zeitschr. f. Biol.* **41.**309 (1901).

[11] K. Spiro and W. Pemsel, *Zeitschr. f. physiol. Chem.* **26.** 233 (1898).

[12] E. Salkowski, *Zeitschr. f. Biol.* **37.** 401 (1899).

What salt one uses for precipitating acid-albumins does not seem to be of much consequence, if one were to believe the accounts generally given. Bülow has observed, however, differences between the an-ions.

The conditions influencing the precipitation of the alkali-albuminates are even less understood, for the precipitation due to the addition of larger amounts of sodium chloride has to compete with the formation of the slightly soluble albuminate of calcium. In this special case the nature of the base plays therefore a great part, but even the exhaustive researches of Pauli[1] have not been able to clear up the matter. Pauli sums up his results thus : The change in the coagulation-temperature of albumins depends on the added effects of the two independent ion-actions, each kind of ion possessing, for nearly every salt, its zone of maximal action. In the case of a single salt its action on the albumin may be so pronounced within a certain zone that the addition of other salts produces no further effect. Thus NaCl within certain limits is practically not affected by the addition of any quantity of $NaNO_3$. If two different acid or metallic ions are present it is impossible to predict the result on the coagulation-temperature, as much depends on the nature of the ions ; but the result generally depends on the effect of one salt predominating within certain concentrations, and then the other salt, if present up to a certain minimum, may be increased five- or sixfold. Sodium chloride and sodium nitrate show two such phases—one for either salt, while the mixtures of NH_4Br and NH_4Cl or $MgCl_2$ and NaCl show only one phase.

That albumin coagulates while albumoses do not, Pauli ascribes to the presense of several albumose radicals in each albumin molecule, or to a special kind of unison between the albumose-groups in the albumin ; and he believes the ions of a salt during heat coagulation to attach themselves, more or less firmly, to different parts of the albumose radicals. Spiro[2] has found that inorganic bases (cholin, pyridin, anilin, piperidin, ortho-toluidin, xylidin), and even the feebly basic urea, thio-urea and urethan form alkali-albuminates,[3] and that for this reason they keep denaturalised albumin in solution. Ramsden's[4] observations on urea are also very interesting. Plurivalent alcohols, glycerine, carbohydrates, esters, ketones, and aldehydes have a similar

[1] W. Pauli, *Pflüger's Arch. f. d. ges. Physiol.* **78.** 315 (1899). See Mann's *Physiological Histology*, pp. 60-65.

[2] K. Spiro, *Zeitschr. f. physiol. Chem.* **30.** 182 (1900).

[3] Renard, *Journ. of Physiol. Proceed.* xxviii. **23.** (1902).

[4] W. Ramsden, *Journ. of Physiol. Proceed.* **28.** 23 (1902).

action. If large amounts of alcohol-salts be present, then an alcoholic albumin solution will not coagulate.[1]

In practice one does not usually attempt to coagulate alkaline solutions, as the calcium-albuminates are not quite insoluble, and as the precipitation of alkali-albuminates requires a great deal of salt and is even then not complete. The best plan to adopt is that suggested by Cohnheim,[2] who adds to the albuminous solution to be coagulated, firstly, sodium chloride or some other neutral salt, and then an excess of acetic acid. If no salt is added, then the minutest trace of acid in excess is sufficient to give rise to acid-albumin which, remaining in solution, is converted into albumoses, if the boiling be prolonged. If for any reason the addition of a neutral salt is contra-indicated, then acetic acid should be employed, but only in minimal quantities; the reaction must be just perceptibly acid, so that the small amounts of salt which are normally present may suffice for the precipitation. But even adopting all these precautions, one encounters almost unsurpassable difficulties in coagulating muscle-albumins and other organ-albumins.[3]

A neutral reaction of the fluid we wish to coagulate is only permissible if the albumin has been freed by prolonged dialysis as thoroughly as possible from salts, acids, and bases (Cohnheim).

Heat-coagulation is the only method we possess for separating an albumin from its primary dissociation-products, and it is therefore employed very frequently. For this very reason it is essential, if we wish to precipitate the whole of the albumin, and if we do not wish to form acid-albumins or alkali-albuminates, to keep the reaction of the solution as feebly acid as possible, or to add a large amount of salt. Disregard of these rules has led many observers, even up to quite recent times, into making the most serious mistakes.

9. Coagulation-Temperature

If coagulation by heat is brought about in slightly acid solutions or after the addition of sodium chloride and larger amounts of acetic acid, it is found that each albumin has its specific coagulation-temperature. After many attempts had been made to define accurately the coagulation-temperatures of egg-white and similar substances at the beginning of last century, Kühne[4] showed, in 1864, that two albumins are present in muscle-plasma, one of which is coagulated at a much

[1] K. Spiro, *Hofmeister's Beiträge*, **4.** 300 (1903).
[2] O. Cohnheim, *Zeitschr. f. physiol. Chem.* **33.** 455 (1901).
[3] W. His and W. Hagen, *ibid.* **30.** 350 (1900).
[4] W. Kühne, *Protoplasma und Kontraktilität*, Leipzig, 1864.

lower temperature than is the other. Subsequently fractional heat-coagulation has been chiefly employed by Frédéricq [1] and Halliburton [2] and his pupils for the isolation and characterisation of individual albumins. They found the coagulation-temperature to be very constant, and to differ only 1 to 2°. Generally speaking, it has been found that the complex, tissue-forming albumins and the more differentiated bodies, such as fibrinogen and myosin, coagulate at a lower temperature than do the simple albumins and globulins. The coagulation-temperature of each albumin, as far as it has been determined, will be given when dealing with the individual albumins.

If the reaction be alkaline we are dealing with much more complicated conditions. Pauli [3] finds that most salts lower the coagulation-temperature if they be present in low concentrations, while they raise it in higher concentrations; and further, that the ions of the salts act, as is usual, collectively. No further definite facts could be ascertained as regards salting-out and similar processes.

That egg-white diluted with nine times its bulk of water does not coagulate was first observed in 1880 by William Roberts.[4]

10. The Formation of Additive Compounds

This question is fully discussed in the author's *Physiological Histology*, pp. 68-70. It will suffice here to point out that the two reagents chiefly used by the author were the aldehydes, which give rise to methylene-compounds, see this book p. 250, and secondly osmium tetroxide, which acts as an oxidiser. Neither aldehydes nor osmium tetroxide are electrolytes, and whenever they have to be used for fixing the morphological appearance of cell-albumins, they should always be made up in normal, *i.e.* isotonic salt solutions.

Attention is drawn to the article by Neubauer,[5] who along with Langstein has found that all substances with a double or treble link between two carbon-atoms will reduce osmium tetroxide, which therefore is a reagent for unsatisfied compounds. The same conclusion

[1] L. Frédéricq, 'Coagulation du sang,' *Bull. d. l'Académie royale de Belgique*, 2 Sér., Bd. **64.** (1877), 7 Juli; also *Ann. de Soc. de Médecine de Gant.* (1877); Bérard and Corin, *Travaux du laboratoire, etc., de Frédéricq II.* 171 (1887); L. Frédéricq, *Zentralbl. f. Physiolog.* **3.** 601 (1890).

[2] W. D. Halliburton, *Journ. of Physiol.* **5.** 155; **8.** 133 (1887); **11.** 454; R. O. Hewlett, *ibid.* **13.** 493 (1892).

[3] W. Pauli, *Pflüger's Arch. f. d. ges. Physiol.* **78.** 315 (1899).

[4] William Roberts, *Lumleian Lectures on Digestive Ferments and Artificially Digested Food* (London: Smith, Elder and Co., 1880).

[5] Neubauer, *Sitzber. d. Münchener morphol.-physiol. Ges.* **19.** 31 (1905).

Altmann had previously arrived at in connection with the staining of the unsatisfied oleic acid.[1]

11. 'Spontaneous' Coagulation of Albumins (see p. 266)

According to Cohnheim, certain albumins, for example, fibrinogen, casein, some cell-plasms, and perhaps also para-myosinogen and myosinogen, may assume 'spontaneously' a peculiar state which is intermediate between the originally soluble and the ultimately precipitated condition. These albumins are chemically altered and precipitated under the influence of certain ferments, but are then still relatively soluble, and may be precipitated still further and become denaturalised by such agencies as heat, formaldehyde, alcohol, and other means. For this second 'coagulation,' a second 'heat-coagulation-temperature' exists, which can be determined at least for fibrin, and which differs from that of the original fibrinogen (see, however, p. 378). Cohnheim thinks it is necessary, therefore, to distinguish, as Arthus[2] has done recently, several distinct processes, namely:—

(1) Precipitation or precipitation without denaturalisation by means of salting out or by acidification.

(2) Caseification or solidification which is induced by ferments, as, for example, when rennet acts on caseinogen.

(3) Coagulation or denaturalisation which destroys the colloidal character of the albumin.

The author does not agree with Cohnheim in this view; the classification of Arthus is a very artificial one, for under 'precipitation' are classed the two entirely different processes of (1) rendering a compound supersaturated by the withdrawal of water as in the case of salting out, and (2) causing neutralisation precipitates by the addition of acids. Under 'caseification' we must remember that salts are absolutely necessary in addition to the albumins and the ferments, and 'coagulation' need by no means destroy the colloidal character of albumins, as is well seen in the case of globulin prepared by Starke's method,[3] namely, by diluting egg-white with ten times its bulk of water (see p. 322), and then dialysing the solution at a temperature of 75-85° C. The globulin formed by this process is insoluble in pure water and in neutral salt solutions, but passes into solution on being treated with very dilute alkalies or with dilute acids (Mann).[4] When in solution, the globulin, formed out of egg-

[1] Mann, *Physiological Histology*, 1902, p. 306.

[2] M. Arthus, *Arch. de Physiologie norm. et pathol.* 1893, p. 673.

[3] J. Starke, *Zeit. f. Biol.* **40**. 419, 494.

[4] Mann, *Physiological Histology*, 1902, p. 58.

albumin, becomes once more colloidal. That no difference exists between colloids and electrolytes, or, to put it differently, that colloids are electrolytes under special conditions, was first pointed out by the author,[1] and also in this book (p. 268). Ramsden is further of the opinion that fibrin and fibrinogen have the same coagulation-temperature (see p. 382).

With the exception of the neutral salts used in the salting-out process, all agents causing precipitation lead to denaturalisation. But even with the neutral salts, according to Spiro,[2] a change is said to occur spontaneously although very slowly, but this by no means bears out the author's experience, for egg-albumin crystals may be kept unchanged for years, provided the ammonium sulphate solution is perfectly neutral and saturated; the glass vessel lined with a high melting paraffin, and the access of air carefully prevented.

Some Properties of Colloidal Albumins

1. Formation of Crystals[3]

Many albumins are known in a crystalline state, the crystals occurring either naturally or having been made artificially. To the naturally occurring crystalline albumins belong most of the phytoglobulins, which are stored either as such, or in the form of their salts in the seeds of plants, and also the vitellines in the eggs of fish. Crystalline albumins have been prepared by artificial means from the egg-white of the hen and other birds, from the blood of the horse and the rabbit, and from the milk of the cow; crystalline globulins have been obtained from the white of the hen's egg, from Bence Jones' albumin; while crystalline proteids are represented by hæmoglobin, hæmocyanin, and the phyko-erythin of algæ; and, finally, crystalline peptones by glutokyrin (see p. 200).

The long-known crystallisation of hæmoglobin and the preparation of edestin and other phytoglobulins by Osborne does not differ principally from other kinds of crystallisation.

It is a different matter with the crystallisation of albumins from

[1] Mann, *Physiological Histology*, pp. 45, etc.

[2] K. Spiro, *Hofmeister's Beiträge*, **4.** 300 (1903).

[3] F. N. Schulz, *Die Kristallisation von Eiweissstoffen*, Jena, G. Fisher (1901), gives a good review of the literature dealing with albumin crystals.

half-saturated ammonium-sulphate solutions according to the method invented by Hofmeister[1] and improved by Hopkins and Pinkus.[2] Hopkins' most recent instructions[3] for the preparation of crystalline egg-albumin are as follows :—

Procure newly-laid eggs and collect the egg-white. Measure it carefully, and add exactly the same amount of a saturated ammonium-sulphate solution. Beat the two together till the whole mass forms a stiff froth, and let it stand overnight.

Filter off the precipitated globulins and mucoids, and to the clear filtrate add very gradually, under constant stirring, a solution of 10 per cent glacial acetic acid till a slightly milky permanent precipitate is formed. To litmus paper the mixture by this time will be slightly acid. Now add to each 100 ccm. of this milky mixture 1 ccm. of the 10 per cent glacial acetic acid, when a bulky amorphous precipitate is formed, which in the course of five hours becomes crystalline. To obtain the full yield of crystals (at least 60 grms. per litre) let the mixture stand till next day.

Pure crystals are obtained thus :—Filter off the precipitate, and wash it in three changes of half-saturated solution of ammonium sulphate containing 1 per 1000 of glacial acetic acid. Dissolve the crystals in a minimal quantity of water ; add very slowly, stirring gently all the while, a saturated solution of ammonium sulphate till a distinct precipitate is formed ; then add, in addition, for each litre of the solution, 2 ccm. of saturated ammonium-sulphate solution. As a rule, the albumin will have recrystallised in twenty-four hours. Should the crystals, however, not form readily, agitate the vessel containing the solution gently, but do not shake violently, as mechanical coagulation is apt to occur.

To remove the ammonium sulphate, wash the crystals repeatedly with a completely saturated solution of pure sodium chloride containing 1 per cent acetic acid.

Krieger[4] recommends for the preparation of serum-albumin crystals sulphuric acid saturated with ammonium sulphate instead of acetic acid.

These methods for preparing albumin crystals have been used extensively during the last few years for the preparation of albumins, for hæmocyanin,[5] hæmoglobin,[6] and phyco-erythin.[7]

[1] F. Hofmeister, *Zeitschr. f. Physiol. Chem.* **14**. 163 (1889) ; **16**. 187 (1891).
[2] F. G. Hopkins and S. W. Pinkus, *Journ. of Physiol.* **23**. 130 (1898).
[3] Hopkins, *ibid.* **25**. 306 (1900).
[4] H. T. Krieger, *Kristallinische Eiweissstoffe, med. Dissertation*, Strassburg, 1899.
[5] M. Henze, *Zeitschr. für physiol. Chemie*, **33**. 370 (1901).
[6] F. N. Schulz, *ibid.* **24**. 449 (1898).
[7] Molisch, *Botanikerzeitung*, 1894, p. 177 ; 1895, p. 131.

Schulz has drawn attention to the fact that this kind of crystallisation differs essentially from other forms of crystallisations, inasmuch as it depends on the principle of salting-out, and Spiro[1] has pointed out that the mixture of albumin, salt, and water, which at first is fluid, becomes crystalline only secondarily. For these reasons the crystals enclose always large amounts of the mother-liquor. To obtain salt-free albumin, the crystals are first dissolved and the albuminous solution is then dialysed, but colloidal impurities cannot be got rid of by this means.

Schulz and Zsigmondy[2] have shown that to remove colloidal contaminations recrystallisation from three to six times is required (see p. 333). Crystals prepared in this way must be carefully protected, as otherwise they may absorb again impurities, for Wichmann has shown that crystals imbibe "like a sponge" all sorts of substances from solutions. That this absorption is not a purely mechanical process as held by Wichmann,[3] but a chemical process of the nature of salt-formation, has been pointed out by the author,[4] who found that albumin-crystals prepared by the method of Hopkins react promptly with the Mylius-Ehrlich test for determining the presence of bases, quite apart from the fact that the amino-nature of albumin allows the latter, according to circumstances, to play the part of an acid or a base.

However important the crystallisation of albumins is for the conception that albumins are uniform, chemical individuals, we must never forget that albumins require a very thorough purification by means of repeated crystallisation. The objection that recrystallisation produces alterations is not valid, according to Schulz, and Schulz and Zsigmondy.

Krieger first suggested and Mörner[5] has proved that albumin-crystals are not free albumin, but either an acetate (Hopkins' method) or a sulphate (Krieger's method).

Albumin-crystals appear at first in very varying forms, such as needles, platelets, tables, etc., and Gürber[6] was therefore of the opinion that at least three distinct fractions were present in serum-albumin. Krieger has shown, however, that the individual shapes of serum-albumin-crystals show all stages of transition, and Wichmann[7]

[1] K. Spiro, *Hofmeister's Beiträge*, **4**. 300 (1903).
[2] F. N. Schulz and R. Zsigmondy, *ibid.* **3**. 137 (1902).
[3] A. Wichmann, *Zeitschr. f. physiol. Chem.* **27**. 575 (1899).
[4] Mann, *Physiological Histology*, 1902, pp. 214, 289.
[5] K. A. H. Mörner, *Zeitschr. f. physiol. Chem.* **34**. 207 (1901).
[6] A. Gürber, *Sitzungsber. d. Würzburger Phys.-med. Ges.* 1894, p. 143.
[7] A. Wichmann, *Zeitschr. f. physiol. Chem.* **27**. 575 (1899).

has proved by very careful work that all albumin-crystals are crystallographically identical or at least isomorphic. They probably belong to the hexagonal system, and are, more or less, positively doubly-refractile. Egg-albumin yields principally six-sided columns, 0·1 to 0·15 mm. long and 0·003 to 0·021 mm. thick, while serum-albumin and milk-albumin show different combinations of proto-prisms and proto-pyramids. In this form the crystals remain soluble for a very long time, but ultimately they become denaturalised; the crystals, as Wichmann puts it, change from the monotropic α-modification into the enantiotropic β-modification; they are changed into pseudo-morphoses and lose simultaneously their optic properties. On being heated in half-saturated or even stronger ammonium-sulphate solution they become coagulated, and are again changed into pseudo-morphoses. When quite dry they may be heated to 150° without undergoing decomposition. The hæmoglobin crystals are described under hæmoglobin.

2. Composition, Molecular Weight, Heat of Combustion, and Rotatory Power

Albumins are not readily analysed, because their combustion is not easily carried out owing to the presence of sulphur and of ash. A further difficulty is one which has already been pointed out in connection with the quantitative determination of the dissociation-products, namely, the difficulty of obtaining uniformly pure material for purposes of analysis. According to Michel,[1] serum-albumin, which we may regard as a typical simple albumin, possesses the following percentage composition :—

C	53·08	per cent.
H	7·10	„
N	15·93	„
S	1·90	„
O	21·99	„

It is remarkable how little other albumins differ in their percentage composition from serum-albumin, notwithstanding the fact that they are built up of amino-acids differing so greatly from one another both qualitatively and quantitatively.

The carbon percentage rises in casein and histone to 54 and 54·97, and may fall in other albumins as low as 52; the nitrogen percentage rises in histone to over 18 and in the phyto-vitellines to over 19, while

[1] Michel, *Würzburger Phys.-med. Ges.* N.F. **29**. 117 (1895).

in egg-albumin, which is perhaps not a simple albumin, it falls to 15. Proteids, which contain, besides albumin, other groups with varying constitutions, differ of course more widely, as do also the protamins and many albuminoids. The sulphur percentage differs more greatly, as in keratin it rises to 4 and even 5 per cent, while in certain albumins rich in sulphur it amounts to 2 per cent, but it falls to 0·4 per cent in hæmoglobin.

The molecular formula, calculated from the percentage composition, must be at least doubled in the case of serum-albumin, as the latter is split up by peptic digestion into at least two sulphur-containing portions; as the sulphur-containing dissociation-product is apparently cystin, which contains two molecules of sulphur, it would appear that the formula given above has to be quadrupled. Hofmeister[1] assumes even a formula with six atoms of sulphur, which he bases on his experiments on iodisation:

$$C_{450}H_{720}N_{116}S_6O_{140}.$$

This would correspond to a molecular weight of 10,166. For egg-albumin he calculates in a similar manner a molecular weight of 5378. The molecular weight of hæmoglobin may be determined by two entirely different methods, which is all the more important because hæmoglobin may be prepared as an undoubtedly pure, uniform material, for it crystallises with great ease. In the first instance it is possible to calculate from the percentage ratio of the iron and sulphur the least molecular weight. In this way in Bunge's laboratory, Zinnofsky[2] has calculated for horse's blood, Jaquet[3] for dog's blood, and subsequently Hüfner and Jaquet[4] for ox-blood, the least molecular weight as 16,669. From this weight Jaquet calculates for dog's blood the formula:

$$C_{758}H_{1203}N_{195}O_{218}FeS_3.$$

The second method of calculating the molecular weight depends on the power hæmoglobin has for binding oxygen and carbon-dioxide. Hüfner[4] finds, as 1 molecule of hæmoglobin binds 1 molecule of CO_2, that he obtains the same molecular weight, immaterial whether he determines it by the CO_2 absorbing power of the hæmoglobin or by the percentage number of the iron present in the hæmoglobin. The

[1] Fr. Hofmeister, *Zeitschr. f. physiol. Chem.* **24**. 159 (1897); D. Kurajeff, *ibid.* **26**. 462 (1898).

[2] O. Zinnofsky, *ibid.* **10**. 16 (1885).

[3] A. Jaquet, *ibid.* **14**. 289 (1889).

[4] G. Hüfner, *Arch. f. (Anat. u.) Physiol.* 1894, p. 130.

molecular weight of globin, *i.e.* the albumin-radical of hæmoglobin, may of course be very much less, as we do not know whether the colour-radical, the hæmatin, is joined up with one or with several molecules of globin. But the ratios of the dissociation-products to one another and the equivalent weights according to Grübler,[1] Laqueur and Sackur,[2] Harnack,[3] and Werigo,[4] also yield a molecular weight of 5000-8000, and even more.

The direct methods of determining molecular weight we cannot make use of, as raising of the boiling-point leads to heat-coagulation. The lowering of the freezing-point method has yielded in the hands of Sabanajew and Alexandrow,[5] for egg-albumin the molecular weight of 14,270. Here again the admixture of inorganic salts, which is quite unavoidable, makes itself felt very badly; taking into consideration the high molecular weight and the slight solubility of albumins, the figures which have been obtained have never been higher than could be accounted for by the presence of the inorganic salts alone. Starling's[6] attempt of estimating directly the osmotic pressure of albuminous solutions is also bound up with too many experimental errors. There can, however, be no doubt that the colloidal albumins possess an extraordinarily high molecular weight, and even the albumoses, judging by their sulphur-content, possess at least a molecular weight of 2000, while that of gluto-kyrin, a peptone, is at least 545.

The heat of combustion has been determined for a number of albumins by Stohmann and Langbein.[7] They found amongst others for 1 grm. serum-albumin, 5917·8 cal.; for hæmoglobin, 5885·1 cal.; for egg-albumin, 5735·2 cal.; for casein, 5867 cal.; and for glutin, 500 to 700 cal. less.

The true albumins, the albumoses, and the peptones are in watery solutions lævo-rotatory, each albumin possessing its own rotatory power, and therefore Frédéricq,[8] Kühne,[9] and others have made the attempt to make use of the rotatory power for the characterisation of the in-

[1] G. Grübler, *Journ. f. prakt. Chem.* [2] **23**. 97 (1881).
[2] E. Laqueur and O. Sackur, *Hofmeister's Beiträge*, **3**. 193 (1902).
[3] E. Harnack, *Zeitschr. f. physiol. Chem.* **5**. 178 (1881).
[4] B. Werigo, *Pflüger's Arch. f. d. ges. Phys.* **48**. 127 (1891).
[5] A. Sabanajew and N. Alexandrow, *Journ. of the Russian Phys.-Chem. Society*, 1891, p. 7; see *Maly's Jahresber. f. Tierchemie*, **21**. 11 (1891).
[6] E. H. Starling, 'Glomerular Function,' *Journ. of Physiol.* **24**. 257 (1899).
[7] F. Stohmann and H. Langbein, *Journ. f. prakt. Chem.* [2] **44**. 336 (1891).
[8] L. Frédéricq, *Arch. de Biol.* **1**. 457 (1880); II. p. 379 (1881).
[9] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **20**. 11 (1884).

dividual albumins. The albumins possess, however, the same property as do the amino-acids, namely, that of rotating the light to a different extent according as whether they are free or in salt-like combination; and a further difficulty is that the salts of the albumins undergo hydrolytic dissociation, and therefore show different rotations according to the concentration of the solution and the nature of the acid or basic radical with which they are combined.[1] As albumins cannot be investigated in strong acids or in alkalies because of their decomposition, only those numbers are available which have been obtained with really pure albumins in perfectly neutral solutions, and such determinations are few.

Some proteids, *e.g.* hæmoglobin and the nucleo-proteids, are dextro-rotatory, as has been discovered by Gamgee,[2] who also points out various peculiarities of hæmoglobin.

3. Osmotic Pressure

The question of osmotic pressure has been discussed in a very interesting way by Moore and Parker,[3] who find that a definite osmotic pressure is exerted by colloids in solution. They point out that the albumin-molecule may be considered as built up of a number of smaller molecules, each of which has a comparatively simple structure. These chemically simple molecules, by aggregating, form the physically-complex albumin-molecule, which the authors prefer to call a 'solution-aggregate.' The rise of these aggregates varies within wide limits according to the temperature and chemical reaction of the solution, and as to whether electrolytes are present or absent. The weight of the solution-aggregate is from four to five times greater in serum-albumin than in egg-albumin. In the case of serum-albumin it becomes reduced approximately to one-fifth its value by alkalisation. "Protoplasm may be built up by a continuation of such a process of aggregation; absorption of materials by the cell may be governed by the formation of varying aggregations with the protoplasm already built up in the cell, and similarly granule formation in the cell may be produced.'

Moore and Parker have discussed the investigations of Sebanejew,[4]

[1] K. Bülow, *Pflüger's Arch. f. d. ges. Phys.* **58**. 207 (1894); F. Framm, *ibid.* **68**. 144 (1897).

[2] A. Gamgee and Croft Hill, *Ber. d. deutsch. chem. Ges.* **36**. I. 913 (1903); A. Gamgee and W. Jones, *ibid.* **36**. I. 914 (1903).

[3] B. Moore and W. H. Parker, *Amer. Journ. of Physiol.* **7**. 261 (1902).

[4] Sebanejew, *Ber. d. deutsch. chem. Ges.* **23**. 87 (1890); **24**. 558 (1891).

Tamman,[1] Ludeking,[2] Dreser,[3] Koeppe,[4] Krafft and Wiglow,[5] Starling,[6] Martin,[7] and Weymouth Reid.[8]

In a more recent paper Weymouth Reid[9] has come to the conclusion that by washing salted-out or crystallised albumins, solutions are obtained giving no osmotic pressure. The same conclusion the author arrived at previously in his *Physiological Histology*, in which he gave the reasons why salt-free albumins cannot exert any osmotic pressure, for albumins in their natural state are chemically inert, as they do not even react with aniline dyes. They are, in short, in the pseudo-acid pseudo-basic state, forming ring-compounds (see this book, p. 219). If albumins contain any radical which leads to their dissociation, which makes them chemically active, they do possess a definite osmotic pressure, as is instanced by hæmoglobin solutions, which Weymouth Reid[10] has found to show no "ultra-microscopic" structure, and thus to differ from serum and egg-albumin, and also to exert a pressure: Taking the molecular weight of hæmoglobin as 16,669 (see p. 328), a one per cent solution, assuming that no dissociation occurs, gives a pressure of about 10·77 mm. of mercury at 15° C.

4. Precipitine Reactions

Bordet,[11] Wassermann,[12] Myers,[13] and others have found that albumins on being introduced into the blood give rise to specific precipitates as do other colloids, and Ascoli,[14] Umber,[15] Michaelis and Oppenheimer,[16] Schütze,[17] Hamburger,[18] v. Dungern,[19] and others have

[1] Tamman, *Zeitschr. f. physik. Chem.* **20**. 180 (1896).

[2] Ludeking, *Ann. d. Phys. u. Chem.* **35**. 552 (1888).

[3] Dreser, *Arch. f. experimen. Pathol. u. Pharmak.* **29**. 314 (1892).

[4] Koeppe, *Pflüger's Arch.* **42**. 571 (1896).

[5] F. Kraft and H. Wiglow, *Ber. d. deutsch. chem. Ges.* **28**. 2566 (1895).

[6] Starling, *Science Progress*, April 1896; *Journ. of Physiol.* **19**. 312 (1896); *ibid.* **24**. 317 (1899); *Schäfer's Textbook of Physiol.* **1**. p. 307.

[7] Martin, *Journ. of Physiol.* **20**. 317 (1896); *ibid.* p. 364.

[8] Weymouth Reid, *ibid.* **27**. 161 (1901).

[9] E. Weymouth Reid, *ibid.* **31**. 438 (1904).

[10] *Idem*, *ibid.* **33**. 12 (1905).

[11] Bordet, *Ann. de l'Institut Pasteur*, 1899, p. 232.

[12] Wassermann, *Kongress f. innere Medizin*, 1900, p. 501; *Münchener medizin. Wochenschr.* 1900, II. p. 986.

[13] W. Myers, *Zentralbl. f. Bakteriol.* **28**. 237 (1900).

[14] M. Ascoli, *Münchener medizin. Wochenschr.* 1902, I. p. 398; 1903, Nr. 5.

[15] F. Umber, *Berliner klin. Wochenschr.* 1902, Nr. 28.

[16] L. Michaelis and C. Oppenheimer, *Arch. f. (Anat. u.) Physiol.* 1902, Suppl. p. 336 (here also the older literature); L. Michaelis, *Deutsche medizin. Wochenschr.* 1902, Nr. 41.

[17] A. Schütze, *ibid.* 1903, Nr. 45.

[18] F. Hamburger, *Wiener klin. Wochenschr.* 1901, Nr. 49; 1902, Nr. 45.

[19] E. v. Dungern, *Die Antikörper*, Jena, Fischer, 1902.

made the attempt to employ specific reactions for the separation and identification of definite albumins. But this specific reaction is a very limited one according to Nolf,[1] Umber,[2] Rostoski,[3] Schütze,[4] Michaelis and Oppenheimer,[5] Obermayer and Pick,[6] Hamburger,[7] Landsteiner and Calvo,[8] Linossier and Lemoine,[9] Kluck and Inada.[10]

It is fairly well marked in the case of the albumins of different animals, and therefore it is possible to distinguish between the serum-albumins of man, ox, and rabbit by means of the 'biological reaction.' Closely related animals show much less reaction, and therefore it is easy to show that monkeys are related to us (Nuttall).[11]

After Umber had immunised rabbits against egg-albumin and egg-globulin, both these substances were precipitated by the anti-globulin-serum, while the anti-albumin-serum failed to precipitate any albumin; Rostoski failed to distinguish with certainty between the serum- and the egg-albumin of hens' eggs. Whether the discrepancy is due to a multiplicity of precipitins, as Ascoli believes in support of Ehrlich's view, or whether the reaction is not due to albuminous substances at all, but to bodies of unknown constitution, as held by Obermayer and Pick, is as yet a moot point, and therefore the precipitin-reaction is as yet of but subordinate interest as far as the chemistry of the albumin-molecule is concerned. It is remarkable that, according to Oppenheimer and Michaelis, the precipitin-formation ceases during either tryptic- or peptic-digestion simultaneously with the disappearance of the last trace of colloidal albumin.

Hunter[12] has recently very carefully reinvestigated this whole question, and the reader's attention is specially drawn to his article. Hunter states that 'albumin, euglobulin, and pseudo-globulin of ox-serum are each capable of leading to the formation of precipitins, and that these precipitins are in a limited degree specific. The precipitins are mixtures of at least four distinct antibodies, of which

[1] Nolf, *Annales de l'Inst. Pasteur*, 1900, p. 297.

[2] F. Umber, *Berliner klin. Wochenschr.* 1902, Nr. 28.

[3] Rostoski, *Münchener medizin. Wochenschr.* 1902, p. 740; and *Verhandl. d. physik. med. Ges. Würzburg*, **35**. (1902).

[4] A. Schütze, *Deutsche medizin. Wochenschr.* 1903, Nr. 45.

[5] L. Michaelis and C. Oppenheimer, *Arch. f. (Anat. u.) Physiol.* 1902, Suppl. p. 336 (here also the older literature); L. Michaelis, *Deutsche medizin. Wochenschr.* 1902, Nr. 41.

[6] Obermayer and Pick, *Wiener klin. Rundschau*, 1902, Nr. 15.

[7] F. Hamburger, *Wiener klin. Wochenschr.* 1901, Nr. 49; 1902, Nr. 45.

[8] Landsteiner and Calvo, *Centralbl. f. Bacteriol.* 1902, p. 781.

[9] Linossier and Lemoine, *Compt. Rend. de la Soc. de Biol.* **54**. Nos. 3, 9, 10, 11 (1902).

[10] H. Kluck and R. Inada, *Deutsch. Arch. f. klin. Med.* **81**. 410 (1904).

[11] Nuttall, *Blood Immunity and Relationship* (Cambridge, 1904).

[12] A. Hunter, *Journ. of Physiol.* **32**. 327 (1905).

albumin yields only one, while euglobulin and pseudo-globulin yield three each. The serum of animals which have been injected does not develop its full precipitating power till five days after the injection (Nuttall, Hunter), and the amount of precipitin in the serum is always in inverse ratio to the number of leucocytes. It is suggested that the precipitins are formed in the leucocytes, and Kraus and Levaditi [1] are likewise of the opinion that leucocytes give rise to the precipitins.

Meyer [2] has found that extracts from mummy-muscles, 2000-5000 years old, gave distinct precipitates with the serum of rabbits, which had been injected with fluid from human pleural transudations, placental blood, or ascitic fluid, while no precipitates were formed in the control experiments.

Merkel [3] found that rabbits which had been injected with human blood, on becoming pregnant, passed on the precipitins to their offspring, and that therefore the young rabbits reacted in the same way as did their mothers.

5. The Gold Number of Albumins

Zsigmondy having found that a colloidal gold-solution is precipitated by electrolytes, *e.g.* by NaCl, but that this precipitation is influenced by the presence of other colloids in a manner which is quite characteristic for each colloid, this question was more fully investigated by Schulz and Zsigmondy.[4]

They designated that number of milligrammes of a colloid which is just insufficient to prevent 10 ccm. of a colloidal gold-solution from showing a change in colour, after the addition of 1 ccm. of a 10 per cent NaCl solution, the gold number of the colloid in question. Schulz and Zsigmondy have determined these gold numbers for the albumins of egg-white :—

Globulin	0·02–0·05.
Ovomucoid	0·04–0·08.
Crystalline egg-albumin . .	2–8.
Other (con-)albumin . . .	0·03–0·05.
Alkali-albuminate . . .	0·006–0·04.

The great difference between the crystalline egg-albumin and the other colloids of egg-white makes it possible to recognise very minute traces of impurity in the egg-albumin with greater precision than by any other method. This method has shown that egg-albumin must be

[1] Kraus and Levaditi, *Compt. Rend.* **138**. 865 (1904).
[2] J. Meyer, *Münchener med. Wochensch.* 1904, p. 663.
[3] H. Merkel, *ibid.* **51**. No. 8 (1904).
[4] F. N. Schulz and R. Zsigmondy, *Hofmeister's Beiträge*, **3**. 137 (1902).

recrystallised three to six times to remove all impurities. If albuminous substances are denaturalised by means of alkali, then all previously existing differences disappear; all alkali-albuminates have the same low gold number.

6. Power of forming Emulsions

Owing to their colloidal[1] nature, albumins possess the power of keeping insoluble substances in solution. In this way lecithin and calcium-soaps are kept in solution in serum and calcium-phosphate in milk. According to Paal,[2] albuminates have further the power of dissolving metallic oxides. In this connection may also be mentioned the older observations of Schadee van der Does,[3] who described how solutions of egg- and serum-albumin may be rendered uncoagulable by being shaken up with freshly prepared metallic silver in fine subdivision, or freshly prepared or not too old silver oxide. The author[4] explains this fact by assuming the silver oxide to act similarly to osmium tetroxide, after the addition of which egg-albumin does not coagulate on heating (Bethe and Mönckeberg).[5] In the case of osmium, the OsO_4 is probably changed into $Os(OH)_4$, which replaces the displaceable hydrogen-atoms of the albumin-molecule. The author arrived at his interpretation by assuming that during heat-coagulation a potential hydrogen-ion is liberated, and that this H-atom in the albumin-molecule is oxidised by OsO_4. That such a replaceable H-atom really does exist has been shown since by the researches of Heffter[6] (see p. 97). The author's explanation is thus a chemical one, and shows that the solution of metallic oxides does not depend on the 'colloidal' nature of albumins as defined by Cohnheim.

It is quite different, however, with mixtures of two or more colloids; for one colloid being impermeable to and by another colloid it is easy to understand why albumins and albumoses remain in solution in albuminous fluids, although they are insoluble in non-albuminous fluids, for each colloid interferes mechanically with the precipitation of the other colloid (Mann). This very property makes it so difficult to isolate the albumins. We must, however, always keep in mind the fact that both salts and other radicals of the chemically active albumins play a part in this process. Related to these phenomena is, further, the power possessed by casein and other

[1] 'Colloidal' includes definite chemical action, as explained on pp. 259 and 268.

[2] C. Paal, *Ber. d. deutsch. chem. Ges.* **35**. II. 2206 (1902).

[3] S. v. d. Does, *Zeitschr. f. Physiol. Chem.* **24**. 351 (1897).

[4] Mann, *Physiological Histology*, 1902, p. 67.

[5] Bethe and Mönckeberg, *Arch. f. mikr. Anat.* **54**. 135 (1899).

[6] A. Heffter and Max Hausmann, *Hofmeister's Beiträge*, **5**. 213 (1904).

albumins of forming permanent emulsions with finely divided fat-droplets: on precipitating the casein the whole of the emulsified fat also separates out. An explanation of the formation of the haptogen-membranes has been given by Jamison and Hertz, who worked under Ramsden.[1] They showed that the film or skin on milk is not peculiar to caseinogen or lactalbumin, as similar films are produced on warming any albuminous solution containing emulsified fat or paraffin. In the case of non-coagulable albumins at any temperature, and in the case of heat-coagulable albumins at a temperature beneath that of heat-coagulation, the film is probably formed of unchanged dry albumin. If a coagulable albumin is coagulated by heat, then the film is composed, at any rate partly, of coagulated albumin. Drying is therefore one essential condition for the formation of a film, which latter, in addition to dried albumin, contains also, entangled in the film, globules of fat or of paraffin.

7. Power of clarifying Solutions

The very opposite of the phenomena described in the previous paragraph is the following one:—When albuminous substances are either precipitated, or when they otherwise come into contact with substances which are in solution along with themselves, they carry these other substances down with them by enclosing them, or condensing them on their own surfaces by surface attraction. To this special power used to be attributed the precipitation of ferments, but Jacoby[2] has shown that ferments, as regards precipitation, are subject to the same conditions as are albumins, and that surface attraction only comes into play when ferments are precipitated on fibrin. The dye-stuffs, salts, etc., which albumin-crystals imbibe, according to Wichmann,[3] "like a sponge," are certainly held by chemical forces (Mann);[4] and the same holds good for the ash constituents (Kossel[5] and Harnack[6]), which, so far, it has been impossible to completely remove from albumin. Even the most "ash-free" albumin of Hofmeister[7] contained traces of inorganic constituents, and it is nothing uncommon for pure albumin to contain 0·5–1 per cent of ash. Compare also the views of Ramsden on mechanical conglutination, p. 274.

[1] R. Jamison and A. F. Hertz, *Journal of Physiol.* **27**. 26 (1901).

[2] M. Jacoby, *Zeitschr. f. physiol. Chem.* **30**. 135 (1900); *Arch. für experiment. Path. u. Pharmak.* **46**. 28 (1901).

[3] A. Wichmann, *Zeitschr. f. physiol. Chem.* **27**. 95 (1899).

[4] Mann, *Physiological Histology*, 1902, pp. 289, etc.

[5] A. Kossel, *Zeitschr. f. physiol. Chem.* **3**. 58 (1879).

[6] E. Harnack, *ibid.* **19**. 299 (1894).

[7] F. Hofmeister, *ibid.* **16**. 187 (1891).

Dissociation of Albumin by Means of Acids and Alkalies

Acid-Albumins and Alkali-Albuminates

The dissociation of albumin by means of alkalies and acids is closely related to coagulation, as the albumin is changed primarily into an alkali-albuminate or into an acid-albumin, but sooner or later there are also formed albumoses and peptones. Goldschmidt,[1] Zunz,[2] and others have taken the possibility into consideration, that the acid-albumin-moiety corresponds only to one part of the albumin-molecule, and that during its formation the albumose-complexes are separated off. This view is, however, not supported by the fact that the whole of the albumin is converted into acid-albumin on being boiled for a short time with acids (Erb[3]); a second objection is that the ratio of acid-albumin to albumose varies greatly. Acid-albumin is, without doubt, the primary transformation-product of the entire albumin-molecule, while the other products are only formed secondarily, after the lapse of more or less time.

As already stated on p. 148, so here again we meet with a difference between the readily dissociable hemi-group and the resistant anti-group, and we find that acid-albumin is identical with the anti-albumid coagulum, with plastein, etc. The acid-albumin of the whole albumin-molecule, and the acid-albumin prepared from the anti-group, agree with one another in every particular as regards such external characters as solubility and precipitability, although they differ from one another in their chemical configuration.

Acid-albumin is formed instantly, whenever a solution of an albumin is heated to its coagulation-temperature in the presence of even the minutest trace of an acid. At room-temperature or at body-temperature this change requires, however, much more time, and also a much more concentrated acid; in this respect different albumins behave very differently: serum-albumin has been investigated by Johannsson;[4] serum- and egg-albumin by Goldschmidt; the muscle-albumins by Kühne,[5] and by v. Fürth.[6] The myogen of v. Fürth is so readily converted into acid-albumin—by one drop of a $\frac{1}{10}$ normal

[1] F. Goldschmidt, *Säuren und Eiweiss*, Dissertation, Strassburg, 1898.
[2] E. Zunz, *Zeitschr. f. physiol. Chem.* **28.** 132 (1899).
[3] W. Erb, *Zeitschr. f. Biol.* **41.** 309 (1901).
[4] J. E. Johannsson, *Zeitschr. f. physiol. Chem.* **9.** 310 (1885).
[5] W. Kühne, *Protoplasma und Kontraktilität*, Leipzig, 1864.
[6] O. v. Fürth, *Arch. f. experiment. Pathol. u. Pharmakol.* **36.** 231 (1895).

HCl in a few minutes—that its coagulation by heat is not an easy matter. This acid-albumin prepared from muscle has received the special name 'syntonin,' but by some workers the term syntonin is also used, without exception, for all acid-albumins. Egg-albumin is much more difficult to convert into acid-albumin than is muscle-albumin, and yet, according to Goldschmidt,[1] $\frac{1}{8}$ normal HCl will form in one hour a demonstrable amount of acid-albumin, and even a weaker acid may perhaps do so. Serum-albumin is still more resistant to the action of acids: 0·25 per cent HCl and 2 per cent acetic acid have no action whatever at room-temperature, while at 40° acid-albumin is formed to a slight extent in 14 days; even 2 per cent HCl converts serum-albumin only very slowly at room-temperature. In all these experiments we have to remember, as was pointed out by Danilewsky,[2] that native albumin is a base, and that in neutralising a part of the acid it makes the latter to that extent ineffective. Many of the differences which have been described as existing between certain albumins and certain strengths of acid may be explained in this way.

The transformation of albumin into acid-albumin is enormously hastened, especially at body-temperature, if the acid is assisted by the ferment pepsin. Under these conditions acid-albumin is not only formed much more quickly, but it is also very quickly transformed into albumoses, peptones, and peptids. According to Umber,[3] the relative resistance of different albumins to pepsin + hydrochloric acid is quite different from that shown to hydrochloric acid alone; but in this case anti-ferments play perhaps a part (Cohnheim). The author holds with Umber that the pepsin attacks the albumin-molecule in quite a different way than does the acid. (See also index.)

The natural albumins are changed by alkalies even more readily than by acids, in consequence of which, as a rule, alkali-albuminates are formed more quickly, at a lower temperature, and with feebler concentrations of alkalies than are the corresponding acid-albumins. Alkali-albuminates are formed at once, if albumins are heated to their coagulation-temperature, but even at room-temperature the greater part of serum-albumin is converted into alkali-albuminate in $2\frac{1}{2}$ hours if it is acted upon by 0·2 per cent sodium hydrate, according to Johannsson. A 2 per cent NaOH solution quickly disintegrates serum-albumins in a remarkable way, according to Maas.[4] According to Zoth[5] and Dieudonné,[6] serum-albumin becomes partially converted

[1] F. Goldschmidt, *Säuren und Eiweiss.* Dissertation, Strassburg, 1898.
[2] A. Danilewsky, *Zeitschr. f. physiol. Chem.* **5.** 158 (1881).
[3] F. Umber, *ibid.* **25.** 258 (1898). [4] O. Maas, *ibid.* **30.** 61 (1900).
[5] O. Zoth, *Sitzungsber. der Wiener Akad., Math.-nat. Kl.*, Abteil. III. **100.** 140 (1891).
[6] Dieudonné, *Verh. d. Phys.-med. Ges. zu Würzb. Münch. med. Wochens.* 1903, p. 43.

into alkali-albuminate on being heated for some hours to 40° in a feebly alkaline solution. No other investigations into this question have been made, but the statements of Bernert[1] regarding the oxidation of albumin by means of permanganate and caustic alkalies, as well as Hammarsten's investigations into the acid-albumins: mucin, globulin, fibrinogen, casein, which, as a rule, were dissolved in dilute alkalies, in alkali-carbonates, or in ammonia, all show clearly that albumin is denaturalised with great rapidity when it is brought into contact with alkalies.

This denaturalisation can readily be proved, for the alkali-albuminates differ chemically from the albumins from which they are derived. Nasse[2] and Schmiedeberg[3] found alkali-albuminates poorer in nitrogen than the native albumins, and for this reason Schmiedeberg introduced the term 'desamido-albuminic acid.' Nasse has found acid-albumins to be also poorer in nitrogen. The ultimate dissociation-products resulting from the action of acids and alkalies have been discussed on pp. 90-96.

As already pointed out, the formation of acid-albumins and of alkali-albuminates in the absence of salts is accompanied by no visible change, while in the presence of small amounts of salt a precipitate is formed. Quite different is the behaviour of concentrated albumins when they are brought into contact with very strong acids or alkalies, for more or less stiff jellies are formed, which may show all transitions between glass-like transparency and milk-white opacity. To these jellies were originally restricted the terms 'acid-albumins' and 'alkali-albuminates.'

The first to observe this jelly formation or gelation was Rose[4] in 1833, who prepared an albumin-ferric-chloride jelly. Magendie,[5] Lieberkühn,[6] Johnson[7] were other early observers; while subsequently this question was investigated by Rollet[8] and his pupil Zoth;[9] by Kieseritsky[10] and Rosenberg[11] under the direction of Alexander Schmidt; by Neumeister and the author. Whether a jelly is formed

[1] R. Bernert, *Zeitschr. f. physiol. Chem.* **26.** 272 (1898).

[2] O. Nasse, *Pflüger's Arch. für die gesammte Phys.* **7.** 139 (1872).

[3] O. Schmiedeberg, *Arch. f. experiment. Path. u. Pharmak.* **39.** 1 (1897).

[4] Ferdinand Rose, *Poggendorff's Ann.* **28.** 140 (1833).

[5] Magendie, *Leçons sur le sang*, Paris, 1836, p. 170 (according to Rollet).

[6] N. Lieberkühn, *Arch. f. Anat., Physiol. u. wissenschaftl. Med.* 1884, pp. 285, 323.

[7] Johnson, *Journ. of the Chemical Soc.* N.S. **12.** 734 (according to Rollet).

[8] A. Rollet, *Sitzungsber. d. Wien. Akad., Math.-naturw. Kl.*, Abteil. III. **84.** 332 (1881).

[9] O. Zoth, *ibid.* **100.** 140 (1891).

[10] W. Kieseritsky, *Die Gerinnung des Faserstoffes, Alkalialbuminats und Acid-albumins.* Dissertation, Dorpat, 1882.

[11] A. Rosenberg, Dissertat., Dorpat, 1883.

or is not, and whether the jelly is transparent or is opaque, depends on the concentration of the acid or alkali, and on the amount of inorganic neutral salts present, as has been very thoroughly investigated, after Rose, by Rollet, and Zoth, and Rosenberg, and Kieseritsky.

Generally speaking, acids require to be in much greater concentration than alkalies to lead to the formation of a jelly. Pure glacial acetic acid, which is neutral to litmus paper, acts on pure egg-white as a dehydrating agent according to the author,[1] who finds, on mixing 1 ccm. of egg-white of newly laid summer eggs with 10 ccm. of glacial acetic acid, that there are thrown down large membranes of a white translucent colour, which do not change their appearance even if kept for months; the albumin is hereby so completely precipitated that no opalescence is seen in the mother-liquor; 1 ccm. of egg-white with 10 ccm. of a 25 per cent glacial acetic acid shows a few ill-defined flocculi, while with 50 per cent acid a large number of minute membranes and transparent flocculi float about in a jelly-like mother-liquor, which latter exhibits a slight opalescence.

While, therefore, a jelly-like acid-albumin requires for its formation a strong solution of acetic acid, a jelly-like alkali-albuminate may be formed occasionally quite spontaneously, according to Zoth. If, *e.g.*, serum-albumin is allowed to stand, the amount of alkali which is normally present in the blood-serum may suffice to convert it into a jelly. On increasing the amount of alkali, the fluid solidifies more quickly, and becomes also more transparent, although less firm; a still larger amount of alkali may prevent the solidification altogether. Analogously, gelatinisation of an albumin may be prevented by too high a concentration of an acid, as the latter acts either as a dehydrant, as in the case of acetic acid (Mann, see above), or as a coagulant, as in the case of mineral acids. Organic bases, cholin, and even urea, if in sufficient concentration, also give rise to brawny jellies, according to Spiro[2] and Ramsden.[3]

Kieseritsky and Rosenberg have specially studied the influence of salts, and have found that no jelly is formed if by very prolonged dialysis all salts are removed. They have also shown that the time in which a jelly is formed and the firmness of the jelly are greatly influenced by the presence of salts. These facts lead them to draw an interesting parallel between the coagulation of acid-albumins and alkali-albuminates and the precipitation of colloidal silicic acid in the

[1] Gustav Mann, *Physiological Histology*, 1902, p. 105.

[2] K. Spiro, *Zeitschr. f. physiol. Chem.* **30.** 182 (1900).

[3] W. Ramsden, *Journ. of Physiol. Proceedings*, 28, xxiii. (1902).

presence of salt, which occurs slowly in the cold, and quickly on heating. The smaller the amount of salt which is present, the more transparent, but also the less firm, is the jelly, and *vice versa*. Acid-albumin requires for its gelation less salt than does an alkali-albuminate. Warmth hastens and augments jelly-formation enormously.

A hard-boiled egg is a good example of the formation of a jelly-like alkali-albuminate, for egg-white is a concentrated alkaline albumin-solution. In the hen's egg, and in the eggs of birds which leave their nests as soon as they are hatched, the alkali-albuminate becomes white and opaque on boiling, while in the eggs of all birds which remain for a considerable time in their nests, as is the case with the crow, the swallow, and the lapwing, the egg-white solidifies on boiling into a jelly as clear as glass, as has been pointed out by Lieberkühn, Tarchanoff,[1]—who introduced the term Tata-albumin, in memory of a little Russian girl Tata, who observed that swallows' eggs remained clear on boiling,—and Helbig.[2] This phenomenon depends on the different amounts of salt and of alkali which are present in the egg-white. The white of hens' eggs may also be made to solidify into a clear jelly by placing the eggs for two to three days into 10 per cent KOH solution (Tarchanoff[1] and Zoth[3]). Another application of the transparent, jellified alkali-albuminate is that which Koch[4] has introduced into bacteriology, for blood-serum solidifies to a fairly transparent jelly on being heated for some time to 65°; by altering the concentration of the serum, and the time we take to coagulate the serum, it is possible to somewhat influence the mode of coagulation.[1]

Other Methods of Denaturalisation

Many other causes, in addition to acids and alkalies, will change the normally occurring, colloidal albumin into a denaturalised state.

Dry Heat.—Pure albumin crystals, according to Wichmann,[5] may be heated to 150°, but on being heated for some time in their insoluble state, they become less readily digestible by pepsin and by trypsin, according to Smith,[6] Strohmer,[7] and Rotarski;[8] Laqueur and Sackur,[9]

[1] J. Tarchanoff, *Pflüger's Arch. f. d. ges. Physiol.* **33.** 303 (1884); **39.** 476 and 489 (1886).

[2] E. Helbig, *Arch. f. Hygiene*, **8.** 475 (1888).

[3] O. Zoth, *Sitzungsber. d. Wiener Akad., Math.-naturw. Kl.* III. **100.** 140 (1891).

[4] R. Koch, *Mitteil. a. d. Kaiserlichen Gesundheitsamte*, **2.** 48 (1884).

[5] A. Wichmann, *Zeitschr. f. physiol. Chem.* **27.** 575 (1899).

[6] H. Smith, *Zeitschr. f. Biol.* **19.** 469 (1883).

[7] F. Strohmer, *Chem. Zentralbl.* 1902, II. 971.

[8] T. Rotarski, *Zeitschr. f. physiol. Chem.* **38.** 552 (1903).

[9] E. Laqueur and O. Sackur, *Hofmeister's Beiträge*, **3.** 193 (1902).

on drying casein at 100°, observed that it is disintegrated into phosphoric acid and at least two albuminous substances which differed from one another and from the original casein. It is uncertain as to whether this great susceptibility is due to want of purity or due to the peculiar constitution of casein.

Alcohol.—Salt-free albumin is precipitated by alcohol only with difficulty; and such alcohol-precipitated albumin is at first still soluble, but on the addition of a trace of salt the precipitate becomes very abundant, and this precipitate quickly loses its colloidal nature (Aronstein,[1] Harnack,[2] Alexander Schmidt,[3] Kühne,[4] v. Fürth,[5] and Spiro[6]). According to Kühne and Chittenden,[7] albumoses and peptones are also precipitated much more completely if salts are present, but in this case a possible error may have crept in owing to the presence of alcohol-soluble acid-albumins.

Lilienfeld[8] in 1893 observed that albumin fixed in absolute alcohol has no affinity for either acid or basic dyes. Mathews[9] has also pointed out that egg-white, coagulated by heat or by alcohol, does not stain in neutral solutions of either colour-acids or colour-bases. "It is true it will imbibe a certain amount of colour and will appear stained, but this colour is easily and quickly removed by washing in water." . . . "A most striking contrast is shown by two pieces of coagulated albumin, one of which has been immersed in a neutral, the other in an acid solution of acid fuchsin. After washing, the former will be found to be colourless, the latter a brilliant red." . . . "If two pieces of (alcohol-) coagulated egg-albumin be brought, the one into slightly acid and the other into alkaline solutions of thionin, the stain poured off after a few seconds, and the albumin washed in water, the piece that has been in the alkaline solution will be an intense purple, the other barely tinged with colour." . . . "These reactions clearly indicate that the staining of coagulated albumin depends on chemical combinations similar in all respects to those which the albumoses enter into with the same stains. In neutral solution, neutral coagulated albumin combines neither with acid nor with basic stains; in alkaline solutions it combines only with the basic; in acid solutions, only with

[1] B. Aronstein, *Pflüger's Arch. f. d. ges. Physiol.* **8.** 75 (1874).

[2] E. Harnack, *Ber. d. deutsch. chem. Ges.* **22.** VI. 3046 (1889).

[3] Alexander Schmidt, *Zur Blutlehre*, Leipzig, 1892; *Weitere Beiträge zur Blutlehre* Wiesbaden, 1895.

[4] W. Kühne, *Zeitschr. f. Biol.* **29.** 1 (1892).

[5] O. v. Fürth, *Arch. f. experiment. Pathol. u. Pharmakol.* **36.** 231 (1895).

[6] K. Spiro, *Hofmeister's Beiträge*, **4.** 300 (1903).

[7] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **19.** 188 (1883).

[8] Lilienfeld, *Arch. f. Anat. u. Physiol.* 1893, p. 391, and in *Zeitschr. f. physiol. Chem.* **18.** 473 (1894).

[9] A. Mathews, *Amer. Journ. of Physiol.* **1.** 445 (1898).

the acid stains." Albumin fixed in acetic acid alcohol combines at once with the acid dyes in neutral solutions, probably because the acetic acid radical, which had united with the albumin-molecule at the time of fixation, becomes now replaced by the colour-acid of the staining fluid (Mann).[1]

It is of great interest that alcohol is able to preserve the pseudo-acid—pseudo-basic nature of the amphoteric albumin-molecule. See remarks of the author on p. 219.

O. v. Fürth[2] has noticed that different, alcohol-coagulated albumins become denaturalised with different rapidities; thus myosin (paramyosinogen of Halliburton) and ovalbumin are denaturalised more quickly than are myogen (myosinogen of Halliburton) and serum-albumin.

St. Hilaire[3] has further pointed out that nucleo-histones are decomposed by alcohol.[4]

Aceton behaves similarly to alcohol (Mann,[5] Spiro). The coagulum is at first soluble, but then becomes denaturalised secondarily.

Alkaloidal Reagents and Dyes.—When precipitated with phosphotungstic acid or with aniline dyes according to the methods of Mathews and Heidenhain (see p. 225), albumin remains for a time soluble, but ultimately becomes coagulated (Cohnheim).

Salts of Heavy Metals.—Whether precipitation with the salts of the heavy metals produces denaturalisation at once is not known, but that denaturalisation supervenes later has been shown by Bülow[6] and Werigo.[7] (See pp. 303-315.)

Silver Oxide.—Schadee van der Does[8] has described how solutions of egg- and serum-albumin may be rendered uncoagulable by being shaken up with freshly prepared metallic silver, or freshly prepared or not too old silver oxide. Silver chloride and silver sulphide do not act in this way. It is suggested by him that the silver may possibly replace the sulphur of the albumin-molecule. The author[9] suggests that silver in the metallic state, especially when in fine subdivision, being slightly oxidised, must act in the same way as does the

[1] Mann, *Physiological Histology*, 1902, p. 351.

[2] O. v. Fürth, *Arch. f. experiment. Pathol. u. Pharmakol.* **36.** 231 (1895).

[3] Constantin Saint-Hilaire, *Zeitschr. f. physiol. Chem.* **26.** 102 (1898).

[4] See Mann's *Physiological Histology*, p. 320.

[5] Mann, *ibid.* 1902, pp. 88, 104.

[6] K. Bülow, *Pflüger's Arch. f. d. gesammte Physiol.* **58.** 207 (1894).

[7] B. Werigo, *ibid.* **48.** 127 (1891).

[8] Schadee van der Does, *Zeitschr. f. physiol. Chem.* **24.** 351 (1897); also F. Bayer and Co., 'Patentschrift,' *Chem. Zentralblatt*, 1900, I. 524, and 1901, I. 652.

[9] Mann, *Physiological Histology*, 1902, p. 67.

oxide Ag_2O. This compound, which is soluble to the extent of 1 : 3000 parts of water, imparts to the latter an alkaline reaction, owing to the formation of a hydrate which undergoes hydrolysis. The author holds that the hydroxyl-ions, by uniting with potential hydrogen-ions in the albumin-molecule, render the hydrogen-ions inert, and thus prevent the coagulation by heat (see p. 318). Cohnheim believes silver oxide to produce in the albumin-molecule a change analogous to that induced by formaldehyde, *i.e.* the formation of methylene compounds (see p. 250).

Osmium Tetroxide.—The author[1] discussed the oxidising action of osmium tetroxide at the Anatomical Congress in Kiel in 1898, and to this discussion Mönckeberg and Bethe[2] refer in a paper, in which they point out that osmium tetroxide is not able to form salts, and that for this reason it does not cause coagulation, as do most other reagents. They state, that by acting as an oxidiser, osmium tetroxide becomes reduced to metallic osmium ; that white of egg treated with an equal volume of 2 per cent OsO_4 remains fluid on being boiled, and that it becomes unprecipitable by nitric acid, acetic acid, or alcohol, while it is coagulated by a mixture of sublimate and nitric acid. Osmium tetroxide, not being an electrolyte, has been used extensively by the author for histological purposes, as it causes no structural change,[3] and even prevents sublimate from producing in the cytoplasm the usual change brought about by electrolytes. The author's methods have been adopted by Apáthy and Bethe.[4]

Surface Action.—Hermann[5] was the first to show that certain albuminous substances, such as casein, nucleo-albumins, fibrinogen, the paramyosinogens of different tissues, etc., are precipitated owing to surface action, when burnt clay or animal charcoal is mixed with their solutions. The same cause is at work, and therefore also leads to precipitation, when milk is sucked through porcelain filters, for Zahn,[6] and subsequently Lehmann,[7] have shown that the albumin passes through the filter, while the casein becomes precipitated and is held back by the filter. Picton and Linder[8] have found similarly

[1] Mann, *Verhandl. d. Anat. Gesellsch. Kiel*, 1898, p. 39.

[2] A. Bethe and G. Mönckeberg, *Zeitschr. f. mikroskop. Anat.* **54.** 135 (1899).

[3] Mann, *Zeitschr. f. wiss. Mikr.* **9.** 481 (1894), and *Physiological Histology*, 1902, pp. 69, 82, 102, 107, 128.

[4] A. Bethe and G. Mönckeberg, *Zeitschr. f. mikroskop. Anat.* **54.** 135 (1899) ; A. Bethe, *Allgemeine Anat. u. Physiol. des Nervensystems*, Leipzig, 1903.

[5] L. Hermann, *Pflüger's Arch. f. d. ges. Physiol.* **26.** 442 (1881).

[6] F. W. Zahn, *ibid.* **2.** 598 (1870).

[7] W. Hempel, J. Lehmann's ' Milk Investigations,' *ibid.* **56.** 558 (1894).

[8] H. Picton and S. E. Linder, *Journ. of the Chem. Soc.* **61.** 148 (1892).

that haemoglobin does not pass through porous earthenware filters. The author explains this phenomenon as due to the same causes as those which lead to the mutual precipitation of two colloidal solutions of opposite sign.

The firm albuminous compounds, such as fibrin and the tissue-forming elements, react in the same way as do the soluble albumins; for when coagulated by heat, alcohol, metallic salts, and formaldehyde, they become denaturalised and lose their natural properties. The hardening and fixing of organs for histological purposes by means of the salts of the heavy metals, by acids, aldehydes, alcohols, and osmium tetroxide, depend on 'coagulation' being produced. There is, however, a great difference between the electrolytes, which cause structural changes, and the non-electrolytes, such as osmium tetroxide and formaldehyde, which preserve the minute structure of colloidal substances by forming additive compounds, as is fully discussed in the author's *Physiological Histology*.

Properties of Denaturalised Albumin

As soon as denaturalisation occurs, all albumins lose their specific solubilities, and they resemble one another in their denaturalised state in being insoluble in water and in neutral salt solutions, while they are soluble in alkalies and acids. The acid-albumins and alkali-albuminates resemble one another further in being much more soluble in dilute alcohol than are the native or natural albumins. Other properties, however, such as chemical composition, reactions, salt formation, etc., are not destroyed. As regards histological staining, it is very important that the coagulated tissue-constituents on entering into chemical combination with dye-stuffs are soluble to different extents, and that hydrolysis of these coagulated albumin + dye-compounds takes place quite analogously to that seen with non-coagulated albumin. In what respects coagulated albumin differs from natural albumin, particularly as regards the conversion of pseudo-acids and pseudo-bases into real acids and real bases, is fully discussed in the author's *Physiological Histology*. The molecular weight of denaturalised casein is, according to Laqueur and Sackur,[1] not essentially different from that of native casein.

The 'ash-free albumin' of Harnack[2] has been especially carefully

[1] E. Laqueur and O. Sackur, *Hofmeister's Beiträge*, **3.** 193 (1902).

[2] E. Harnack, *Zeitschr. f. physiol. Chem.* **5.** 198 (1881); also *Ber. d. deutsch. chem. Ges.* **22.** II. 3046 (1889); **23.** I. 40 (1890); **23.** II. 3745 (1890); **31.** II. 1938 (1898).

investigated. It is prepared by precipitating egg-albumin with salts of copper;[1] dissolving the copper-albuminate in alkalies; precipitating it by careful neutralisation, and repeating this process several times. In this way it is possible to obtain a compound containing very little ash. It is, according to Bülow[2] and Werigo[3] an acid-albumin, a chloride of denaturalised albumin. Harnack's albumin illustrates very well the properties of denaturalised albumin. It is soluble in acids and alkalies in the absence of salts, while in acid solutions it is precipitated at once by the addition of salts. Its equivalent numbers are referred to on p. 329. It gives all the reactions of albumin except the lead-sulphide reaction, notwithstanding the fact that it contains the full complement of sulphur. Harnack explains this phenomenon by assuming a partial oxidation, and he calls the ash-free albumin 'anoxidised.' Its heat of combustion is stated by Stohmann and Langbein[4] to be 180 calories lower than that of native albumin, but these figures are of little value considering that neither normal albumin nor ash-free albumin are quite pure bodies.

Other examples of denaturalised albumins are the halogen compounds, the oxyprotein and oxyprotsulphonic acid, the methylene- and ethylene-albumins, all of which it is impossible to coagulate (see index). The substances just mentioned resemble Harnack's albumin in not giving the lead-sulphide reaction at all, or only to a slight extent. What causes this change in the sulphur-radical is not known, but the change is not directly dependent on denaturalisation, for Schulz and others have made their ordinary sulphur determinations mostly on coagulated albumins.

[1] See p. 304 under copper albuminates.'

[2] K. Bülow, *Pflüger's Arch. f. d. gesamte Physiol.* **58.** 207 (1894).

[3] Br. Werigo, *ibid.* **48.** 127 (1891).

[4] F. Stohmann and H. Langbein, *Zeitschr. f. prakt. Chem.* (2) **44.** 336 (1891).

SPECIAL PART

Classification of Albumins

(By Cohnheim)

A rational classification of albumins should start with the simplest substances, the peptids and the peptones, and should then show how by progressive combination of these simple substances there are built up the albumoses and finally the albumins. This classification should be based on a qualitative and quantitative synthesis of amino-acids. Such a classification has indeed been attempted by Kossel,[1] who considers the nucleus of the albumin-molecule to be represented by the union of urea with a diamino-acid, as met with in arginin, and that to this nucleus the other diamino-acids, mono-amino-acids, etc., are linked on. According to this plan four distinct groups may be distinguished :—

1. The protamins, which are rich in arginin. They differ from one another in the amounts of other bases and of mono-amino-acids.
2. The histones, which still possess a relatively high percentage of arginin and of other bases.
3. Certain vegetable albumins very poor in arginin, and containing no lysin.[2]
4. All other albumins, containing all three hexone bases and the greater number of amino-acids. This group cannot as yet be subdivided any further. Compare p. 348.

A second classification originating with Kühne,[3] and worked out

[1] A. Kossel, *Ber. d. deutsch. chem. Ges.* **34.** III. 3214 (1901) ; *Bull. de la Soc. chimique de Paris*, 3rd Ser. T. 29, No. 14, 20th July 1903 ; *Sitzungsber. d. Marburger Ges. z. Bef. d. ges. Naturwiss.* 1900, p. 21.

[2] Kossel and F. Kutscher, *Zeitschr. f. physiol. Chem.* **31.** 165 (1900).

[3] W. Kühne, *Heidelberger Naturh.-medizin. Verein*, N. F. **1.** 236 (1876) ; Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **19.** 159 (1883) ; **22.** 423 (1885).

especially by Pick,[1] has received a very strong confirmation through the researches of E. Fischer and Abderhalden.[2] This classification assumes the existence of two distinct radicals in the albumin-molecule, namely, the hemi- and the anti-group, and has already been dealt with in full, pp. 148-154. It shows that casein and prot-albumose form the hemi-end of a chain, the anti-end of which is represented by gelatine and hetero-albumose. Globin and the Bence-Jones' albumin resemble casein, while edestin and serum-globulin approach gelatine in their constitution.

All other albumins cannot be classified according to this scheme, and other classifications, as, *e.g.*, according to the amount of glutaminic acid,[3] or cystin,[4] or tyrosin,[5] are arbitrary, and with our present knowledge would account for only a certain number of albumins.

It is to be regretted, but at present we have to be conservative, and must retain the old classification which is based on the solubilities and on the distribution of the different albumins, a classification which goes back to the days of Hoppe-Seyler[6] and Drechsel,[7] and which has also been adopted by Hammarsten.[8]

According to this classification, the naturally occurring, or native, genuine albuminous substances, or the albumins in the restricted sense, form the central group. They are characterised by possessing a colloidal character when in solution, and by becoming denaturalised under certain conditions. From these albumins are derived certain compounds possessing definite features, namely, the acid-albumins and the alkali-albuminates; the albumoses, peptones and peptids; the halogen-albumins, etc. A third group contains the proteids, which are compounds of an albumin with another radical, the so-called 'prosthetic group.'[9] The albuminous portion of the proteids, namely, the histones and the protamins, may be brought into line with the true albumins. A fourth group is represented by the 'albuminoids' or albumins which form the firm supporting structures in the animal body. The expression 'albuminoid' is not based on a chemical but on an anatomical foundation, and is therefore, from a chemical point of view, inadmissible.

[1] E. P. Pick, *Zeitschr. f. physiol. Chem.* **28.** 219 (1899).

[2] E. Fischer and E. Abderhalden, *ibid.* **39.** 81 (1903).

[3] F. Kutscher, *ibid.* **38.** 111 (1903).

[4] K. A. H. Mörner, *ibid.* **34.** 207 (1901).

[5] F. Reach, *Virchow's Archiv*, **158.** 288 (1899).

[6] F. Hoppe-Seyler, *Handbuch der physiologisch- und pathologisch-chemischen Analyse*, 6. Aufl. p. 243 (1893).

[7] E. Drechsel, article 'Eiweisskörper' in *Ladenburg's Handwörterbuch der Chemie*, **3.** 534 (1885).

[8] O. Hammarsten, *Text-book of Physiol. Chemistry*, 4. Aufl. 1899, p. 17.

[9] A. Kossel, *Arch. f.* (*Anat. u.*) *Physiol.* 1893, p. 157.

This classification into native albumins, dissociation-products, proteids, and albuminoids does not include, however, such bodies as the nucleo-albumins. When dealing with these later on, it will be shown that they have nothing whatever to do with the nucleo-proteids, with which they were formerly confounded, and from which they have received their name, except that they contain phosphorus. The nucleo-albumins form a special, physiologically and chemically well-defined class; it is immaterial whether we regard them as proteids or as phosphorus-containing albumins. The same holds good of the mucins and allied substances, which may be classified as albumins with a specially high amount of carbohydrate, or as glyco-proteids. Following Hammarsten, the nucleo-albumins have been described under the albumins, while the mucins and mucoids are counted amongst the proteids. It is best to drop the expression 'nucleo-albumin' altogether, as these substances have nothing to do with the nucleus, and to use instead the term "phospho-globulin," proposed by Cohnheim.

The former classification of native albumins into the water-soluble albumins and into the water-insoluble globulins, which latter are, however, soluble in acids, alkalies, and salts, seems to have received a certain amount of justification through more recent work. Although albumins differ greatly in their composition, they resemble one another in possessing the power of crystallising; the globulins also agree well with one another as far as salting-out is concerned. The group of coagulable albumins is certainly not a homogeneous one, and has therefore been subdivided. The important reserve-albumins of seeds, which sometimes go under the name of vitellines, and at other times are called globulins, have been classed as a special group.

Between the individual groups of the scheme shown below there are transition forms which it is sometimes very difficult to class. Many albumins have been examined not as such, but in the form of their salts, a factor which must never be lost sight of when dealing with their solubilities.

I. Albumins proper

1. Albumins: serum-albumin, egg-albumin,[1] lact-albumin.
2. Globulins: serum-globulin, egg-globulin, lacto-globulin, cell-globulins.
3. Plant-globulins and -vitellines.
4. Fibrinogen.
5. Myosin and allied substances.
6. Phosphorus-containing albumins (nucleo-albumins), caseins,

[1] The author does not consider egg-albumin to be a typical albumin, see p. 352.

vitellines, nucleo-albumins of the cell-protoplasm, mucoid nucleo-albumins.

7. Histones.
8. Protamins.

II. Transformation-products

1. Acid-albumins and alkali-albuminates.
2. Albumoses, peptones, and peptids.
3. Halogen-albumins, oxyprotein, oxyprotsulphonic acid, and allied substances.

III. Proteids

1. Nucleo-proteids.
2. Hæmoglobin and allied substances.
3. Glyco-proteids, mucins, mucoids, helico-proteid.

IV. Albuminoids

1. Collagen.
2. Keratin.
3. Elastin.
4. Fibroin.
5. Spongin, etc.
6. Amyloid.
7. Albumoid.
8. Colouring-matters derived from albumin.

The transformation-products have already been discussed in the first part of this book, while the native albumins will be discussed in this second part.

In what respect albumins differ from other substances has already been stated, and the reasons why peptids and protamins may be regarded as albumins have also been given.

Cohnheim[1] says: 'No definite answer can be given to the question whether corresponding albumins of different animals are identical or not. The difference between the caseins of cow's milk and woman's milk is so great as regards composition and reaction as to leave no doubt that these two caseins are different substances. Hæmoglobins, on the other hand, agree almost completely apart from their solubilities. The serum-albumins and serum-globulins, the muscle-albumins, etc., show more or less distinct and constant differences as regards percentage-composition, coagulation-temperature, and rotatory power. The serum-albumins of the horse and of the rabbit crystallise, while those of all

[1] The author's views are given on p. 351.

other animals up till now have been obtained only in an amorphous form. These differences are, however, not sufficient to prove a chemical difference, especially if we take into consideration that the substances in question have by no means been prepared in a pure state. Even amongst the well-known mono- and di-saccharids are seen small differences in general appearance, in the shape of the crystals, or the degree of purity, if the same substance be prepared from different plants, and it requires much trouble and time to purify these sugars to such an extent as to obtain them in a pure state with uniform characteristics. This difficulty becomes very great indeed when we have to deal with the colloidal polysaccharids, for the differences between the starches obtained from potatoes, rice, and maize are very considerable; but at present it is impossible to tell whether we are dealing with starches differing from one another chemically, or whether the apparent differences are due to the way in which the molecules are arranged in the amylum-granules.

If the difficulty of preparing pure substances is great amongst the sugars, it is still far greater amongst the albumins, for we have not yet succeeded in preparing pure albumins. The recently discovered precipitin-reaction seemed to be able to throw light on this question of identity or non-identity of the corresponding albumins of different animals, as this reaction permits us to recognise and thereby to distinguish with certainty blood, milk, etc., of different animals. It has, however, already been pointed out that we are not quite certain as to whether the reaction depends at all on albuminous substances.

At present all the relations are so complicated that we cannot state definitely that the specificity of a species depends on the chemical structure of its albumins. The same holds good for the differences between the albumins of different organs. We know that it is possible to isolate nucleo-albumins, globulins, and probably also myosin-like substances, from the protoplasm of all glandular organs, but we do not know whether these individual chemical substances determine the functions of the organs, or whether the plasm in each case is built up of the same chemical products, but arranged in each individual case in some specific manner.

There is, however, no doubt that certain older views as to the existence of a special structure characteristic of 'living albumin' are wrong. The 'unstable (labile) groups of atoms' are merely ferments or other substances which are contained in the protoplasm, but which for that reason need not be albumins. The view of Kraus [1] and of Umber,[2]

[1] Kraus, *Deutsche med. Wochensch.* 1903, No. 14.

[2] F. Umber, *Berliner klin. Wochensch.* 1903, No. 39; Kraus, *ibid.* 1904, No. 1.

that the albumins of an organism may change chemically owing to partial decomposition during metabolism, has been disproved by the self-same authors." (Cohnheim.)

Cohnheim's views are not shared by the author, for the systematic classification of animals and of plants recognises the existence of a few large units, which are then subdivided into more numerous sub-units, and these ultimately through 'general' and 'special' into 'individual' units. Every one of these units is characterised by two sets of definite features, the one of which is common to all, while the other is specific of each unit. Thus all plants and animals have nuclei and cell-plasm in common, the activities of which are inversely proportional to one another. Amongst all animals from Vorticella to Man certain contractile tissues have an alternating arrangement of clear and dim stripes, and the unformed blood of an octopus reacts to certain micro-chemical methods, as does the formed blood of vertebrates;[1] the nerve-cells of amphibians and mammals closely resemble one another in possessing certain aggregations of nucleo-proteid matter, the so-called Nissl-granules, in their cell-plasm, and so on. Therefore the resemblance of animals to one another as far as certain histological characters are concerned is very great, and yet we must not overlook the differences.

As lower organisms by evolution give rise to higher ones, so does, for example, simple collagen by evolution give rise to the stages of cartilage, calcified cartilage, and bone. On the assumption, which Cohnheim believes to be allowable, that the gelatin-basis of the tissues just enumerated is the same, the differences in the final product must be due to the presence of chondro-sulphuric acid, as in cartilage, or to that of metaphosphoric acid, as in bone. The question naturally suggests itself: Whence comes chondro-sulphuric acid or the metaphosphoric acid? These radicals must have been formed somewhere; if albumins play no part in their production, then they must be derivatives of nuclear activity. In any case the special selective activity shown by collagen in certain regions of the body must be due to a difference between this particular collagen and that occurring elsewhere—there must exist chemically different collagens.

We may safely assume that in their coarse chemical framework all groups of plants and of animals agree, and that those characteristics which are peculiar to each individual species are produced by transformations and substitutions in 'side-chains'; and further, that this change is only possible by a new arrangement of the different amino-acids both as regards the relative quantities in which each

[1] Mann, *Physiological Histology*, 1902, p. 217.

acid is present, and also the order in which they are linked to one another.

Nobody realises more than does the author the absolute necessity of preparing the different chemical constituents of the body in as pure a form as possible, but do not let us forget that such 'purification' means stripping a compound, by means of recrystallisation and other processes, of certain perfectly normal constituents, which give the specific character to the compound in question. Purification in many cases may be compared to macerating, for example, three heads, covered with white, red, and black hair, till nothing but the skull is left, and then saying these three heads were all alike. Over-purification must lead to the survival of the more resistant radicals at the expense of the more unstable or perishable ones. At present we are 'learning our bones,' but let us also remember the soft parts.

CHAPTER IX

THE ALBUMINS PROPER

When we read in older descriptions about 'albumins,' and especially about their physical properties, we must remember that the simple coagulable albumins are meant, or, in other words, what we now call "the albumins proper" and the globulins.

I. The Albumins

Two albumins are known: serum-albumin and lact-albumin, but it is customary to also include egg-albumin, although the latter ought to be classified amongst the glycoproteids. How these albumins differ from one another in different animals has been discussed on pp. 355, 360, 361, 363, etc.

Albumins are coagulable substances, which are soluble in salt-free water. The author's view as to why albumins are soluble in salt-free water is given on p. 295. Their pure solutions have a neutral reaction. As a rule, they are less readily precipitable than are the globulins and many of the proteids, and this circumstance is made use of in isolating them. They are not rendered insoluble by coming into contact with animal charcoal or with porous earthenware — as is, for example, caseinogen, — and therefore they may be filtered through porous plates without being precipitated.

They are not precipitated by saturating their neutral solutions, even at 40°,[1] with sodium chloride[1] or with magnesium sulphate,[2] while, according to Schäfer[3] and Starke,[4] they are thrown down by

[1] J. Lewith, *Arch. f. experiment. Path. und Pharm.* **24.** 1 (1887).

[2] Tolmatscheff, *Hoppe-Seyler's med.-chem. Untersuchungen*, p. 272 (1867); O. Hammarsten, *Zeitschr. f. physiol. Chem.* **8.** 467 (1884); E. Johannsson, *ibid.* **9.** 310 (1885); J. Lewith, *Arch. f. experiment. Path. und Pharm.* **24.** 1 (1887); K. V. Starke, *Maly's Jahresber. f. Tierchem.* **11.** 17 (1881).

[3] E. A. Schäfer, *Journ. of Physiol.* **3.** 181 (1880).

[4] K. V. Starke, *Maly's Jahresber. f. Tierchem.* **11.** 17 (1881).

magnesium and sodium sulphates if these are used combinedly in saturated solution, and also by sodium chloride[1] or magnesium sulphate[2] if the reaction be acid. The precipitation-limits for ammonium sulphate lie, according to Hofmeister, between 6·4 and 9 (see p. 292), and therefore very high; for this reason they are not salted out by half-saturated solutions, and consequently half-saturation is used for separating albumins from the globulins with which they are constantly found together. If albumin solutions have an acid reaction, the precipitation-limits for ammonium sulphate are lowered.

The three albumins mentioned above have been prepared in a crystalline form, and they differ in their crystalline character from most of the other simple albuminous substances. The crystals have already been discussed on p. 326, where it was pointed out that they are probably not free albumins, but salt-like combinations, being, for example, either sulphates or acetates.

Albumins give all the colour- and precipitation-tests of albuminous substances.

1. Serum-Albumin

It is found in varying amounts in the blood-serum of vertebrates,[3] and also in the lymph. It is therefore met with in all organs which have not been freed thoroughly from blood and lymph. In inflammations of the kidney it passes into the urine, and is also found in pathological transudations. Gürber[4] was the first to prepare it in a crystalline form from the blood of the horse. According to Krieger[5] it is best to employ ammonium sulphate and sulphuric acid (see p. 324), for only sulphuric-acid solutions will give constant results with the blood of the horse, while other acids fail occasionally according to Gürber and Krieger. Gürber has also obtained crystals from the plasma of the rabbit, while he and Schulz[6] failed in obtaining crystals

[1] P. Panum, *Virchow's Archiv*, **4.** 419 (1851); E. Salkowski, *Zentralbl. f. d. med. Wissenschaften*, 1880, p. 38 (*Maly's Jahresber.* **10.** 16).

[2] Tolmatscheff, *Hoppe-Seyler's med.-chem. Untersuchungen*, p. 272 (1867); O. Hammarsten, *Zeitschr. f. physiol. Chem.* **8.** 467 (1884); E. Johannsson, *ibid.* **9.** 310 (1885).

[3] O. Hammarsten, *ibid.* **8.** 467 (1884); G. Salvioli, *Archiv f.* (*Anat. u.*) *Physiol.* 1881, p. 289; J. Joachim, *Wiener klinische Wochenschrift*, 1902, No. 21; G. Meyer, *Medical Dissertation*, Würzburg, 1896.

[4] A. Gürber, *Würzburger physiol.-medizin. Ges.* 1894, p. 113; 1895, p. 26; A. Michel, *Würzburger physiol.-medizin. Ges.* **29.** 117 (1895) (additional note by Gürber, p. 139); G. Meyer, *Medical Dissertation*, Würzburg, 1896; E. Middeldorf, *Dissertation*, Würzburg, 1898.

[5] H. T. Krieger, *Dissertation*, Strassburg, 1899.

[6] F. N. Schulz, *Kristallisation von Eiweissstoffen*, Jena, G. Fischer, 1901.

from the blood of other animals. Wichmann[1] has studied the crystallography of serum-albumin. Gürber was of the opinion that the once generally accepted view, namely, that the serum-albumins of different animals are similar, could not be maintained because of crystallographic differences, but his pupils Meyer, Wichmann,[1] and Schulz[2] have disproved his objections. The endeavour of Oppenheimer[3] to split up serum-albumin into several fractions by means of ammonium sulphate has not led to definite results, and therefore, at present, serum-albumin must be regarded to be a uniform and specific compound. The great agreement between the analyses of even amorphous preparations also points to there being only one serum-albumin.

C	H	N	S	O		
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.		
53·08	6·96	15·93	1·9	21·99	Horse, crystals.	Michel.[4]
53·04	7·1	15·71	1·86	22·29	,, amorphous.	,,
52·93	7·05	15·89	1·82	22·31	,, crystals.	Abderhalden.[5]
...	...	...	1·88	...	,, ,,	Middeldorf.[6]
52·95	6·96	...	1·94	...	,, ,,	Schulz.[7]
53·05	6·85	16·04	1·77	22·29	,, amorphous.	Starke.[8]
...	...	...	1·73	...	,, crystals.	Mörner.[9]
...	...	...	2·31	...	Man, amorphous.	,,
...	...	16·26	...	...	Ox, amorphous.	Blum.[10]

From their analyses Hofmeister and Kurajeff[11] calculate the formula

$$C_{450}H_{720}N_{116}S_6O_{140}$$

and the molecular weight as 10,166.

The coagulation-temperature has been determined by Frédéricq[12] as 67°, and with it the figures of Starke,[8] Sebelien,[13] and Michel[4] agree well. The specific rotation is -57 to $-64°$, according to Frédéricq,[14]

[1] A. Wichmann, *Zeitschr. f. physiol. Chem.* **27.** 575 (1899).
[2] F. N. Schulz, *Kristallisation von Eiweissstoffen*, Jena, G. Fischer, 1901.
[3] C. Oppenheimer, *Archiv f. (Anat. u.) Physiol.* 1903, p. 201.
[4] A. Michel, *Würzburger phys.-med. Ges.* N.F. **29.** 117 (1895).
[5] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **37.** 495 (1903).
[6] Middeldorf, *Würzburger phys.-med. Ges.* N.F. **31.** (1897).
[7] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **25.** 16 (1898).
[8] K. V. Starke, *Maly's Jahresbericht*, **11.** 17 (1881).
[9] K. A. Mörner, *Zeitschr. f. physiol. Chem.* **34.** 207 (1901).
[10] F. Blum, *ibid.* **28.** 288 (1899).
[11] D. Kurajeff, *ibid.* **26.** 462 (1899).
[12] L. Frédéricq, *Bull. de l'Acad. r. de Belgique*, 2 sér. **64.** 7 (1877); *Ann. de Soc. d. médecine de Gent*, 1877 (reprint).
[13] J. Sebelien, *Zeitschr. f. physiol. Chem.* **9.** 445 (1885).
[14] L. Frédéricq, *Arch. de Biolog.* I. 457 (1880).

Michel,[1] Gürber,[2] Starke,[3] and Sebelien.[4] Iodised serum-albumin contains 12 per cent iodine, according to Kurajeff.[5] It combines with 1 to 0·2 grm. hydrochloric acid, according to Erb.[6] The capacity for alkali for the same concentrations is, according to Spiro and Pemsel,[7] much less. The dissociation-products are mentioned in the table, p. 70, No. 2. Glycocoll is absent, otherwise the products are the usual ones. Its bases have not been investigated. Sulphur exists only in the form of cystin, according to Mörner.[8] Serum-albumin yields some glucosamin, according to Langstein,[9] and perhaps also still another carbohydrate acid, but only in infinitesimal quantities (see p. 160). Dissociation by means of pepsin has been investigated by Umber;[10] while that by trypsin takes place only slowly and very incompletely, according to Oppenheimer and Aron,[11] probably because of its antitrypsin-content (Cohnheim).

According to Starke[3] it is only slowly denaturalised by alcohol and ether, and thus differs from other albumins; Johannsson[12] has also noticed a remarkable resistance of serum-albumin towards dissociation by means of dilute acids; even 2 per cent HCl attacks it at room-temperature only after twenty-four hours, while 0·25 per cent hydrochloric and 2 per cent acetic acid have no action.

After Starke[13] had succeeded, for the first time, in converting egg-albumin into a globulin by means of heat, Mott[14] has also accomplished the conversion of serum-albumin over a pseudo-globulin stage into euglobulin by either heating normal serum for half an hour up to 56°, or heating 1 to 3 per cent. of crystalline serum-albumin dissolved in $\frac{n}{132}$ caustic soda solution. The more concentrated an albumin-solution, the greater is the yield of the globulin. What makes the investigation of Mott so important is that the artificial globulin agrees with the normally occurring euglobulin not only in such physical

[1] A. Michel, *Sitzungsber. der Würzburger phys.-med. Ges.* N.F. **29.** 117 (1895).
[2] A. Gürber, *ibid.* **29.** 139 (1895).
[3] K. V. Starke, *Maly's Jahresbericht*, **11.** 17 (1881).
[4] J. Sebelien, *Zeitschr. f. physiol. Chem.* **9.** 445 (1885).
[5] D. Kurajeff, *ibid.* **26.** 462 (1899).
[6] W. Erb, *Zeitschr. f. Biolog.* **41.** 309 (1901).
[7] K. Spiro and W. Pemsel, *Zeitschr. f. physiol. Chem.* **26.** 231 (1898).
[8] K. A. Mörner, *ibid.* **34.** 207 (1901).
[9] L. Langstein, *Hofmeister's Beiträge*, **1.** 259 (1901).
[10] F. Umber, *Zeitschr. f. physiol. Chem.* **25.** 258 (1898).
[11] C. Oppenheimer and H. Aron, *Hofmeister's Beiträge*, **4.** 279 (1903).
[12] J. E. Johannsson, *Zeitschr. f. physiol. Chem.* **9.** 310 (1885).
[13] J. Starke, *Zeitschr. f. Biol.* **40.** 419 and 494.
[14] L. Mott, *Hofmeister's Beiträge*, **4.** 563 (1904).

characters as solubility in salt solutions, but also in having the same percentage-composition.

2. Egg-Albumin

Egg-albumin forms the chief constituent of that concentrated albuminous solution known as egg-white or white of egg. The latter contains, in addition to egg-albumin, also a globulin and a mucoid. The mucoid has been investigated only recently by Mörner[1] and Zanetti,[2] and has been shown to be a distinct substance. For this reason all the older and many of the more recent accounts of egg-albumin do not deal with pure egg-albumin, but with mixtures of the latter with various other albuminous substances.[3] Because of the ease with which it may be obtained, egg-albumin has been more frequently investigated than any other albumin.

Hofmeister[4] was the first to prepare an albumin in a crystalline form from a half-saturated ammonium-sulphate solution, and this albumin was egg-albumin. The method now used by Hopkins[5] is described on p. 325. Instead of acetic acid, any other acid, such as H_2SO_4, HCl, may be used, according to Schulz[6] and Hopkins.[7]

Analysis has yielded these values :—

C	H	N	S	O	
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
53·28	7·26	15·0	1·18	23·28	Hofmeister.[8]
52·26	7·4	15·19	1·23	23·92	Schulz.[9]
52·07	6·95	15·11	1·614	24·26	Bondzynsky and Zoja.[10]
52·44	7·26	15·58	1·7	23·02	
52·75	7·12	15·43	1·57	23·13	Hopkins.[7]
52·75	7·10	15·51	1·62	22·90	Osborne and Campbell.[11]
52·46	7·19	15·29	1·34	23·72	Langstein.[3]

This table shows that the analyses vary in quite an irregular manner, as is specially noticeable in the sulphur-content, and the dis-

[1] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **18.** 525 (1893).

[2] C. U. Zanetti, *Ann. Chim. di. e. Farmac.* **12.** (1897) (according to *Maly's Jahresbericht*, **27.** 31 (1897)).

[3] L. Langstein, 'Über die gerinnbaren Stoffe des Eierklars,' *Hofmeister's Beiträge*, **1.** 83 (1901). (Here the older literature and many analyses.)

[4] F. Hofmeister, *Zeitschr. f. physiol. Chem.* **14.** 163 (1889).

[5] F. G. Hopkins, *Journ. of Phys.* **25.** 306 (1900).

[6] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **29.** 86 (1899).

[7] F. G. Hopkins, *Journ. of Physiol.* **25.** 306 (1900).

[8] F. Hofmeister, *Zeitschr. f. physiol. Chem.* **16.** 187 (1891); **24.** 159 (1897); F. N. Schulz, *ibid.* **25.** 16 (1898).

[9] F. N. Schulz, *ibid.* **29.** 86 (1899).

[10] St. Bondzynsky and L. Zoja, *ibid.* **19.** 1 (1893).

[11] T. B. Osborne and F. G. Campbell, *Journ. Americ. Chem. Soc.* **21.** 477 (1899); **22.** 422 (1900).

crepancies are too great to be attributed to experimental error. Bondzynsky and Zoja[1] explain the discrepancies by assuming the existence of several crystalline albumins, while Hofmeister,[2] Hopkins,[3] Osborne and Campbell[4] maintain that in addition to the crystalline albumin there is present in egg-white a second non-crystallisable albumin, which Osborne and Campbell and Langstein[5] call con-albumin. This con-albumin is characterised by a higher nitrogen- and sulphur-content, and in this it resembles the older non-crystallisable preparations of Hammarsten.[6] As, further, the ovo-mucoid—which can only be separated with great difficulty from the ov-albumin—contains still more sulphur but less C and N than the con-albumin, it is readily seen how by its admixture great discrepancies in the analytical numbers may be induced. Schulz and Zsigmondy[7] have shown by means of the gold number (see p. 333) that it is exceedingly difficult to free egg-albumin from colloidal impurities, even sixfold recrystallisation not being sufficient in some cases, and they found in addition to the ovo-mucoid a "contaminating substance" which may not be an albumin at all and which may possess an entirely different chemical constitution. It is this impurity which adheres so strongly to egg-albumin. Under these conditions the analytical differences may readily be attributed to contaminating substances, and there is no necessity to assume a multiplicity of albumins. According to Spiro[8] the precipitation and crystallisation of albumins by means of ammonium sulphate is never complete, and therefore the non-crystallisable fraction or con-albumin need not be a separate substance.

The precipitation-limits for pure egg-albumin by means of ammonium sulphate lie, according to Langstein,[5] between 6·2 and 6·8 (see p. 292); the limits are therefore very narrow, a fact which also supports the view that egg-albumin is a uniform substance. It is precipitated by sodium chloride in acid solutions, provided the solution is really saturated with sodium chloride, according to Hopkins.[3]

The coagulation-temperature has been determined by Starke[9] as

[1] St. Bondzynsky and L. Zoja, *Zeitschrift f. physiol. Chem.* **19**. 1. (1893).

[2] F. Hofmeister, *ibid.* **16**. 187 (1891); **24**. 159 (1897); F. N. Schulz, *ibid.* **25**. 16 (1898).

[3] F. G. Hopkins, *Journ. of Physiol.* **25**. 306 (1900).

[4] T. B. Osborne and F. G. Campbell, *Journ. Amer. Chem. Soc.* **21**. 477 (1899); **22**. 422 (1900).

[5] L. Langstein, *Hofmeister's Beiträge*, **1**. 83 (1901).

[6] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **9**. 273 (1885); K. V. Starke, *Maly's Jahresber.* **11**. 17 (1881).

[7] F. N. Schulz and R. Zsigmondy, *Hofmeister's Beiträge*, **3**. 137 (1902).

[8] K. Spiro, *ibid.* **4**. 300 (1903).

[9] K. V. Starke, *Maly's Jahresber.* **11**. 17 (1881).

56°, while other authors give higher figures (compare with Ramsden's figures for fibrinogen, p. 382). Hopkins[1] found

$$a_D = -30{\cdot}70.$$

The dissociation-products have been given on p. 70, No. 5. Its high glucosamin-content is of special importance, and has been definitely established after a long discussion by Müller and Seemann[2] and Langstein.[3] The latter found at least 10 to 11 per cent glucosamin, while Hofmeister[4] estimates it at 15 per cent. According to Steudel,[5] glucosamin is contained in egg-white, as it is in the mucins, as a higher complex. According to Mörner,[6] only the smaller part of the sulphur occurs as cystin. There is also present a volatile substance, which is perhaps ethyl sulphide, and if this be so, the ethyl sulphide is derived from cystin, see p. 168.

Iodo-egg-albumin contains about 9 per cent iodine, according to Hofmeister.[4] Blum and Vaubel[7] have not employed pure preparations, but the mixture of egg-white; Hopkins[8] and Pinkus found a Br-capacity of 15·8 per cent for crystalline albumin. The HCl-capacity has been determined by Erb[9] as at most 0·234 grm. pro 1 grm.; that of egg-white has been investigated by Sjöqvist[10] and Bugarszky and Liebermann.[11] The behaviour of egg-albumin during coagulation has been frequently investigated, especially by Pauli (see p. 285). The so-called "ash-free" albumin (see p. 344) is denaturalised albumin. If hens' eggs are placed for two to three days into 10 per cent KOH, then the egg-white no longer coagulates in the usual way on being boiled, but sets into a vitreous transparent jelly, as the salts are diminished while the alkalies are increased in amount, according to Tarchanoff[12] and Zoth.[13] Alcohol and ether denaturalise egg-albumin much more rapidly than serum-albumin, according to Starke.[14] Dissociation by

[1] F. G. Hopkins, *Journ. of Physiol.* **25**. 306 (1900).

[2] F. Müller and J. Seemann, *Deutsche medizin. Wochenschrift*, 1899, p. 209; J. Seemann, *Medizin. Dissertation*, Marburg, 1898; F. Müller, *Zeitschr. f. Biol.* **42**. 468 (1901).

[3] L. Langstein, *Zeitschr. f. physiol. Chem.* **31**. 49 (1900).

[4] F. Hofmeister, *ibid.* **24**. 158 (1897).

[5] H. Steudel, *ibid.* **34**. 353 (1901).

[6] K. A. H. Mörner, *ibid.* **34**. 207 (1901).

[7] F. Blum and W. Vaubel, *Journ. f. prakt. Chem.* N.F. **57**. 365 (1898); F. Blum, *Zeitschr. f. physiol. Chem.* **28**. 288 (1893).

[8] F. G. Hopkins, *Ber. d. deutsch. chem. Ges.* **30**. II. 1860 (1897); F. G. Hopkins and S. N. Pinkus, *ibid.* **31**. II. 1311 (1898).

[9] W. Erb, *Zeitschr. f. Biol.* **41**. 309 (1901).

[10] J. Sjöqvist, 'Über Salzsäure,' Skandin., *Arch. f. Physiol.* **5**. 276 (1894).

[11] St. Bugarszky and Liebermann, *Pflüger's Arch. f. d. ges. Physiol.* **72**. 51 (1898).

[12] J. Tarchanoff, *ibid.* **33**. 303 (1884); **39**. 476 and 489 (1886).

[13] O. Zoth, *Wiener Akad. mathemat. Kl.* III. **100**. 140 (1891).

[14] K. V. Starke, *Maly's Jahresber.* **11**. 17 (1881).

means of pepsin has been studied by Umber[1] and Langstein.[2] Introduced into the circulation egg-albumin behaves as a foreign substance. If it be eaten in large quantities it is absorbed undigested and is excreted into the urine, damaging simultaneously the renal epithelium.[3]

Crystallised albumin has been found by Panormoff[4] in the egg-white of pigeons, and by Worms[5] in the egg-white of the seed-crow; Schulz[6] was unsuccessful in obtaining crystallisation with either pigeon-eggs or goose-eggs. The egg-white of the crow, swallow, lapwing, and other birds which do not leave their nests immediately after hatching, remains as clear as glass on being boiled, according to Lieberkühn[7] and Tarchanoff.[8] For explanation of Tata-albumin, see Index.

On diluting egg-white with ten times its bulk of water and then dialysing it at a temperature of 75-85° Starke[9] obtained a substance giving all the characteristic reactions of ordinary globulins, for it is insoluble in distilled water and in perfectly neutral solutions, but becomes soluble on being treated with very dilute alkalies. If neutral salts are present, then the same amount of alkali will lead to more of the globulin passing into solution, the explanation offered by Mann being[10] that the neutral salts drive out the CO_2 normally present in the water, and that therefore none of the added alkali is bound by the CO_2 and that therefore more alkali is available for the conversion of the globulin into a soluble compound. Attention has already been drawn on p. 356 to the fact that serum behaves quite analogously to egg-white in also giving rise to a globulin on being heated to 56°.

3. Lact-Albumin

Lact-albumin is a constant constituent of all kinds of milk, but compared with casein it is present only in small quantities and is therefore frequently neglected during investigations. A pure preparation was

[1] F. Umber, *Zeitschr. f. physiol. Chem.* **25**. 258 (1898).

[2] L. Langstein, *Hofmeister's Beiträge*, **2**. 229 (1902).

[3] Stokvis, *Zentralbl. f. d. med. Wissenschaften*, 1864, p. 596; M. Ascoli, *Münchener medizinische Wochenschrift*, 1902, I. p. 398; 1903, I. p. 201.

[4] A. Panormoff, *Maly's Jahresber.* **27**. 4 (1897).

[5] W. W. Worms, *Journ. d. russisch. phys.-chem. Ges.* **33**. 448 (according to *Chem. Zentralbl.* 1901, II. p. 1229).

[6] F. N. Schulz, *Kristallisation von Eiweissstoffen*, Jena, G. Fischer, 1901.

[7] Lieberkühn, *Arch. f. Anat., Phys. u. ration. Med.* 1848, p. 323.

[8] J. Tarchanoff, *Pflüger's Arch. f. d. ges. Physiol.* **31**. 368 (1883); **39**. 476 and 485 (1886); C. T. Helbig, *Arch. f. Hygiene*, **8**. 475.

[9] J. Starke, *Zeitschr. f. Biol.* **40**. 419 and 494.

[10] Mann, *Physiological Histology*, p. 58.

investigated for the first time by Sebelien [1] in Hammarsten's laboratory. Wichmann [2] succeeded in preparing crystalline lact-albumin by Gürber's method; the crystals resemble those of the other albumins. The data at present are insufficient for drawing any conclusion as to the inter-relationship of lact-albumin and serum-albumin.

According to Sebelien lact-albumen has the percentage composition

$$C_{52\cdot19}\quad H_{7\cdot18}\quad N_{15\cdot77}\quad S_{1\cdot73}\quad O.$$

Other albumins are not known. The albumin found by C. T. Mörner [3] in the eye and by Kühne [4] in muscle are derived from traces of blood or lymph (v. Fürth).[5] No albumins could be found in the liver and in the thyroid by Plósz,[6] Oswald,[7] and others. The so-called yolk-albumin, from which Mayer and Neuberg prepared glucosamin, if judged by its solubility, is not an albumin but rather a vitelline (see p. 405). Infusoria (Sosnowski [8]) and mussels (Cohnheim) also contain no albumin. H. Buchner [9] states that he obtained 'albumin' from the juice pressed out of bacteria, but as he uses this term only to show that the substance in question is not a proteid it may well be a globulin. Palladin [10] mentions a vegetable albumin, without, however, describing it more fully.

II. The Globulins

The formation of globulins by heating albumins is referred to on pages 356 and 360.

The globulins are simple coagulable albumins, which differ from albumins in being insoluble in pure water and in dilute acids, but they are soluble in dilute alkalies and in solutions of neutral salts (see p. 297). They are, therefore, precipitated from salt-solutions by dilution with water, and more completely by removing the salts through dialysis; on adding salts they pass again into solution. An explanation of their physical behaviour has been offered by the author on p. 296. They are also precipitated by acidifying their solutions and even by passing a constant stream of CO_2 through the solution, and are rendered

[1] J. Sebelien, *Zeitschr. f. physiol. Chem.* **9**. 445 (1885) (here the older literature).
[2] A. Wichmann, *ibid.* **27**. 575 (1899).
[3] C. T. Mörner, *ibid.* **18**. 61 (1893).
[4] W. Kühne, *Archiv f. Anat. u. Physiol.* 1859, p. 748.
[5] O. v. Fürth, *Schmiedeberg's Arch.* **36**. 231 (1895).
[6] P. Plósz, *Pflüger's Archiv*, **7**. 371 (1873).
[7] A. Oswald, *Zeitschr. f. physiol. Chem.* **27**. 14 (1899).
[8] J. Sosnowski, *Zentralbl. f. Physiol.* **13**. 267 (1899).
[9] H. Buchner, *Münchener medizin. Wochenschr.* 1897, No. 12.
[10] W. Palladin, *Zeitschr. f. Biolog.* **31**. 191 (1894).

neutral again by neutralising the solution. They become much more readily insoluble, *i.e.* denaturalised, than the albumins by precipitation with acids or by dialysis, and therefore they are only completely re-soluble immediately after being precipitated.

According to Osborne,[1] the hydrogen-ions of the water are the cause of the globulins becoming insoluble, and this would account for coagulation occurring more quickly in the presence of CO_2 or small amounts of other acids than in pure water. Osborne calls the globulin edestin after it has become insoluble, edestan, and proposes to call the derivatives of other globulins by analogous names. Edestan is said to possess a different acid-capacity from edestin.

The precipitability of globulins by means of acids and their solubility in alkalies depend on the fact that they themselves are acids, which with litmus give an acid reaction and which liberate CO_2 (J. Starke).[2] They must not, however, be confounded with the acid-albumins (J. Starke).[3]

Kutscher[4] observed that deutero-albumoses cause a precipitate in a solution of sodium globulinate, and Hammarsten[5] has further shown that sodium globulinates are precipitated by neutral salts and small amounts of sodium carbonate.

Globulins are salted out completely by saturated magnesium-sulphate solutions, and partially also by saturated sodium-chloride solutions; for neutral ammonium sulphate the limits for serum-globulin lie between 2·9 and 4·6, and other globulins behave similarly; all globulins are completely precipitated by half-saturated ammonium-sulphate solutions, and occupy in this respect a position midway between the albumins which are more difficult to precipitate, and fibrinogen and casein,[6] which are more readily precipitable.

Globulins are obtained as a precipitate by either saturating a solution containing globulins, with neutral magnesium sulphate, according to Hammarsten, or by adding to the serum an equal quantity of cold-saturated, neutral ammonium-sulphate solution, according to Hofmeister. This precipitate is dissolved in water—some sodium chloride being added, if necessary—and the globulin-solution is then either greatly diluted with distilled water, or the salts are removed by

[1] T. B. Osborne, *Zeitschr. f. physiol. Chem.* **33**. 225 (1901).

[2] J. Starke, *Zeitschr. f. Biol.* **40**. 419 (1900).

[3] J. Starke, *ibid.* **40**. 494 (1900); L. Moll, *Hofmeister's Beiträge*, **4**. 563 (1903).

[4] F. Kutscher, *Zeitschr. f. physiol. Chem.* **23**. 115 (1897).

[5] O. Hammarsten, *Pflüger's Arch. f. d. ges. Physiol.* **18**. 38 (1880); *Zeitschr. f. physiol. Chem.* **8**. 467 (1884).

[6] G. Kauder, *Arch. f. experim. Path. u. Pharm.* **20**. 411 (1886); J. Pohl *ibid.* **20**. 426 (1886).

dialysis, and finally the globulin is precipitated by means of dilute acetic acid. The yield is, however, bad if dialysis or acidification is employed, and therefore, if the presence of salt does not matter, it is better to prepare globulin by repeated salting-out. As the globulin in its precipitated state becomes very rapidly insoluble (see p. 298 for an explanation), it is necessary to be as quick as possible in all one's manipulations, according to Cohnheim; but Starke[1] is of the opinion that globulin which has become insoluble in neutral salt solutions, may be rendered soluble again by the addition of a trace of alkali.

The presence of globulins in a solution may be assumed whenever a coagulable, phosphor-free albumin is precipitated by diluting or acidifying an albuminous solution, if in addition the precipitate gives the above indicated precipitation-limits for ammonium sulphate.

Fibrinogen, myosin, and some related substances resemble the globulins as far as their acid properties and solubilities are concerned, but they must be considered as belonging to a special group; and the vegetable albumins, which in part are true globulins, forms also a group by themselves.

The rotatory power of 1 per cent globulins in 10 per cent NaCl has been investigated by Alexander.[2] (See also pp. 364, 367, and 373.)

serum-globulin	– 48	hemp-seed-globulin	– 41·5
egg-globulin	– 40	brazil-nut-globulin	– 40·5
fibrinogen	– 45 to – 50	flax-seed-globulin	– 38·5

1. Serum-Globulin

After Panum[3] had shown that an albumin occurred in serum, which could be precipitated by diluting or by acidifying the serum, and after Weyl[4] had proved that this substance was a definite body, to which he gave the name of serum-globulin, it was thoroughly investigated by Hammarsten.[5] The older views of Alexander Schmidt,[6] who believed that serum-globulin played a part in blood-coagulation and who gave to it the name of "paraglobulin," are no longer tenable.

[1] J. Starke, *Zeitschr. f. Biol.* **40**. 419, 494.

[2] A. C. Alexander, *Journ. of Experimental Medicine*, **1**. 186 (1896).

[3] P. Panum, *Virchow's Arch.* **4**. 419 (1851).

[4] Th. Weyl, *Pflüger's Arch. f. d. ges. Physiol.* **12**. 635 (1876); and in *Zeitschr. f. physiol. Chem.* **1**. 72 (1877).

[5] O. Hammarsten, *Pflüger's Arch. f. d. ges. Physiol.* **17**. 413; **18**. 38 (1878); and in *Zeitschr. f. physiol. Chem.* **8**. 467 (1884); and in *Ergebnisse der Physiologie von Asher-Spiro*, **1**. I. 330 (1902).

[6] Alexander Schmidt, *Zur Blutlehre*, Leipzig, 1892 (Review).

Serum-globulin forms a fraction of the albumins occurring in blood-serum. According to Meyer,[1] Krieger,[2] and Joachim,[3] its percentage varies for different species of animals, and also for different animals of the same species; the author believes its amount to be determined by the alkalinity of its tissue-juices. Globulin passes into the urine [4] during kidney-disease, into such transudations as the liquor amnii,[5] and into the lymph. Ludwig and Salvioli [6] have further shown that the ratio of the albumin to the globulin is the same in lymph as in blood.

It has not been prepared in a crystalline form.

Its analysis has yielded these results:

C	H	N	S	O	
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
52·71	7·01	15·85	1·11	23·32	Hammarsten.[7]
...	...	15·83	...	...	Hausmann.[8]
...	...	15·82	...	...	Blum.[9]
.	...	...	1·38	...	Schulz.[10]
...	...	...	0·97	...	Mörner.[11]

The coagulation-temperature has been found to be the same by all investigators.[12] It amounts to 75°, and is fairly independent of the amount of salt present. According to Frédéricq [13]

$$a_D = -47{\cdot}8.$$

The dissociation-products are enumerated on p. 70, No. 3. The high glycocoll-content is especially remarkable, and this is characteristic of the antigroup. For the same reason it is very resistant to ferments, as pointed out by Umber,[14] E. Fischer and Abderhalden,[15]

[1] G. Meyer, *Med. Dissert.*, Würzburg, 1896.

[2] H. Krieger, *Dissert.*, Strassburg, 1899.

[3] J. Joachim, *Wien. klin. Wochenschr.* 1902, No. 21.

[4] J. Pohl, *Arch. f. experiment. Pathol. u. Pharm.* **20**. 426 (1886).

[5] Th. Weyl, *Arch. f.* (*Anat. u.*) *Physiol.* 1876, p. 543.

[6] G. Salvioli, *ibid.* 1881, p. 269.

[7] O. Hammarsten, 'Über das Fibrinogen,' *Pflüger's Arch. f. d. ges. Physiol.* **22**. 431 (1880).

[8] W. Hausmann, *Zeitschr. f. physiol. Chem.* **27**. 95 (1899).

[9] F. Blum, *ibid.* **28**. 288 (1899).

[10] F. N. Schulz, *ibid.* **25**. 16 (1898).

[11] K. A. H. Mörner, *ibid.* **34**. 207 (1901).

[12] O. Hammarsten, *Pflüger's Arch. f. d. ges. Physiol.* **17**. 413; **18**. 38 (1878); and in *Zeitschr. f. physiol. Chem.* **8**. 467 (1884); and in *Ergebnisse der Physiologie von Asher-Spiro*, **1**. I. 330 (1902); L. Frédéricq, *Ann. de la Soc. de Méd. de Gent*, 1877; Th. Weyl, see No. 4, last page.

[13] L. Frédéricq, *Ann. de la Soc. de Méd. de Gent*, 1877; Th. Weyl, see No. 4, last page.

[14] F. Umber, *Zeitschr. f. physiol. Chem.* **25**. 258 (1898).

[15] E. Fischer and E. Abderhalden, *ibid.* **39**. 81 (1903).

and Oppenheimer and Aron.[1] According to the two last observers, this is only partially due to the presence of antiferment. Sulphur, according to Mörner,[2] is entirely in the form of cystin. Langstein[3] has found in globulin dextrose and still other carbohydrates, but the possibility must be kept in mind that the amorphous globulin may be contaminated with serum. Iodo-serum-globulin contains 8·45-8·99 per cent iodine, according to Blum.[4] Umber[5] has investigated its peptic digestion ; the albumoses of the antigroup are especially very abundant. The conditions as to solubility are the same as for all other globulins ; indeed, most determinations on the solubility of globulins have been made on serum-globulin.

In preparing serum-globulin, the serum has to be freed in the first instance from all remains of fibrinogen by the addition of 43 ccm. of saturated ammonium-sulphate solution to 100 ccm. of serum.

The question as to whether serum-globulin is a uniform substance is very much discussed. Serum-globulin is precipitated incompletely by diluting serum with water, or dialysing it, and by adding acid, but Hammarsten[6] has shown that the precipitated globulin as well as the portion which escaped precipitation are both again partially precipitable, and therefore it is not allowable, as Marcus[7] and Freund and Joachim[8] have done, to assume the existence of two distinct globulins in serum.

Fuld and Spiro[9] and Porges and Spiro[10] have attempted to break up serum-globulin into several fractions by means of fractional salting-out with ammonium sulphate or potassium acetate. They obtained a 'euglobulin' having with ammonium sulphate the precipitation-limits of 2·8 to 3·3, and a pseudoglobulin with the limits of 3·4 to 4·6 ; half-saturation with potassium acetate gave limits which did not correspond with those obtained with ammonium acetate. Rostoski[11] succeeded in fractionising serum-globulin still more, and Porges and Spiro found confirmatory evidence, inasmuch as considerable differences exist in the composition of their fractions, and they further

[1] C. Oppenheimer and A. Aron, *Hofmeister's Beiträge*, **4**. 279 (1903).
[2] K. A. H. Mörner, *Zeitschr. f. physiol. Chem.* **34**. 207 (1901).
[3] L. Langstein, *Wien. Akad. math.-nat. Kl.* **112**. IIb, May 1903.
[4] F. Blum, *Zeitschr. f. physiol. Chem.* **28**. 288 (1899).
[5] F. Umber, *ibid.* **25**. 258 (1898).
[6] O. Hammarsten, *Pflüger's Arch. f. d. ges. Physiol.* **18**. 38 (1880) ; *Zeitschr. f. physiol. Chem.* **8**. 467 (1884).
[7] E. Marcus, *ibid.* **28**. 559 (1899).
[8] E. Freund and J. Joachim, *ibid.* **36**. 407 (1902).
[9] E. Fuld and K. Spiro, *ibid.* **31**. 132 (1900).
[10] O. Porges and K. Spiro, *Hofmeister's Beiträge*, **3**. 277 (1902) (here the literature).
[11] Rostoski, *Münch. med. Wochenschr.* 1902, p. 740.

found that certain ferments, antiferments, and antitoxins occur constantly only in one of their fractions.[1] But at present it is quite impossible to say to what extent these differences are due to admixtures of foreign bodies and how many globulins there really exist.[2]

2. Cell-Globulins

Globulin-like bodies have been prepared from many organs. Kühne[3] obtained globulin from muscle-plasma; Gottwald[4] and Lönnberg,[5] from the kidney; Plósz[6] and Halliburton,[7] from the liver; Laptschinsky,[8] from the lens of the eye; Halliburton[9] and Lilienfeld,[10] from leucocytes, in large amounts; Halliburton, from the central nervous system[11] and from red corpuscles;[12] and Cohnheim from the pancreas. Some of these globulins have the same coagulation-temperature as has serum-globulin, and there is a certain suspicion that many so-called cell-globulins are in reality true serum-globulin owing to the incomplete removal of blood or lymph from the tissues, for v. Fürth[13] has not found any globulin in muscle which had been thoroughly washed out.

Halliburton,[7] on the other hand, has found in many organs 'cell-globulins α.' These substances have coagulation-temperatures of 48° and 52°, and are precipitated by even not completely saturated solutions of magnesium sulphate and sodium chloride. It is possible that these substances belong to the class of the myosins. Other chemical properties of these bodies are not known.

Only one of these cell-globulins has been examined thoroughly, namely, the thyreo-globulin, which Oswald[14] extracted from the thyroid gland. It has the same precipitation-limits for ammonium sulphate

[1] Fuld and Spiro, Marcus, see p. 365; K. Spiro, *Hofmeister's Beiträge*, **1**. 78 (1901); K. Glässner, *ibid.* **4**. 79 (1903); N. Asakawa, *Zeitschr. f. Hyg. u. Infektionskrankh.* **45**. 93 (1903).

[2] O. Hammarsten, *Ergebnisse der Physiologie von Asher-Spiro*, I. **1**. 330 (1902).

[3] W. Kühne, *Untersuchungen über das Protoplasma und die Kontraktilität*, Leipzig, 1864.

[4] E. Gottwald, *Zeitschr. f. physiol. Chem.* **4**. 437 (1880).

[5] J. Lönnberg, *Skandinav. Arch. f. Physiol.* **3**. 1 (1890).

[6] P. Plósz, *Pflüger's Arch. f. d. ges. Physiol.* **7**. 371 (1873).

[7] W. D. Halliburton, *Journ. of Physiol.* **13**. 806 (1892).

[8] M. Laptschinsky, *Pflüger's Arch. f. d. ges. Physiol.* **13**. 631 (1876).

[9] W. D. Halliburton, *Journ. of Physiol.* **9**. 229 (1880).

[10] L. Lilienfeld, *Zeitschr. f. physiol. Chem.* **18**. 473 (1893).

[11] W. D. Halliburton, *Journ. of Physiol.* **15**. 90 (1894).

[12] W. D. Halliburton and W. M. Friend, *ibid.* **10**. 532 (1889).

[13] O. v. Fürth, *Arch. f. experim. Pathol. u. Pharmak.* **36**. 231 (1895).

[14] A. Oswald, *Zeitschr. f. physiol. Chem.* **27**. 14 (1899); **32**. 121 (1901).

as has serum-globulin, but it is completely precipitated by acids. Its iodine-content is remarkable. The human thyreo-globulin has the following percentage composition :

$$C_{51\cdot81} \quad H_{6\cdot88} \quad N_{15\cdot49} \quad S_{1\cdot87} \quad I_{0\cdot34} \quad O_{23\cdot57}.$$

The composition of the thyreo-globulin of other animals is nearly the same as that of man. Its coagulation-temperature is 65°.

It is not a salt, but an iodo-albumin. On it depends the physiologically and pharmacologically specific activity of the thyroid gland. It forms the chief constituent of the colloid, and is enormously increased in goitre. The iodine-percentage varies according to the age of the person and also under other conditions, and does not seem to play a great part in the function of the gland.

3. Crystallin

This expression was first used by Berzelius for the albuminous substances of the crystalline lens. Laptschinsky [1] showed that it was composed of a globulin (or vitellin) and an albumin. Subsequently it was investigated by Mörner,[2] who distinguishes :

1. The α-crystallin. It gives the same reactions as does serum-globulin, but it is not precipitated by sodium chloride, and only by high concentrations of ammonium sulphate. It also differs from globulin in not being precipitated on being diluted with water. It does not give the lead-sulphide reaction.

$$\alpha_D = -46\cdot9.$$

Its coagulation-temperature is 72°.

It occurs principally in the outer layers of the lens.

2. The β-crystallin. It is only with difficulty precipitated by acetic acid and carbonic acid, but otherwise it gives the ordinary globulin reactions.

$$\alpha_D = -43\cdot3.$$

Its coagulation-temperature is 63°.

It occurs principally in the inner, firmer portions of the lens.

4. Egg-Globulin

The long-known egg-globulin has been investigated and analysed most recently by Langstein,[3] who found amongst the dissociation-

[1] M. Laptschinsky, *Pflüger's Arch. f. d. ges. Physiol.* **13**. 631 (1876).

[2] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **18**. 61 (1893).

[3] L. Langstein, *Hofmeister's Beiträge*, **1**. 83 (1901).

products considerable amounts of glucosamin and a correspondingly well-marked reaction of Molisch. All the other colour-tests gave also positive results. Its solubility is the same as that of serum-globulin, and the question as to its uniform nature is as little settled as in the case of serum-globulin. Umber[1] obtained egg-globulin, by an accident, once in a crystalline form. Many experiments on the action of salts on albumins refer to this globulin, as the experiments were made with impure egg-albumin, the globulin fraction of which is more readily precipitated than is the albumin fraction.[2]

The percentage composition varies considerably, owing to the difficulty of preparing a pure ovo-globulin.

	C	H	N	S	S	O		
Ovomucin 1	50·69	6·71	14·99	2·28	traces	25·83	0·53	Osborne and Campbell
Ovomucin 2	50·95	6·85	14·82	1·933	–	25·442	1·41	
Soluble globulin	52·67	7·12	15·48 15·58	1·687	0·123	22·77	0·41	
Soluble portion	51·43	7·04	15·16	1·66	–	24·7	–	Langstein
Total globulin	51·91	7·04	15·13	2·000	–	22·886	–	

5. Lact-Globulin

It was discovered by Sebelien[3] in milk, and was subsequently also found by Hewlett.[4] Milk contains only a few milligrammes per litre. It resembles serum-globulin both as regards precipitation-limits and coagulation-temperature, 72°. Lact-globulin is much more abundant in colostrum than it is in milk.[5]

6. Crystalline Globulin from Urine

Noel Paton[6] found once in the urine of a man suffering from an unknown disease very large quantities of a crystalline globulin in addition to ordinary albumin. Huppert[7] has subsequently brought forward the view that Paton's globulin is in reality a hetero-albumose

[1] F. Umber, *Berl. klin. Wochenschr.* 1902, No. 28.

[2] W. Pauli, *Pflüger's Arch. f. d. ges. Physiol.* **78**. 315 (1899); *Hofmeister's Beiträge*, **3**. 225 (1902); F. Mylius, *Ber. d. deutsch. chem. Ges.* **36**. I. 775 (1903).

[3] J. Sebelien, *Zeitschr. f. physiol. Chem.* **9**. 445 (1885); and in *Journ. of Physiology*, **12**. 95 (1891).

[4] Hewlett, *Journ. of Physiol.* **13**. 798 (1892).

[5] J. Sebelien, *Zeitschr. f. physiol. Chem.* **13**. 135 (1888).

[6] Noel Paton, *Proc. of the Roy. Soc. of Edinburgh*, 1891-92, p. 102.

[7] Huppert, *Zeitschr. f. physiol. Chem.* **22**. 500 (1896).

or hetero-globulose. The nature of this substance is therefore as yet doubtful. It crystallised from the very strongly acid urine, which contained relatively little salt, after a few hours or only after some weeks in the shape of broad needles belonging to the rhombic system.

Its analysis yielded :

$$C_{51\cdot89}\ H_{6\cdot88}\ N_{16\cdot06}\ S_{1\cdot26}\ O_{23\cdot93}.$$

The coagulation-temperature was between 58° and 59°, but coagulation was never very complete. Its reactions were the same as those of globulin, except that it was completely salted out by sodium chloride.

This globulin is probably identical with Bence Jones' albumin.

7. Bence Jones' Albumin

This substance used to be taken for an albumose, but is a native albumin according to more recent accounts. It is found in the urine.

Bence Jones[1] observed in 1848 the elimination of a substance which in 1869 was rediscovered and described by Kühne.[2] Subsequently observations have been made by Matthes,[3] Ellinger,[4] Magnus-Levy,[5] Jochmann and Schumm,[6] Grutterinck and de Graaff,[7] and by Parkes.[8] In all cases sufficiently carefully examined, the patients were suffering from sarcoma of the marrow, and only in the case of Jochmann and Schumm from a true osteomalacia. The patients excreted Bence Jones' albumin in abundant, and in some cases even very abundant amounts, either during the whole period of the disease or at least during some time of it.

Ellinger has succeeded in obtaining this body from the diseased bone-marrow, although only in very small quantities. There is nothing else known as to its origin.

The most accurate description of it we owe to Magnus-Levy ; he, as well as Grutterinck and de Graaf, succeeded in obtaining Bence Jones' albumin in crystals. Complete analyses have not been made.

[1] Bence Jones, *Philosophical Transact.* 1848, I. p. 55.

[2] W. Kühne, *Zeitschr. f. Biolog.* **19**. 209 (1883).

[3] M. Matthes, *Verhandl. d.* 14. *Kongresses f. inn. Medizin*, Wiesbaden, 1896, p. 476.

[4] A. Ellinger, *Deutsch. Arch. f. klin. Medizin*, **62**. 255 (1899) (here a complete account of the literature).

[5] A. Magnus-Levy, *Zeitschr. f. physiol. Chem.* **30**. 200 (1900).

[6] G. Jochmann and O. Schumm, *Münch. med. Wochenschr.* 1901, p. 1340.

[7] A. Grutterinck and C. J. de Graaff, *Zeitschr. f. physiol. Chem.* **34**. 393 (1901).

[8] Weber F. Parkes, *Journ. of Pathol. and Bacteriol.* **9**. 172 (1904) (here is given a complete account of all the clinical cases).

Ellinger found 15·59 and Magnus-Levy 15·57 per cent nitrogen. Amongst its dissociation-products Spiro found leucin, tyrosin, glutaminic acid, and ammonia,[1] but no glycocoll. Judging by the colour-tests it also contains tryptophane, detachable sulphur, and a carbohydrate. Phosphorus is absent.

On being heated Bence Jones' albumin behaves in a characteristic way. It becomes coagulated between 50° and 58°, but on being heated still more it passes into solution again if there be present an abundance of ammonia-salts or urea. The nitric acid and alcohol precipitates also redissolve in the presence of ammonium chloride, and on cooling the coagulum reappears. As urine constantly contains urea and ammonium chloride, the coagulum disappears on being heated beyond 58°, and this happens sometimes even with the 'apparently' pure substance, for which reason Kühne held the albumin to be an albumose closely related to hetero-albumose. Magnus-Levy, however, has shown that the body coagulates if it be really pure; further, that it is denaturalised by alcohol and other precipitating agents; and that it is converted into acid-albumin or alkali-albuminate when it is acted upon by fairly strong acids or alkalies. On being digested with pepsin it yields the ordinary albumoses and peptones, but no hetero-albumose. It must therefore be a genuine albumin. In other respects this body shows the usual reactions towards precipitating agents. The limits for ammonium sulphate are between 4 and 6, and they vary somewhat according to the purity of the preparation. Magnesium sulphate does not precipitate, while sodium chloride does so both in neutral and in acid solutions. Grutterinck and de Graaff obtained this albumin in a crystalline form on using a 10 per cent ammonium-sulphate solution which was subsequently acidified with sulphuric acid. The ammonium sulphate could be replaced by magnesium- or zinc-sulphate, sodium chloride or ammonium chloride, and the sulphuric acid by hydrochloric acid. In 66 per cent alcohol this albumin is insoluble. It is very readily digested owing to the absence of hetero-albumose and of glycocoll.

III. The Albumins of Seeds

The percentage-amount of albuminous substances in plants, taking these as a whole, is so low that these albumins have received very little attention from chemists, if we exclude the investigations of Bokorny.[2] The seeds, on the other hand, with their large amount of stored albumin,

[1] A. Magnus-Levy, *Zeitschr. f. physiol. Chem.* **30.** 200 (1900).

[2] Th. Bokorny, *Pflüger's Arch. f. d. ges. Physiol.* **80.** 48 (1900).

have been investigated very thoroughly. Starting with Liebig, a large number of papers have been published dealing with these readily accessible and economically very important bodies. We owe to Ritthausen [1] a special debt of gratitude for the careful way in which he analysed, and also determined the solubilities of, a large number of different albuminous substances belonging to the cereals and to pulse (beans, peas, lupines, etc.). As the methods employed by Ritthausen did not guarantee that he had isolated chemical individuals, his observations received for a long time little attention; but through the work of E. Schulze and Kossel, who analysed Ritthaussen's substances, the importance of the latter's investigations was proved. The dissociation products are given in the Tables, pp. 71-72, Nos. 13 to 24.

Better defined from a chemical point of view are a series of albumins which occur in a crystalline form [2] in hemp, the Para-nut, in the seeds of the cucumber, and in the castor-oil plant. These albumins have also been prepared in a crystalline form from their solutions,[3] and they were the only crystalline albumins known till egg- and serum-albumin were crystallised. At present edestin and oxyhæmoglobin are probably the two purest albuminous substances we possess. More recently this group of substances has been investigated especially by Osborne (see later).

Most of the vegetable albumins are globulins, *i.e.* acid-albumins which are insoluble in pure water, but soluble in salt-solutions, and precipitable by dilution and acidification; they are known collectively as phyto-globulins. Some of the vegetable albumins contain phosphorus, and not merely as an admixture, in their molecule, according to Liebig,[4] Ritthausen, Palladin,[5] and Wiman.[6] They resemble the true nucleo-albumins in being insoluble in neutral salt-solutions, but they are soluble in alkalies. Liebig gave to these substances the name of vegetable casein, but they are now, following Weyl,[7] usually called

[1] H. Ritthausen, *Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Ölsamen*, Bonn, 1872 (abstract of his own work and that of his pupils, published mostly in the *Journ. f. prakt. Chem.*; here also the older literature). See also *Pflüger's Arch. f. d. ges. Physiol.* **15.** 269 (1877); **16.** 293 and 301 (1878); **18.** 236 (1878); *Journ. f. prakt. Chem.* (2) **23.** 412 (1881); **24.** 221 and 257 (1881); **25.** 130 (1882).

[2] Hartig, *Botanikerztg.* **13.** 881 (1855); **14.** 257, 297, and 313 (1856); Maschke, *ibid.* **17.** 409 and 417 (1859).

[3] G. Grübler, *Journ. f. prakt. Chem.* **131.** 97 (1881); O. Schmiedeberg, *Zeitschr. f. physiol. Chem.* **1.** 205 (1877).

[4] J. v. Liebig, *Liebig's Annalen*, **39.** 128 (1841).

[5] W. Palladin, *Zeitschr. f. Biol.* **31.** 191 (1895).

[6] A. Wiman, *Maly's Jahresber.* **27.** 21 (1897).

[7] Th. Weyl, *Pflüger's Arch. f. d. ges. Physiol.* **12.** 635 (1876); *Zeitschr. f. physiol. Chem.* **1.** 72 (1877).

phyto-vitellins. What makes the investigation of these compounds especially difficult is the presence of considerable amounts of salts such as potassium-, calcium-, or magnesium-phosphates, or of alkalies in combination with organic acids. These salts are frequently the real cause of the acid or alkaline reactions given by phyto-vitellines. Tannin is frequently also present, and will precipitate albumins whenever the reaction becomes acid.[1] In many instances the salts of the albumins have been studied instead of the free albumins,[2] and in other cases special deductions had to be made, to allow for the ash constituents which have been carried down mechanically during coagulation. For all these reasons it is, in many cases, impossible to tell whether we are dealing with globulins or with vitellines, and for this reason all these albuminous substances, being biologically allied, have been classed together. Palladin[2] has shown that the so-called plant-myosin is merely the calcium salt of vitelline (Cohnheim).

1. Edestin

Weyl,[3] Schmiedeberg,[4] Drechsel,[5] Chittenden and Hartwell,[6] and Osborne[7] have found in the Para-nut (Bertholletia) an albumin, the lime or magnesium salts of which crystallise out in well-formed octohedra. Grübler[8] and Ritthausen[9] found a similar albumin in cucumber seeds. Subsequently Ritthausen,[9] Osborne,[10] Chittenden and Mendel,[11] Leipziger,[12] and others prepared from hemp-seed a substance which, in its composition and all its properties, fully agrees with the globulin prepared from the Para-nut, and which has been called 'edestin' by Osborne. Later on Osborne obtained edestin also from the seeds of the castor-oil plant,[10] the flax,[10] the oat,[13] cucumber,[10] maize,[14] different

[1] H. Ritthausen, *Journ. f. prakt. Chem.* (2) **24.** 257 (1881).

[2] W. Palladin, *Zeitschr. f. Biol.* **31.** 191 (1897); T. B. Osborne, *Journ. of the Amer. Chem. Soc.* **21.** 486 (1899).

[3] Th. Weyl, *Pluger's Arch. f. d. ges. Physiol.* **12.** 635 (1876); *Zeitschr. f. physiol. Chem.* **1.** 72 (1877).

[4] G. Grübler, *Journ. f. prakt. Chem.* **131.** 97 (1881); O. Schmiedeberg, *Zeitschr. f. physiol. Chem.* **1.** 205 (1877).

[5] E. Drechsel, *Journ. f. prakt. Chem.* (2) **19.** 331 (1879).

[6] R. H. Chittenden and J. A. Hartwell, *Journ. of Physiol.* **11.** 434 (1890).

[7] T. B. Osborne, *Amer. Chem. Journ.* **14.** No. 8 (1893).

[8] G. Grübler, *Journ. f. prakt. Chem.* **131.** 97 (1881).

[9] H. Ritthausen, *ibid.* (2) **23.** 412 (1881); (2) **25.** 130 (1882).

[10] T. B. Osborne, *Amer. Chem. Journ.* **14.** No. 8 (1893).

[11] R. H. Chittenden and L. B. Mendel, *Journ. of Physiol.* **17.** 48 (1894).

[12] R. Leipziger, *Pflüger's Arch. f. d. ges. Physiol.* **78.** 402 (1899).

[13] T. B. Osborne, *Amer. Chem. Journ.* **14.** 212 (1893).

[14] T. B. Osborne, *Journ. of the Americ. Chem. Soc.* **19.** 525 (1897).

nuts,[1] sunflowers,[2] lentils,[3] wheat,[4] cotton,[5] and malt.[6] As in the case of the serum-albumins, so here it is very difficult to say whether we are always dealing with one and the same substance, as the distinguishing features are very ill defined. The edestin from hemp-seed has been most fully investigated. Its analysis gives these figures :—

C	H	N	S	O	
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
50·92	6·91	18·71	0·82	22·64	Ritthaussen.[7]
51·63	6·90	18·78	0·90	21·79	Chittenden and Mendel.[8]
51·27	6·85	18·76	0·91	22·22	Osborne.[9]
...	...	...	0·88	...	Osborne.[10]
51·21	6·87	18·64	0·95	22·37	Abderhalden.[11]
...	...	18·53	...	...	Hausmann.[12]

The specific rotation, according to Chittenden and Mendel,[8] is − 43·48. Its dissociation-products are given in the table on p. 71, No. 12. All the amino-acids are present. Edestin, next to globulin and some of the protamins, is the best analysed substance. According to Erb[13] and Osborne[14] all the colour-tests, except that of Molisch, give positive results. The precipitation-tests give the same results as in the case of the globulins, and the numbers obtained with the salting-out method are also the same as for globulin, according to Osborne.[15] The hydrochlorides of edestin have been investigated by Erb[16] and Osborne,[17] and the lime and magnesium salts by Schmiedeberg,[18] Drechsel,[19] and Grübler.[20] As already mentioned, the composition and the reactions of edestins prepared from different seeds do not differ from one another

[1] T. B. Osborne and J. F. Harris, *Journ. Amer. Chem. Soc.* **25.** 848 (1903).
[2] T. B. Osborne and G. F. Campbell, *ibid.* **19.** 487 (1897).
[3] T. B. Osborne, *ibid.* **20.** 362 (1892).
[4] T. B. Osborne and C. G. Vorhees, *Amer. Chem. Journ.* **15.** No. 6 (1894) ; T. B. Osborne and C. G. Vorhees, *Conn. Agric. Exper. Station*, 1893, p. 175.
[5] Osborne and Vorhees, *ibid.* 1893, p. 211.
[6] T. B. Osborne and G. F. Campbell, *ibid.* 1895, p. 239.
[7] H. Ritthausen, *Journ. f. prakt. Chem.* (2) **23.** 412 (1881) ; (2) **25.** 140 (1882).
[8] R. H. Chittenden and L. B. Mendel, *Journ. of Physiol.* **17.** 48 (1891).
[9] T. B. Osborne, *Amer. Chem. Journ.* **14.** No. 8 (1893).
[10] T. B. Osborne, *Conn. Agric. Exper. Station*, 1901, p. 443.
[11] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **37.** 499 (1903).
[12] W. Hausmann, *ibid.* **29.** 136 (1900).
[13] W. Erb, *Zeitschr. f. Biol.* **41.** 309 (1901).
[14] T. B. Osborne and J. F. Harris, *Journ. Amer. Chem. Soc.* **25.** 474 (1903).
[15] T. B. Osborne and J. F. Harris, *ibid.* **25.** 837 (1903).
[16] W. Erb. *Zeitschr. f. Biol.* **41.** 309 (1901).
[17] T. B. Osborne, *Zeitschr. f. physiol. Chem.* **33.** 225 and 240 (1901).
[18] O. Schmiedeberg, *ibid.* **1.** 205 (1887).
[19] E. Drechsel, *Journ. f. prakt. Chem.* (2) **19.** 331 (1879).
[20] G. Grübler, *ibid.* **131.** 97 (1881).

very considerably. The most recent estimations by Abderhalden [1] are as follows :—

	Edestin from Cotton Seed.	Edestin from Sunflowers.
	Percentage.	Percentage.
Glycocoll	1·2	2·5
Alanin	4·5	4·5
Aminovalerianic Acid .	+	0·6
α-Prolin	2·3	2·8
Leucin	15·5	12·9
Glutaminic Acid . .	17·2	13·0
Aspartic Acid . . .	2·9	3·2
Phenylalanin . . .	3·9	4·0
Serin	0·4	0·2
Tyrosin	2·3	2·0
Tryptophane . . .	+	+

2. Plant Caseins. Phyto-vitellines. Legumins. Conglutins

Under this heading a number of substances have been classified, which are insoluble in water and in salt-solutions, but soluble in dilute alkalies, and precipitable from their alkaline solutions by means of acids. According to Wiman,[2] the legumin of peas contains 0·35 per cent phosphorus, and yields on peptic digestion a pseudo-nuclein. It is therefore a nucleo-albumin, and seems to be identical with the albumins of lupines and of other plants described by Palladin [3] and Vines.[4] A phosphorus-containing albumin is also contained in wheat, according to Morishima.[5]

Legumin gives the reactions of nucleo-albumins ; it can be coagulated only partially, and is denaturalised by alcohol only very slowly. Sulphuric acid, if in excess or on heating, redissolves the precipitate ; the biuret-reaction is not violet, but red. Because of this colour-reaction, their nitric-acid reaction, and their partial coagulability, these substances have occasionally [6] been classified with the albumoses, but this is not justifiable. Weyl's [7] plant-myosin, which coagulates between 55° and 60°, is, according to Palladin,[3] the lime salt of vitelline. The following vitellines have been investigated more thoroughly :—

(*a*) The gluten-casein of wheat. It forms the, in alcohol, water, and salt-solutions, insoluble fraction of wheat-gluten. It has been

[1] E. Abderhalden, *Zeitsch. f. physiol. Chem.* **44**. 265 (1905).
[2] A. Wiman, *Maly's Jahresber. f. Tierchem.* **27**. 21 (1897).
[3] W. Palladin, *Zeitschr. f. Biolog.* **31**. 191 (1895).
[4] S. H. Vines, *Journ. of Physiol.* **3**. 93 (1880).
[5] K. Morishima, *Arch. f. experim. Path. u. Pharm.* **41**. 345 (1898).
[6] S. H. Martin, *Journ. of Physiol.* **6**. 336 (1885).
[7] T. Weyl, *Zeitschr. f. physiol. Chem.* **1**. 72 (1877).

analysed by v. Ritthausen[1] and Osborne.[2] The dissociation-products are given on p. 71, No. 17.

(*b*) Plant-caseins from other cereals. Similar substances have also been found in rye,[3] maize,[4] spelt,[5] and barley.[6] They constitute nearly 50 per cent of the total albumin.

(*c*) Legumins from pulse. Ritthausen[5] has found albumins in oats, buckwheat, and especially in the different forms of pulse: peas, vetches, beans, lentils, etc. The albumins called legumins by Ritthausen are globulins, according to Osborne, who has examined a large number of these substances.[7] The dissociation-products are given on p. 72, No. 19.

(*d*) Conglutins. This name has been introduced by Ritthausen for the albumins of lupines, almonds, nuts, etc. Analyses have been made by Ritthausen and Osborne.[8] The dissociation-products are given on p. 72, No. 18. The conglutins are of especial interest because of the physiological researches which E. Schulze made into the germination and metabolism of lupines.

3. Alcohol-soluble Albumins of Cereals

(*Gluten-fibrin, Mucedin, Zein*)

Ritthausen was the first to point out that the seeds of wheat, rye, barley, maize, and oats contain, in addition to plant-caseins, other albumins which are insoluble in water and in salt-solutions, but readily soluble in dilute alcohol. These alcohol-soluble bodies, along with the insoluble gluten-casein, form the so-called gluten, an adhesive, glue-like material, which gives to dough its toughness. Because gluten resembles fibrin, Weyl and Bischoff[9] have assumed an albumin

[1] H. Ritthausen, see p. 371.

[2] T. B. Osborne and C. G. Vorhees, *Amer. Chem. Journ.* **15**. 392 (1893).

[3] H. Ritthausen, see above, p. 371; T. B. Osborne, *Journ. Amer. Chem. Soc.* **17**. 429 (1895).

[4] H. Ritthausen, see above, p. 371; R. H. Chittenden and T. B. Osborne, *Amer. Chem. Journ.* **13**. No. 7, and **14**. No. 1 (1892); T. B. Osborne, *Journ. Amer. Chem. Soc.* **19**. 525 (1897).

[5] H. Ritthausen, see above, p. 371.

[6] H. Ritthausen, see above, p. 371; T. B. Osborne, *Journ. Amer. Chem. Soc.* **17**. 539 (1895).

[7] T. B. Osborne, *ibid.*: '*Vigna Catjang*,' **19**. 494; '*Phaseolus radiatus*,' **19**. 509 (1897); 'Pea,' **20**. 348; 'Vetch,' **20**. 406; '*Vicia Faba*,' **20**. 393; '*Glycine hispida*,' **20**. 419; 'Pea, Lentil, Bean, Vetch,' **20**. 410 (1898); '*Phaseolus vulgaris*,' *Conn. Agric. Exper. Station*, 1893, p. 186; 'Cotton,' *ibid.* 1893, p. 211.

[8] T. B. Osborne, *ibid.* **19**. 454 (1897).

[9] T. Weyl and Bischoff, *Ber. d. deutsch. chem. Ges.* **13**. I. 367 (1880).

to exist in wheat comparable to fibrinogen, which is coagulated by ferment action. Johannsen [1] and Osborne [2] have, however, shown the incorrectness of this view. In other cereals the formation of gluten is less pronounced or is absent altogether. The alcohol-soluble wheat-gluten, the plant-glue of the older authors, Ritthausen thought he could fractionate in respect of their alcohol-solubilities into three substances, namely, gluten-fibrin, gliadin, and mucedin. König and Rintelen [3] have confirmed Ritthausen as to existence of three alcohol-soluble proteids. Kossel and Kutscher [4] then showed that lysin is absent in the alcohol-soluble albumin of wheat-gluten, while it is present in Ritthausen's water-soluble gluten-casein. Gliadin and mucedin, according to Kutscher,[5] yielded the same dissociation-products in the same quantities, and were therefore probably identical; while gluten-fibrin was different, as much less glutaminic acid could be obtained from it. The view of Morishima [6] that gluten is composed of one substance 'artolin' is certainly wrong.[7] Osborne and Voorhees [8] and Osborne and Harris [9] state, however, that there is only one alcohol-soluble proteid present in wheat, which they call gliadin and which is characterised by an exceedingly high percentage of glutaminic acid, for the mineral average of the latter in four preparations obtained by treating gliadin with HCL amounted to 36 per cent, the highest figure being 37·3 per cent, and after hydrolysis with H_2SO_4 to 25·3 per cent. According to Osborne and Harris, the statement of Nasmith [10] that the alcohol-soluble proteids contain phosphorus is wrong.

By digesting the hydrochloride of the wheat-gluten 'artolin' with pepsin Hayashi [11] found it to become converted after a short digestion into an albumose, the 'artose' which, apart from a higher water percentage, possessed the same composition as artoline, namely,

[1] W. Johannsen, *Travaux du laboratoire de Carlsberg*, **2.** 199 (1888).

[2] T. B. Osborne and C. S. Vorhees, *Conn. Agric. Exper. Station*, 1893, p. 175.

[3] König and Rintelen, *Zeitschr. f. Unters. d. Nahrungs- u. Genuss-mittel*, **8.** 401 (1904).

[4] Kossel and Kutscher, *Zeitschr. f. Physiol. Chem.* **31.** 165 (1901).

[5] F. Kutscher, *ibid.* **38.** 111 (1903).

[6] K. Morishima, *Arch. f. experim. Path. u. Pharm.* **41.** 345 (1898).

[7] A. Kossel and F. Kutscher, *Zeitschr. f. physiol. Chem.* **31.** 165 (1900); F. Kutscher, *ibid.* **38.** 111 (1903).

[8] Osborne and Voorhees, *Amer. Chem. Journ.* **15.** 392 (1893); T. B. Osborne, 'Wheat,' *ibid.* **15.** No. 6 (1894); *Conn. Agric. Exper. Station*, 1893, p. 175; 'Rye,' *Journ. Amer. Chem. Soc.* **17.** 429 (1895); 'Barley,' *ibid.* **17.** 541 (1895).

[9] T. B. Osborne and F. Harris, *Amer. Journ. of Physiol.* **13.** 35 (1905).

[10] Nasmith, *Trans. of the Canadian Inst.* 1983, vii.

[11] H. Hayashi, *Arch. f. experim. Path. u. Pharm.* **52.** 289 (1904-5).

$$C_{185}H_{288}N_{50}SO_{58} + 2\ HCL.$$

After four days' digestion a jelly-like compound called metartose was split off. It is poor in sulphur, acid in character, and not acted upon by gastric juice.

$$C_{315}H_{504}N_{90}SO_{106}.$$

In addition to the latter a water-soluble complex with more sulphur is formed, namely, parartose:

$$C_{120}H_{192}N_{30}SO_{40},$$

also traces of a water-insoluble hetero-artose,

$$\underset{\text{Artose}}{3\ (C_{185}H_{288}N_{50}SO_{58})} + 12\ H_2O = 2\ \underset{\text{Parartose.}}{(C_{120}H_{192}N_{30}SO_{40})} + \underset{\text{Metartose.}}{C_{315}H_{504}N_{90}SO_{106}}$$

After eight days' digestion are formed the indigestible metartose and three derivatives of parartose, namely:

proto-artose, $C_{185}H_{300}N_{50}S_2O_{61}$ (rich in S.)
hetero- $C_{74}H_{130}N_{20}SO_{24}$
deutero- $C_{156}H_{244}N_{40}SO_{56}$ (poorer in S.)

and finally,

artolin-antipeptone = $C_{11}H_{19}N_3O_5$ (no S.).

The alcohol-soluble albumins have been analysed by Ritthausen and by Osborne.[1]

The dissociation products are given on p. 71, Nos. 14 to 16.

In maize is found zein, which is characterised by its solubility in even strong alcohol. In absolute alcohol it is, according to Osborne,[2] insoluble, but is readily soluble in 96 per cent alcohol, and may be precipitated by the addition of ether. Szumowski[3] has made use of this solubility of zein, which distinguishes it from all other albumins, proteids, and albumoses, in tracing its course through the body. Zein is further characterised by becoming very readily quite insoluble in contact with water, and then it is also not readily attacked by digestive enzymes. Analyses are given by Ritthausen and Chittenden and Osborne.[4] Its dissociation-products are given on p. 71, No. 13. Zein does not contain any lysin.

[1] T. B. Osborne, 'Wheat,' *Amer. Chem. Journ.* **15.** No. 6 (1894); *Conn. Agri. Exper. Station*, 1893, p. 175; 'Rye,' *Journ. Amer. Chem. Soc.* **17.** 429 (1895); 'Barley,' *ibid.* **17.** 541 (1895).

[2] T. B. Osborne, *ibid.* **19.** 525 (1897).

[3] W. Szumowski, *Zeitschr. f. physiol. Chem.* **36.** 198 (1902).

[4] R. H. Chittenden and T. B. Osborne, *Amer. Chem. Journ.* 1892.

IV. Fibrinogen and Fibrin

Fibrinogen

Fibrinogen, myosin, and casein assume a firm state of aggregation when acted upon by certain ferments.[1] These firm compounds are intermediate between the soluble condition—in which the substances may be obtained by the salting-out process—and the completely coagulated state into which they pass on becoming denaturalised. When semi-coagulated they are insoluble in water and in salt-solutions, but converted into completely coagulated, denaturalised compounds by such agencies as heat, alcohol, and formaldehyde.[2]

Ramsden[3] compares coagulated albumins with the mechanical aggregates (see p. 274) which are produced by shaking, and which are also semi-coagulated. Cohnheim is of the opinion that this view is not permissible, because, according to Hammarsten,[4] fibrinogen which has gradually become insoluble differs essentially from fibrin. That Ramsden does not agree to this is shown on p. 382.

Fibrinogen is contained in the blood plasma of all vertebrates. As soon as the blood leaves the arteries, and under pathological conditions even in the blood-vessels, it is changed by the fibrin ferment into fibrin, and to the latter the clotting of blood is due. The fluid portion of the blood before coagulation is called plasma, and after coagulation, when it no longer contains fibrinogen, it is termed serum. The first accurate observations on blood-coagulation were made by Denis[5] and by Alexander Schmidt,[6] who discovered the fibrin ferment. A great step forward was taken when Hammarsten[7] discovered the function of the soluble lime salts during coagulation; when he taught us how to prepare fibrinogen in a pure state, and when he showed that fibrinogen is the only albumin which is concerned in coagulation. After Hammarsten, the part played by the lime salts has been

[1] The most recent papers on blood ferments are by P. Morawitz, *Hofmeister's Beiträge*, **5**. 133 (1904); J. Bordet and O. Gengou, *Ann. de l'Inst. Pasteur*, **18**. 26 (1904).

[2] A. Benedicenti, *Arch. f.* (*Anat. u.*) *Physiol.*, Physiol. Abteilung, 1897, p. 219.

[3] W. Ramsden, *ibid.* 1894, p. 517.

[4] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **22**. 333 (1896).

[5] Denis, *Mémoires sur le sang*, Paris, 1859.

[6] Al. Schmidt, *Arch. f.* (*Anat. u.*) *Physiol.* 1861, p. 682; E. Samson-Himmelstjerna, *Dissert.*, Dorpat, 1882; Al. Schmidt, *Die Lehre von den fermentativen Gerinnungsvorgängen*, Dorpat, 1876; collected papers abstracted in *Zur Blutlehre*, Leipzig, 1892, and *Weitere Beiträge zur Blutlehre*, Wiesbaden, 1895.

[7] O. Hammarsten, *Untersuchungen über die Faserstoffgerinnung*, *Nova acta societ. scientiar. Upsaliensis*, ser. iii. vol. x. 1 (1875); *Pflüger's Arch. f. d. ges. Physiol.* **14**. 211 (1876); **19**. 563 (1879); **22**. 431 (1880); **30**. 437 (1883); *Zeitschr. f. physiol. Chem.* **22**. 333 (1896); **28**. 98 (1899).

especially investigated by Arthus.[1] According to the latest statements of Hammarsten, fibrinogen is preformed in the blood, and becomes coagulated equally well whether present in the blood or after isolation in a pure form. It is coagulated by a ferment which is derived from the formed elements of the blood. The leucocytes or red corpuscles do not, however, give rise to the ferment directly, but to its zymogen, which is converted in the plasma into the active ferment. Space forbids to enter more fully into the question of coagulation, and into the question as to how pro-ferments are converted into ferments.[2] One fact, however, Hammarsten has definitely proved, namely, that the fibrin-ferment, if once formed, is capable of converting fibrinogen into fibrin, even in the absence of lime. Fibrin is neither a fibrinogen-lime compound, nor has lime anything to do with fibrinogen or with fibrin. The slight amounts of lime, namely, 0·006 per cent, which Hammarsten found in preparations made from oxalate-plasma solutions, are not specific for fibrin, but only correspond to the ash, which is present also in other albumins.

Fibrin-ferment has the properties of other ferments: it is active in very small amounts; Hammarsten prepared an exceedingly active solution, which contained only 0·3 per 1000 of solid ingredients; it is destroyed by heat, and also on being kept for some time in alcohol; it cannot, of course, be prepared in a pure state. Fibrin-ferment or substances which call forth the coagulation of blood, or at least hasten it, and which cause clotting even in the circulating blood, have been found by Alexander Schmidt and by Wooldridge[3] in all organs rich in cells.

On the other hand, leeches, crab-muscle, and other bodies contain substances which enormously retard coagulation on being introduced into the circulation. Pick and Spiro[4] found a body giving analogous reactions in the digestive organs, namely, the "peptozyme." This enzyme, by attaching itself to the albumoses and peptones formed during digestion, is the real cause which prevents the coagulation of blood if albumoses, Witte's peptone, and other peptone-preparations are injected into the circulation. This anti-coagulative property of the products of digestion was discovered by Schmidt-Mühlheim[5] and

[1] M. Arthus and C. Pagès, *Arch. de Physiol. normale et pathologique*, 1890, p. 739; M. Arthus, *ibid.* 1894, p. 552; 1896, p. 47; and Thèse, 1890. Good account of literature. See also *Compt. rend. Soc. Biol.* **53**. 962 and 1024 (1901). A very complete account of the literature is given by Lilienfeld, *Zeitschr. f. physiol. Chem.* **20**. 89 (1894).

[2] P. Morawitz, *Hofmeister's Beiträge*, **4**. 381 (1903); *Deutsch. Arch. f. klin. Med.* **79**. 1 (1903); and *Hofmeister's Beiträge*, **5**. 133 (1904); E. Fuld and K. Spiro, *ibid.* **5**. 171 (1904).

[3] L. C. Wooldridge, *Archiv. f.* (*Anat. u.*) *Physiol.* 1886, p. 397.

[4] E. P. Pick and K. Spiro, *Zeitschr. f. physiol. Chem.* **31**. 235 (1900).

[5] A. Schmidt-Mülheim, *Archiv f.* (*Anat. u.*) *Physiol.* 1880, p. 33.

Fano.[1] That the protamins act in a similar manner has been shown by Thompson.[2] The nucleo-histone of the thymus (Lilienfeld[3]) as well as other albuminous substances, and derivatives from the most diverse organs all act as anti-coagulators.

Coagulation-temperature.—The coagulation-temperature of fibrinogen, according to Frédéricq,[4] is 56°. It is of special interest that he obtained three distinct coagulation-temperatures, at 56°, 67°, and 75°, belonging to the three distinct albumins in the case of plasma; while with serum no sharp demarcation occurs between 64° and 75°. The reason for this behaviour is that fibrinogen gives rise to fibrin-globulin coagulating at 64°, and that certain cell-albumins are liberated by the disintegration of blood-corpuscles during clotting.

Frédéricq[5] found in blood-plasma 0·4299 per cent fibrinogen, while Reye[6] obtained 0·3479 per cent. Fibrinogen occurs also in lymph and in pathological transudations. Fibrinogen has not been prepared in a crystalline form.[7] Analysis gives the following figures:—

C	H	N	S	O	
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
52·93	6·9	16·66	1·25	22·26	Hammarsten.[8]
...	...	16·4	...	...	Cramer.[9]
...	...	...	1·13	...	Mörner.[10]

The dissociation-products are given on p. 71, No. 9.

Sulphur does not only occur as cystin, according to Mörner.[10]

Salts of fibrinogen and halogen compounds are not known.

Fibrinogen shows the general characteristics of the globulins; it is insoluble in water, but soluble in salt-solutions. It is further soluble in dilute alkalies and in the alkali-carbonates; it is, however, precipitated by the addition of very minute traces of neutral salts, and passes again into solution on the addition of further quantities of salt.[8] It is also precipitated by dilution with water, by dialysis, by the passage of a stream of carbon-dioxide, and by acetic acid. Precipitation is, however, as in the case of globulins, not a complete one.

[1] Fano, *Archiv. f.* (*Anat. u.*) *Physiol.* 1881, p. 277.

[2] W. H. Thompson, *Zeitschr. f. physiol. Chem.* **29**. 1 (1899).

[3] L. Lilienfeld, *ibid.* **20**. 89 (1894).

[4] L. Frédéricq, *Bull. de l'Acad. royale de Belgique*, 2nd ser. **64**. 7 (1877) (reprint); *Ann. de Soc. de Médecine de Gant*, 1877 (reprint).

[5] L. Frédéricq, *Bull. de l'Acad. royale de Belgique*, 2nd ser. **64**. 7 (1877) (reprint).

[6] W. Reye, *Dissertation*, Strassburg, 1898.

[7] S. Dziergowski, *Zeitschr. f. physiol. Chem.* **28**. 65 (1899).

[8] O. Hammarsten, *Pflüger's Arch. f. d. ges. Physiol.* **22**. 431 (1880).

[9] C. D. Cramer, *Zeitschr. f. physiol. Chem.* **23**. 74 (1897).

[10] K. A. H. Mörner, *ibid.* **34**. 207 (1901).

The salting-out limits for ammonium sulphate are given by Reye [1] as lying between 1·7 to 1·9 and 2·5 to 2·8, according to the concentration of the solution. As the lower limit for globulins lies between 2·7 and 3·1, Reye obtains fibrinogen by adding to 100 parts of plasma 40 parts of saturated ammonium-sulphate solution. Magnesium sulphate and sodium chloride [2] salt out fibrinogen even before complete saturation. Hammarsten obtains his pure fibrinogen by the addition of an equal volume of saturated salt-solution to plasma, and obtains in this way a pure preparation, but only in small amounts. Horse's blood is most suitable, but other blood may be used if its coagulation has been prevented by the addition of 1 : 1000 of sodium oxalate or sodium fluoride.

If one wishes to examine pure coagulated fibrin, it is best to heat blood-plasma very carefully to 56°. The fibrin prepared in the usual way by beating blood with twigs always contains cell-remnants, hæmoglobin, and large amounts of globulin; it must, in addition to water, be also washed out very thoroughly with 3 per cent sodium chloride, but it is even then very impure.

Precipitated fibrinogen is a tough, very elastic, glutinous body of the consistency of coagulated blood. It becomes even more rapidly insoluble than do all the other globulins, it being quite immaterial whether it be precipitated by water or acids or salts; this denaturalisation occurs with special rapidity in the presence of lime salts,[1] a phenomenon met with to a certain extent in all albumins. The insoluble fibrinogen is absolutely distinct from the coagulated fibrin, it is a true denaturalised albumin.[3] Many erroneous statements [3] are due to no distinction having been made between this coagulated fibrinogen fibrin and the readily formed insoluble fibrinogen-lime compound. Even when in solution [3] fibrinogen rapidly deteriorates and becomes uncoagulable if dialysis be prolonged too much.

Fibrin

Under the influence of the fibrin-ferment fibrinogen is changed into fibrin. How this is brought about is not known, but whenever fibrin is formed, an albumin, the so-called fibrin-globulin,[4] remains in solution, immaterial whether fibrinogen is coagulated by being warmed

[1] W. Reye, *Medical Dissertation*, Strassburg, 1898.

[2] O. Hammarsten, *Pflüger's Arch. f. d. ges. Physiol.* **22.** 431 (1880).

[3] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **22.** 333 (1896).

[4] O. Hammarsten, *Pflüger's Arch. f. d. ges. Physiol.* **22.** 431 (1880); **30.** 437 (1883); *Zeitschr. f. physiol. Chem.* **28.** 98 (1899); L. Frédéricq, *Bull. de l'Acad. royale de Belgique*, 2nd ser. **64.** 7 (1877); O. Hammarsten, *Zeitschr. f. physiol. Chem.* **22.** 333 (1896).

to 56°, or is precipitated by acetic acid, or is acted upon by the fibrin-ferment.

The process of fibrin formation is represented by Halliburton[1] in the following tabular way :—

In the plasma a proteid substance exists, called : Fibrinogen.	From the colourless corpuscles a nucleoproteid is shed out, called : Prothrombin. By the action of calcium salts prothrombin is converted into fibrin-ferment, or Thrombin.

Thrombin acts on fibrinogen in such a way that two new substances are formed.

One of these is unimportant, viz. a globulin (fibrino-globulin) which remains in solution. Its amount is very small.	The other is important, viz. fibrin, which entangles the corpuscles, and so forms the clot.

This fibrin-globulin has the same solubilities as have other globulins, the same precipitation-limits for ammonium sulphate as has fibrinogen, and a coagulation-temperature of 64°. The percentage composition of fibrinogen, fibrin, and fibrin-globulin is somewhat different. Serum always contains fibrin-globulin.

Lilienfield,[2] Frederikse,[3] Schmiedeberg[4] and Heubner[5] are of the opinion that coagulation depends on a hydrolytic dissociation of fibrinogen into fibrin and fibrin-globulin; Hammarsten at one time also believed in this view, but now he believes[6] that fibrinogen is completely changed into fibrin by the fibrin-ferment, but that only a portion of the fibrin is precipitated, while the rest remains in solution. All investigations into this matter are very difficult, as fibrin carries down other albumins with it when it is being formed. According to Hammarsten,[6] 77 to 80 per cent of fibrinogen is converted into fibrin, while Heubner gives lower figures.

Ramsden, in a paper not yet published, states that fibrinogen-solutions, free from fibrin-ferment, can be made to yield 'mechanical surface aggregates' indistinguishable from typical fibrin, and that

[1] Halliburton, *Handbook of Physiology*, 1904, p. 414.
[2] L. Lilienfeld, *Zeitschr. f. physiol. Chem.* **20**. 89 (1894).
[3] J. J. Frederikse, *ibid.* **19**. 143 (1894).
[4] O. Schmiedeberg, *Arch. f. experiment. Pathol. und Pharmak.* **39**. 1 (1897).
[5] W. Heubner, *ibid.* **49**. 229 (1903).
[6] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **28**. 98 (1899).

fibrinogen, mechanically-produced fibrin, and ferment-produced fibrin have the same heat-coagulation temperature, 53°-58°.

The necessity of 'fibrin-ferment' for the normal coagulation of bloodplasma is beyond question, but there is no evidence that the change produced by it is chemical. The facts at present known are consistent with the view that the change from fibrinogen to fibrin, however produced, is purely physical in nature, and analogous to the coagulation-change in certain inorganic colloids (Ramsden).

The observations on which Lilienfeld based his theory of blood-coagulation are of great interest in this connection, as showing that fibrin can be obtained in the absence of any fibrin-ferment or prothrombin by the mere addition of calcium chloride to alkaline solutions of fibrinogen poor in sodium chloride. It is maintained by Ramsden that typical fibrin may be obtained in at least four different ways, and that in none of them is there evidence of chemical change:—

(1) By the action of fibrin-ferment on fibrinogen-solutions.
(2) By the action of appropriate electrolytes—Lilienfeld fibrin.
(3) By the mechanical aggregations and impactions of the surface coating of fibrinogen-solutions—mechanical fibrin.
(4) By the spontaneous change of precipitated fibrinogen.

How the agglutinins and precipitins of the pathologist produce their specific effect on the surface tension of the suspended bacteria or proteid particles in colloid solutions, in virtue of which bacteria or colloid particles clump together or agglutinate, is unknown. If the change in surface tension should prove to be independent of any precedent chemical change in the bacterial envelope or the suspended colloidal particles, it will be possible, Ramsden points out, to regard precipitins, agglutinins, fibrin-ferment, and electrolytes as all playing essentially similar parts in producing precipitation, 'clumping,' or gelation by altering the surface tension of fine particles in suspension.[1] Fibrin-ferment might thus be regarded as a specific precipitin for fibrinogen, differing from other precipitins only in the fact that it is developed in the organism in response to the presence of a normal constituent of the blood, whereas the precipitins investigated by the pathologist are produced only in response to the presence of substances which are not normal constituents of the blood.

Fibrin is a tough, strongly elastic, jelly-like substance. It is very voluminous, as, notwithstanding its small amount, it converts the whole of the blood into a solid. It is denaturalised by heat, alcohol, formaldehyde,[2] and prolonged action of salts. Changed in this way

[1] The author believes all alterations in surface tensions to depend on chemical change.

[2] A. Benedicenti, *Arch. f.* (*Anat. u.*) *Physiol.* 1897, p. 219.

it behaves as does any other coagulated albumin. As long as it is not coagulated, it is to a certain extent soluble in acids[1] and alkalies, according to Limbourg,[2] and also in urea, according to Spiro.[3] This passing into solution depends, however, probably on the formation of acid-albumins and of alkali-albuminates, while its solution in very dilute acids and alkalies, as well as in salts (Fermi, Limbourg), must be explained as due to the presence of proteolytic ferments or their zymogens, which are absorbed into the blood, and which are precipitated on the fibrin whenever the latter is formed. Fibrin also frequently encloses considerable amounts of serum-globulin, which is liberated when fibrin is digested, and this liberated globulin led the older investigators to state that globulin was the first product of fibrin-digestion. Pepsin and trypsin attack fibrin with great readiness, and attention has already been drawn to the fact that fibrin has been used for many experiments on digestion. Witte-peptone is said to be digested fibrin.

Halliburton[4] prepared from the blood of crayfish a fibrinogen, which, apart from having a coagulation-temperature of 65°, behaves in every other respect as does that of vertebrates. Loeb[5] has studied the resemblances and the differences in the coagulation of the blood of vertebrates (guinea-pig, birds) and invertebrates (arthropods).

V. The Muscle-Albumins

The fluid contents of the sarcolemma-sheaths of striped muscle, or the sarcoplasma, contains peculiar albumins in solution, which were first investigated by Kühne,[6] and then by Halliburton[7] and v. Fürth.[8] Kühne prepared a muscle-plasma by freezing frog-muscles, and then pounding them to break up the sarcolemma-sheaths. From the plasma so obtained he isolated myosin, which, by coagulating spontaneously, passes over into a fibrin-like modification. Rigor mortis depends on the coagulation of this myosin. The coagulated

[1] C. Fermi, *Zeitschr. f. Biol.* **28**. 229 (1891); G. Wolffhügel, *Pflüger's Arch.* **7**. 188 (1873).

[2] P. Limbourg, *Zeitschr. f. physiol. Chem.* **13**. 450 (1889).

[3] K. Spiro, *ibid.* **30**. 182 (1900).

[4] W. D. Halliburton, *Journ. of Physiol.* **6**. 300 (1885).

[5] Leo Loeb, *Hofmeister's Beiträge*, **5**. 191 and 534 (1904).

[6] W. Kühne, *Arch. f. (Anat. u.) Physiol.* 1859, p. 748; *Text-book of Physiological Chemistry*, p. 272 (1868).

[7] W. D. Halliburton, *Journ. of Physiol.* **8**. 133 (1887); German translation of text-book of physiology by K. Kaiser, 1892, p. 425.

[8] O. v. Fürth, *Arch. f. experim. Pathol. u. Pharm.* **36**. 231 (1895); *Zeitschr. f. physiol. Chem.* **31**. 338 (1900); *Ergebnisse der Physiologie*, I. **1**. 110 (1902); *Hofmeister's Beiträge*, **3**. 543 (1903).

myosin Kühne believed to be partially soluble in salt-solutions, and this solution to possess a coagulation-temperature of 56°. In the fluid which remained after the myosin had coagulated, the so-called muscle-serum, Kühne found, in addition to other, not well-defined, albuminous substances, an albumin which coagulated at 47°, and which he held to be uncoagulated myosin. Halliburton, on investigating the albumins of mammalian muscle, obtained four different proteids in the muscle-plasma by extracting muscle with 5 per cent magnesium sulphate :—

1. A globulin precipitable by heat at 47° C. (paramyosinogen).
2. A globulin precipitable by heat at 56° C. (myosinogen).
3. A globulin precipitable by heat at 63° C. (myoglobulin), occurring in the serum.
4. Traces of an albumin (myoalbumin).

Halliburton now holds with v. Fürth [1] that myoglobulin is some myosinogen which has escaped coagulation, and that the myoalbumin is derived from adherent blood and lymph. v. Fürth maintains that mammalian muscle freed from blood and lymph, and extracted with normal salt-solution, only contains 'myosin' or paramyosinogen, coagulating between 47°-50°, and 'myogen' or myosinogen, coagulating between 55°-60°. Stewart and Sollmann [2] say, "there exist in dead muscle two proteids, a true globulin, paramyosinogen, coagulating at about 45°-50°, and an atypical globulin : myosinogen, coagulating at about 50°-65°. The latter very readily passes into a modification very similar to, if not identical with, the former." Paramyosinogen seems to be more abundant in dead muscle than myosinogen, or at least more is extracted by such saline solutions as 5 per cent magnesium sulphate. Vincent and Lewis [3] also favour the view that paramyosinogen and myosinogen appear, in fact, to be interchangeable one with the other, or to be possibly both formed from some common precursor present in living muscular tissue which on heating coagulates at about 47°. Vincent and Lewis further applied the graphic method introduced by Brodie and Richardson [4] for registering the contraction of muscles during different temperatures. "Both striped and unstriped mammalian muscle, on being subjected to a gradually rising temperature, show two marked sudden contractions—

[1] Halliburton, *Handbook of Physiology*, 1904, p. 156.

[2] Stewart and Sollmann, *Journal of Physiol.* **24.** 427 (1899).

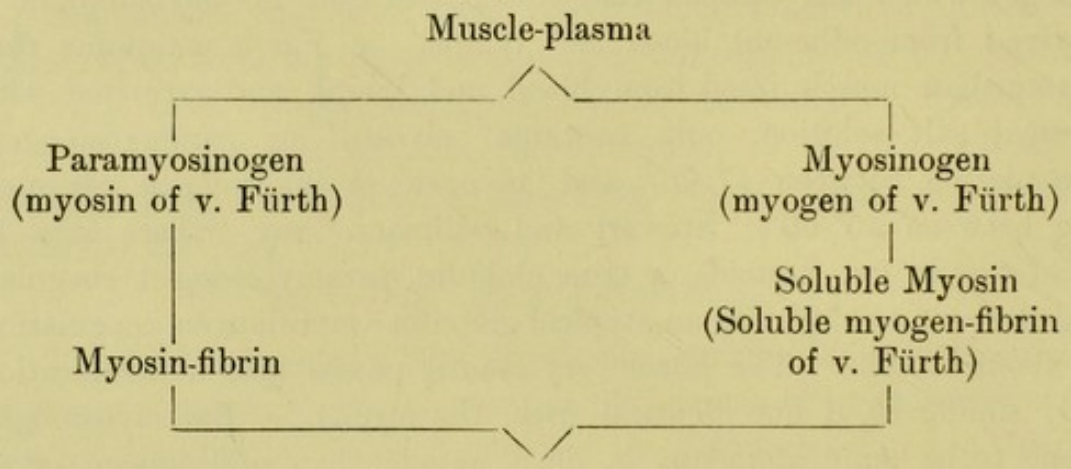
[3] Swale Vincent and Thomas Lewis, *ibid.* **26.** 445 (1901).

[4] Brodie and Richardson, *ibid.* **21.** 353 (1897), and *Phil. Trans.* **191.** 127 (1899). See also H. M. Veron, *Journal of Physiol.* **24.** 239 (1899).

(1) at 47°-50°, (2) at about 63°; and also a tendency to contract at about 56°. The first of these, we conclude, is due to the coagulation by heat of the proteid substance present in the muscle-fibre during life ('paramyosinogen'), the second to changes in the connective-tissue elements of the muscle;[1] the slight change at 56° we attribute to the presence of small quantities of 'myosinogen,' this slight change being the more marked, but never larger, in muscle in partial rigor."

"Amphibian muscle shows marked differences from the above when submitted to heat rigor experiments. The striped muscle of these animals gives a contraction at 38°-40°, due to coagulation of soluble myogen-fibrin, and another at 45°-50°. The unstriped muscle gives only a marked contraction at 54°, sometimes a slight one at 47°. The former contraction, we think, is due to the connective tissue."

Kühne's soluble myosin Halliburton believes to be the mother-substance of myosin and calls it myosinogen. This change is expressed diagrammatically by Halliburton[2] thus :—



There is thus a complete analogy between fibrinogen- and myosinogen-coagulation. v. Fürth agrees with Halliburton in assuming two coagulable substances to exist, namely, the myosin and the myogen. Przibram[3] and Steyrer[4] have also adopted Halliburton's view in all essential points.

Przibram has studied the distribution of paramyosinogen and myosinogen amongst different classes of animals, and has arrived at results which Halliburton has summarised as follows :—

Invertebrates : paramyosinogen present ; myosinogen absent.

Vertebrates : paramyosinogen and myosinogen both present.

[1] Brodie and Richardson. See footnote, p. 385.

[2] Halliburton, *Handbook of Physiology*, 1904, p. 156 ; and *Biochemistry of Muscle and Nerve*, 1904, p. 10. Murray, London.

[3] H. Przibram, *Hofmeister's Beiträge*, **2**. 143 (1902).

[4] A. Steyrer, *ibid.* **4**. 234 (1902).

Fishes: in addition to these two principal proteids, soluble myogen-fibrin and myoproteid (in large quantities) occur.

Amphibians: like fishes, except that myoproteid is only present in traces.

Reptiles, birds, animals: myoproteid is absent, and soluble myogen-fibrin is only present where rigor mortis commences.

According to Steyrer,[1] paramyosinogen increases in muscle which is degenerating after the division of its motor nerve, while it decreases after prolonged tetanisation.

There are, however, as yet, a whole number of unexplained points in the chemistry of the muscle-albumins, and nowhere does the insufficiency of our methods for separating different albumins make itself so much felt as just in the case of the muscle-albumins, for they are very apt to change their properties in the course of the investigation.

Not even the existence of two coagulable substances is beyond dispute, for the differences which have been observed may well be explained on the assumption that one of the substances is a free albumin, while the other is a salt of the albumin. Palladin has shown (see p. 374) that the so-called myosin of many seeds is simply a lime-salt of vitelline, and that the coagulation-temperature of vitelline is lowered 15° by the conversion into a lime salt. It is quite possible that analogous conditions prevail in muscle. If, therefore, later on, paramyosinogen and myosinogen are enumerated separately, this is done with due reserve (Cohnheim).

A series of investigations has been made into that mixture of soluble muscle-albumins which is extracted by acids and to which the name of syntonin has been given. The dissociation-products of myosin have been studied by Hart and Cohnheim (see table p. 71, No. 8), while its acid and metallic salts were investigated by Danilevsky[2] and Chittenden and Whitehouse.[3] By peptic digestion myosin is rapidly made soluble, because it is readily converted into acid-albumin, but having once been rendered soluble, all further change is slow, because a large proportion separates out as 'anti-albumid.'[4] This also occurs during peptic digestion. According to Danilevsky

[1] A. Steyrer, *Hofmeister's Beiträge*, **4**. 234 (1902).

[2] A. Danilevsky, *Zentralbl. f. d. med. Wissensch.* 1880, p. 929; *Zeitschr. f. physiol. Chem.* **5**. 158 (1881).

[3] R. H. Chittenden and H. Whitehouse, *Yale Univers.* **2**. 95 [according to *Maly's Jahresber. f. Tierchemie*, **17**. 11 (1887)].

[4] W. Kühne and R. H. Chittenden, *Zeitschr. f. Biol.* **25**. 358 (1889); R. H. Chittenden and R. Goodwin, *Journ. of Physiol.* **12**. 34 (1891).

and Schipiloff[1] solutions of myosin are doubly refractile, both in solution and when allowed to dry in thin layers.

Halliburton and v. Fürth distinguish, as already stated, two distinct albuminous substances:—

1. Paramyosinogen or Myosin.

The substance which Kühne found to coagulate at 47° Halliburton calls paramyosinogen, while v. Fürth has given to it the name of myosin. It possesses, according to v. Fürth, all the essential properties of the globulins: it is insoluble in water, readily soluble in dilute salt-solutions, from which it is precipitated by being dropped into water or by dialysis. It is also precipitated by dilute acids and by a stream of carbon dioxide, but is very soluble in an excess of the acid. It is readily salted out by diverse salts; for sodium chloride the limits lie between 15 and 26 per cent, for magnesium sulphate between 30 and 50 per cent, for ammonium sulphate between 2·2 and 3·1 (or 3·6). After having been precipitated by dialysis, salting-out, acidification, or alcohol, it very rapidly becomes insoluble, even more readily than does fibrinogen. Its most remarkable property, if kept in solution, consists, however, in its becoming very readily insoluble by passing into a state resembling fibrin. The higher the temperature the more readily does it become insoluble; thus at 40° it changes very rapidly, while at 32°-35° the whole of the myosin may be coagulated in twenty-four hours. This coagulated paramyosinogen Halliburton called paramyosin, while v. Fürth calls it myosin-fibrin. Paramyosin is contained in the coagulum which forms in dead muscle, and also in the expressed muscle-plasma; whether there is also present in addition soluble paramyosinogen in the muscle or in the serum depends on the time after death and on the temperature. According to v. Fürth paramyosinogen amounts to 20 per cent of the soluble muscle-albumins.

Its coagulation-temperature is 47°, but to ensure complete coagulation it is, as a rule, necessary to heat to 50° or 52°. Myosin, therefore, has amongst all the albumins the lowest coagulation-temperature.

2. Myosinogen or Myogen.

Halliburton calls the substance which coagulates at 56° myosinogen, and v. Fürth applies to the same substance the term 'myogen.' This substance Kühne believed to be myosin which had coagulated

[1] Cathérine Schipiloff and A. Danilevsky, *Zeitschr. f. physiol. Chem.* **5**. 349 (1881).

and which had redissolved in salt-solutions. It constitutes about 80 per cent of the muscle-albumin, and is more readily prepared than is paramyosinogen. In certain respects it resembles globulin, as v. Fürth has pointed out, for it is precipitated by acids and by being diluted with water or by dialysis; but it differs from the globulins in being only partially precipitated by dialysis and in being fairly soluble in pure water, with a neutral reaction. By mineral acids it is precipitated, but it dissolves with great ease if an excess of acid be employed. As has been known since the time of Liebig, it is especially readily converted into acid-albumin. It is precipitated by acetic acid only in the presence of neutral salts, as otherwise it is transformed at once into acid-albumin. On the other hand, myosinogen is also precipitated by alkalies and by ammonia if salts are present, and resembles in this respect the basic histones. Myosinogen is precipitated by sodium chloride and magnesium sulphate only if the solutions are quite saturated, the precipitation being even then not complete according to v. Fürth. The precipitation-limits for ammonium sulphate are 3·6 and 5·2; a certain portion is, however, only precipitated by saturated solutions.

Myosinogen does not become rapidly insoluble after precipitation as does paramyosinogen, and it is denaturalised so very slowly by alcohol that v. Fürth employed alcohol for obtaining it in a pure state. It is precipitated by the salts of the heavy metals only in the presence of neutral salts.

The coagulation-temperature of unchanged myosinogen is unanimously given at 56°, but it is very difficult to bring about a complete coagulation, particularly in solutions poor in salts, because myosinogen is very apt to be partially converted into acid-albumin and thereby to escape coagulation.

On standing, myosinogen-solutions are apt to become changed analogously to paramyosinogen-solutions. There is formed a coagulum of myogen-fibrin. During the transition from myosinogen to myogen-fibrin v. Fürth has noticed a state during which the coagulation-temperature falls to 40°. This transition-product of myogen he calls 'soluble myogen-fibrin.'

According to Kühne and Halliburton muscle-albumins are supposed to coagulate completely or nearly so, whether they are left *in situ* or whether they are pressed out of the muscle, and it is also believed that they may be extracted completely from muscle in rigor mortis by 10 per cent sodium-chloride solutions or 15 per cent ammonia.

v. Fürth, on the other hand, is of the opinion that the coagulated albumins are quite insoluble, and that the salt-soluble fraction is Hallibur-

ton's myosinogen (v. Fürth's myogen), which has escaped coagulation, and which has been carried down mechanically by the paramyosinogen (v. Fürth's myosin). In addition we must remember that only a certain proportion of the muscle-albumins separate out as insoluble substances, and v. Fürth makes the just observation, that a solidifying of the muscle-plasma, comparable to blood-coagulation, is only seen in cold-blooded animals. In warm-blooded animals the process amounts simply to the formation of a little coagulum. We must, however, not forget that during the preparation of the muscle-albumins a certain amount of the paramyosinogen and of the myosinogen become insoluble and that they are left behind in the muscle. v. Fürth has further observed that—amongst other respects—the coagulation *in situ* differs from that in the test-tube in the rapidity with which it occurs.

What really determines the coagulation of paramyosinogen and thereby gives rise to rigor mortis is not known. Halliburton regarded it as being of a fermentative nature caused by the formation of 'myosin-ferment' in the dying muscle; but now[1] he is doubtful as to whether a specific myosin-ferment brings about the change.

v. Fürth could not find such a ferment, but does not exclude the possibility of its occurrence. On the other hand, he showed that coagulation is considerably hastened by lime salts, sodium thio-cyanate, sodium salicylate, by an acid reaction, and by other factors. What causes the disappearance of rigor mortis is also completely unknown. Kühne and many of the older investigators assumed that the solution of the paramyosinogen was brought about by the formation of lactic acid in the dead muscle, but v. Fürth found the quantity of lactic acid liberated to be so small as to altogether exclude this explanation. Vogel[2] and Schmidt-Nielsen[3] advance the hypothesis of autolytic action; but again Fürth found no proteolytic ferments, while Salkowski[4] found them only in the minutest traces. A possible explanation is that the coagulum contracts within the sarcolemma, and that it squeezes out the fluid contents in a manner analogous to that occurring in coagulated blood. The observation of Mangold[5] that muscle is still contractile after having passed through rigor mortis seems to show that the chemical processes occurring during rigor are not very profound.

[1] Halliburton, *Handbook of Physiology*, 1904, p. 156.
[2] R. Vogel, *Deutsch. Arch. f. klin. Med.* **72**. 291 (1902).
[3] S. Schmidt-Nielsen, *Hofmeister's Beiträge*, **4**. 182 (1903).
[4] E. Salkowski, *Zeitschr. f. klin. Med.* **17**. Suppl. 77 (1890).
[5] E. Mangold, *Pflüger's Arch.* **96**. 498 (1903).

The author draws attention to the fact that changes analogous to rigor mortis occur in every colloidal solution which is being precipitated, for example, if a suitable electrolyte, such as acetic acid, be slowly added to a strong globulin solution, the latter becomes more and more viscous and may become semi-solid. On the further addition of acetic acid, or on waiting for some days, the viscosity disappears and the globulin solution becomes limpid.

As, according to the author's view, life is given to the albumins by the salts with which they are associated,[1] and as muscle resembles red blood-corpuscles in being very rich in potash salts, the following table of Katz[2] has been included. It is based on a very extensive research on the flesh of different animals :—

K	2·41 to 4·65
Na	0·32 to 1·56
Mg	0·18 to 0·37
Ca	0·02 to 0·39
P total	1·36 to 2·58
from phosphates . .	1·22 to 2·04
from lecithin . . .	0·13 to 0·48
from nuclein . . .	0·09 to 0·32
Cl	0·32 to 0·8
S	1·35 to 2·92
Fe	0·04 to 0·25

Heart-muscle has been specially studied by Boruttan[3] and by Bottazzi and Ducceschi.[4]

Smooth muscle has been investigated by Vincent and Lewis[5] and Velichi.[6] Vincent and Lewis investigated the sheep's stomach, and found, in confirmation of Bottazzi, that nonstriped muscle shows rigor mortis, as does striped muscle, if it be kept for some time at body-temperature. "Unstriped muscle and its extracts in dilute neutral saline solutions are neutral or alkaline, while those of striped muscle are almost always acid. Fresh extracts of unstriped muscle made with 5 per cent magnesium sulphate appear to contain little if any

[1] See p. 211, and also p. 215, where the ring formation of amino-acids is discussed.

[2] J. Katz, *Pflüger's Arch.* **63**. 1 (1896).

[3] Boruttan, *Zeit. f. physiol. Chem.* Strassburg, **18**. 513 (1894).

[4] Bottazzi and Ducceschi, *Il Morgagni*, **39**. No. 10, and also in *Arch. ital. de Biol.* **28**. 395 (1897).

[5] Swale Vincent and Thomas Lewis, *Proc. Physiol. Soc.* Jan. 26, 1901. See *Journal of Physiol.* **26**. p. 445 (1900-1).

[6] Velichi, *Centralbl. f. Physiol.* **12**. 351 (1898).

'paramyosinogen' as indicated by heat-coagulation at 47°-50°, while there is evidence of the presence of abundance of 'myosinogen' coagulating between 55° and 65°." Velichi states that he isolated from the nonstriped muscle of the stomach two proteids :—

1. A globulin, apt to coagulate spontaneously and clotting when heated to 54°-60°.
2. An albumin, precipitated by heating to 46°-50°.

Amongst invertebrates v. Fürth [1] and Przibram [2] found substances resembling myosinogen in their reactions, but possessing, as a rule, a somewhat lower heat-coagulation-temperature. In fish-muscles v. Fürth found 'myoproteid' which is soluble in water, non-coagulable by boiling, but precipitable by strong acids; magnesium sulphate and sodium chloride salt it out, while the precipitation-limits for ammonium sulphate are 4·0 and 10·0. Sodium-hydrate solution does not precipitate even in the presence of salt. It does not contain an appreciable amount of phosphorus, and contains neither a reducing substance nor pseudo-nuclein.

The muscle of the octopus has been studied by v. Fürth,[3] and by Henze,[4] who has paid special attention to the extractives and the reserve-materials with the view of establishing some connection between these and the urinary secretion. Octopus muscle contains 77·3 per cent water and 13·13 per cent nitrogen; the watery extracts contain neither glycogen, urea, hexone-bases or amino-acids, such as glycocoll, nor creatin or creatinin. On the other hand taurin is very abundant, amounting to 0·5 per cent; the purin-bases are represented almost exclusively by hypoxanthin (0·03 per cent), the total nitrogen of the purin-bases amounting to 0·0456 per cent. Sarcolactic acid is absent, but small amounts, 0·01 per cent, of fermentable lactic acid were found. Potash salts preponderate over the sodium salts, and the amount of sulphur is about 2·5 per cent, and therefore three times as great as in vertebræ muscle.

No other soluble albumins in addition to paramyosinogen (and myosinogen) are found in the muscle substance proper, according to v. Fürth and Stewart and Sollmann; the albumin which Kühne once described is a derivative of lymph, while the myoglobulin of Halliburton represents myosinogen which has escaped coagulation.

Mays [5] has, however, found non-coagulating albumins in muscle,

[1] O. v. Fürth, *Zeitschr. f. physiol. Chem.* **31**. 338 (1900).
[2] H. Przibram, *Hofmeister's Beiträge*, **2**. 143 (1902).
[3] v. Fürth, *Zeitschr. f. physiol. Chem.* **31**. 338 (1900).
[4] M. Henze, *ibid.* **43**. 477 (1905).
[5] K. Mays, *Zeitschr. f. Biol.* **34**. 268 (1896).

and Siegfried's[1] observation as to the occurrence of carnic- or sarctic-acid in muscle must be mentioned, although it is very uncertain as to whether this acid occurs normally in muscle.[2] The observations of Mays and Siegfried have, perhaps, some bearing on those of Pekelharing[3] and Kossel,[3] who obtained a nucleo-proteid from the nuclei of muscle. Holmgren[4] has finally described a very insoluble albumin, which can only be extracted as an alkali-albuminate, and of which it is difficult to say whether it is a coagulated albumin or whether it is an albuminoid, forming a part of the muscle-stroma. It will be discussed later amongst the albuminoids.

Whether albuminous substances resembling paramyosinogen (v. Fürth's myosin) occur also in other organs is a difficult question. All tissues undergo in a certain sense rigor mortis, and therefore it is legitimate to say that substances coagulating spontaneously are present in every cell. Reinke and Rodewald[5] found such a body in the protoplasm of *Æthalium septicum*. Plósz[6] obtained from the liver an albumin having the coagulation-temperature of myosin (47°); Lilienfeld[7] a similar compound in the leucocytes of the thymus gland; Chittenden[8] obtained paramyosinogen from the retina; and Halliburton[9] isolated from many organs rich in cells (spleen, thyroid, etc.) albumins having the coagulation-temperature of myosin; he failed, however, in finding this albumin in the brain and in red corpuscles. It is also possible to extract from the pancreas an albumin which coagulates spontaneously at body-temperature, and which also resembles paramyosinogen in other respects; it possesses a coagulation-temperature of 50° or somewhat less; its salting-out limits for ammonium sulphate lie between 1·5 and 3, or as low as in the case of fibrinogen and paramyosinogen. Spontaneously coagulable albumins are also found in mucous membranes. For all these reasons one may regard myosin as forming an integral part of every kind of protoplasmic molecule.

The nucleo-proteids are most abundant in nonstriped muscle, then

[1] M. Siegfried, *Arch. f. Anat. u. Physiol.*, Physiol. Abteil. 1894, p. 401; *Zeitschr. f. physiol. Chem.* **21**. 360 (1895); **28**. 524 (1899); J. Macleod, *ibid.* **28**. 535 (1899).

[2] See on this point Halliburton, *Biochemistry of Muscle and Nerve* (1904), p. 46.

[3] C. A. Pekelharing, *Zeitschr. f. physiol. Chem.* **22**. 245 (1896); A. Kossel, *ibid.* **7**. 7 (1882).

[4] J. F. v. Holmgren, from the Swedish original by Hammarsten in *Maly's Jahresber. f. Tierchemie*, **23**. 360 (1893).

[5] J. Reinke and H. Rodewald, *Botanikerztg.* **38**. (1880).

[6] P. Plósz, *Pflüger's Arch.* **7**. 371 (1873).

[7] L. Lilienfeld, *Zeitschr. f. physiol. Chem.* **18**. 473 (1893).

[8] R. H. Chittenden, "Histochèmie des Sehepithels," *Untersuchungen aus dem Heidelberger physiologischen Institute*, **2**. 438 (1879).

[9] See p. 406.

comes heart-muscle, and finally skeletal muscle, according to Bottazzi and Ducceschi,[1] and Vincent and Lewis.[2]

VI. The Nucleo-Albumins or Phospho-Globulins

The nucleo-albumins contain phosphorus, and for this reason they were classed at first with the 'nucleo-proteids,' with which they also have in common that the complex to which the phosphorus is attached becomes split off during a certain stage of peptic digestion as an insoluble compound, while the main bulk of the albumin-molecule passes into solution. This complex, which is insoluble at first, and which becomes dissolved later on, Kossel[3] calls paranuclein, and Hammarsten[4] pseudo-nuclein.

The nucleo-albumins differ, however, completely from the nucleo-proteids, as neither xanthin-bases, nor pyrimidin-derivatives, nor pentoses occur amongst their dissociation-products.[5] Kossel and Hammarsten, to whom we owe our knowledge regarding the composition of the nucleo-proteids, originally made an attempt to bring the nucleo-albumins and nucleo-proteids closer together by drawing an analogy between the so-called thyminic acid (which is the complex remaining after the xanthin-bases have been split off from nucleic acid) and the pseudo-nuclein or pseudo-nucleic acid. More accurate investigations have shown, however, that the nucleo-albumins and the nucleo-proteids are not at all closely related,[6] and as the nucleo-albumins have nothing to do with the cell-nuclei, Cohnheim suggests to discontinue the use of the term 'nucleo-albumin' and to substitute for it the expression 'phospho-globulin.'

To this group of bodies belong casein, vitellin, and a number of cell-phospho-globulins. Ichthulin is not included here, but classified as a phospho-glyco-proteid amongst the proteids, according to Hammarsten (see p. 405). To the phospho-globulins belong further the phyto-vitellins, such as legumin, and perhaps also plant-casein, which have been discussed above under No. III. along with the phyto-globulins.

The phospho-globulins are distinctly acid; they redden litmus

[1] Bottazzi and Ducceschi, *Arch. ital. de Biol.* **28**. 395 (1897).

[2] Swale Vincent and Thomas Lewis, *Journ. of Physiol.* **26**. 445 (1901).

[3] A. Kossel, *Verh. d. Berl. physiol. Ges., Arch. f. Anat. u. Physiol.*, Physiol. Abteil. 1891, p. 181; L. Lilienfeld, *ibid.* 1892, p. 128.

[4] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **19**. 19 (1893).

[5] A. Kossel, *ibid.* **10**. 248 (1886).

[6] A. Kossel and A. Neumann, *ibid.* **22**. 74 (1896); A. Neumann, *Arch. f. Anat. und Physiol.*, Physiol. Abteil. 1898, p. 374 (*Verh. d. Berl. physiol. Ges.*).

paper and are insoluble in water, while their alkali- or ammonia-salts are very soluble; by acids they are precipitated from their salt-solutions. The solutions of their salts do not coagulate, and may therefore be boiled without undergoing a change. If, however, phospho-globulin-solutions are acidified to an extent less than that required for precipitation and the solution be then heated, they undergo at a certain temperature a distinct coagulation, but otherwise they give the ordinary precipitation-tests of the albumins. When kept in an undissolved state they do not become insoluble; they are also relatively resistant towards acids, but they are very readily decomposed by alkalies.

The special feature of this group is its behaviour towards pepsin-hydrochloric acid, first noticed by Lubavin,[1] and subsequently frequently studied in the case of caseinogen. Pepsin-hydrochloric acid reacts in three stages, according to Salkowski [2] and Willdenow: [3]—

(1) The phospho-globulin is dissolved and partially converted into albumoses.
(2) A phosphorus-containing radical is split off and separated.
(3) The phosphorus radical is dissolved while the peptonisation of the casein-remainder proceeds.

According to the strength of the pepsin the amount of the phosphorus-containing radical which is separated off as an insoluble mass varies greatly. If the pepsin is strong, but little separation of a transient nature takes place, while if the pepsin is only slightly active the separated phosphorus-moiety is abundant and may not pass into solution. This phosphorus-containing portion is called paranucleic acid, and contains necessarily more phosphorus than does the mother-substance; it is also markedly acid in its character, being readily soluble in alkalies and being precipitated by acids. According to Giertz [4] it is readily soluble in barium-hydrate solution—and in this it differs from the true nucleins,—but it is very quickly dissociated by barium hydrate, even at a low temperature, into acid-albumin, albumoses, and phosphoric acid. Paranucleic acid is also rapidly decomposed by other alkaline solutions. According to Salkowski and Hahn [2] orthophosphoric acid is not split off by peptic

[1] N. Lubavin, *Hoppe-Seyler's Med.-chem. Untersuchungen*, p. 463 (1871).

[2] E. Salkowski, *Zentralbl. f. d. med. Wissensch.* 1893, Nos. 23 and 28; *Pflüger's Arch. f. d. ges. Physiol.* **63**. 401 (1896); *Zeitschr. f. physiol. Chem.* **27**. 297 (1899); E. Salkowski and M. Hahn, *Pflüger's Archiv f. d. ges. Physiol.* **59**. 225 (1895); E. Salkowski, *Zeitschr. f. physiol. Chem.* **32**. 245 (1901); compare also W. v. Moraczewski, *ibid.* **20**. 28 (1894). [3] Clara Willdenow, Dissertation, Bern, 1893.

[4] K. H. Giertz, *Zeitschr. f. physiol. Chem.* **28**. 115 (1899).

digestion, while according to Biffi[1] it is separated off by tryptic digestion.

The precipitation of paranucleic acid by peptic digestion is never complete, and this explains the statements made by Alexander[2] and others regarding the phosphorus-content of casein-albumoses. Salkowski has precipitated the paranucleic acid of casein by means of ferri-ammonium sulphate, and has then decomposed the precipitate with sodium-hydrate solution. Levene and Alsberg[3] dissolved the nucleo-albumin with ammonia, acidified and then precipitated the albumin-moiety with picric acid and the paranucleic acid with alcohol.

Paranucleic acid precipitates albumin,[4] and is precipitated by the salts of the heavy metals and by some of the alkaloidal reagents. In its pure, non-albuminous state paranucleic acid is not known, and its dissociation-products are also unknown.

Attempts to make artificial nucleo-albumins by combining thyminic acid or other preparations with albumins have given negative results.[5] The pseudo-nucleins formed in this way are readily dissolved by trypsin, easily absorbed by the intestine, and excreted as phosphoric acid by the kidneys.[6]

The sulphur-component has been studied by v. Moraczewski,[7] by digesting different strengths of casein-solutions for different lengths of time with different amounts of pepsin and hydrochloric acid. He found that the sulphur-content of the paranuclein varies but little under different conditions. There is thus a marked difference in the behaviour of the sulphur and the phosphorus. Whether the paranuclein percentage of the original casein was 2 or was 12 per cent, the sulphur-content varied between 0·32 and 0·40 per cent of sulphur. During digestion a part of the sulphur volatilises, and the more intense the digestion, the greater also is the amount of the sulphur which is lost.

[1] U. Biffi, *Virchow's Archiv*, **152**. 130 (1888).

[2] F. Alexander, *ibid.* **25**. 411 (1898).

[3] P. A. Levene and C. Alsberg, *ibid.* **31**. 543 (1900).

[4] E. Salkowski, *Zentralbl. f. d. med. Wissensch.* 1893, Nos. 23 and 28; *Pflüger's Arch. f. d. ges. Physiol.* **63**. 401 (1896); *Zeitschr. f. physiol. Chem.* **27**. 297 (1899); E. Salkowski and M. Hahn, *Pflüger's Archiv f. d. ges. Physiol.* **59**. 225 (1895); E. Salkowski, *Zeitschr. f. physiol. Chem.* **32**. 245 (1901); compare also W. v. Moraczewski, *ibid.* **20**. 28 (1894); T. H. Milroy, *Zeitschr. f. physiol. Chem.* **22**. 307 (1896).

[5] T. Milroy, *ibid.* **22**. 307 (1896).

[6] W. Sandmeyer, *ibid.* **21**. 87 (1895); J. Sebelien, *ibid.* **20**. 443 (1895).

[7] W. v. Moraczewski, *Hofmeister's Beiträge*, **5**. 489 (1904).

1. Caseinogen [1]

Caseinogen is the chief and most characteristic albumin of milk. Because of its acid properties it was held for long to be an albuminate, and was classed with the alkali-albuminates which are obtained by the denaturalisation of other albuminous substances. Hoppe-Seyler [2] and then Hammarsten [3] in particular taught us that caseinogen is a distinct substance. The caseinogens of different animals differ from one another.[4]

Caseinogen of Cow's Milk

Analysis of cow's milk has given these percentage figures :—

C	H	N	S	P	
52·96	7·05	15·65	0·758	0·847	Hammarsten.[5]
53·3	7·07	15·91	0·82	...	Chittenden and Painter.[6]
54·0	7·04	15·6	0·771	0·847	Lehmann and Hempel.[7]
53·07	7·13	15·64	0·76	0·8	Ellenberger.[8]

The dissociation-products are given on p. 70, No. 7. The absence of glycocoll and the carbohydrate radical and the high tyrosin- and tryptophane-contents make caseinogen especially readily digestible [9] (see p. 148). For the same reason no hetero-albumose is formed during peptic digestion.[9]

It is the only native albumin which is attacked by erepsin,[10] and plays in ordinary metabolism a special part owing to its ready dissociation.[11] In addition to tyrosin and tryptophane it also contains

[1] The author has adopted Halliburton's nomenclature. The mother-substance is called caseinogen (=Kasein of the Germans), and the derived substance casein (=Parakasein of the Germans).

[2] F. Hoppe-Seyler, *Virchow's Arch.* **17**. 417 (1859).

[3] O. Hammarsten, auto-abstract after the Swedish original in *Maly's Jahresber. f. Tierchemie*, **2**. 118 (1872); *Königl. Ges. d. Wissensch. zu Upsala*, 1877; *Zeitschr. f. physiol. Chem.* **7**. 227 (1883).

[4] A. Dogiel, *ibid.* **9**. 591 (1885); Ellenberger, *Arch. f. (Anat. und) Physiol.* 1899, p. 33; 1902, Suppl. p. 313; F. Soxhlet, *Münchener medizin. Wochenschr.* 1893, No. 4; A. Wroblewski, Dissertation, Bern, 1894; C. Storch, *Monatsh. f. Chem.* **23**. 712 (1902).

[5] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **7**. 227 (1883); **9**. 273 (1885).

[6] R. H. Chittenden and H. M. Painter, *Studies from the Yale Univers.* **2**. 156 [according to *Maly's Jahresber. f. Tierchemie*, **17**. 16 (1887)].

[7] W. Hempel, *Pflüger's Arch. f. d. ges. Physiol.* **56**. 558 (1894).

[8] Ellenberger, *Arch. f. (Anat. u.) Physiol.* 1902, Suppl. p. 313.

[9] F. Alexander, *Zeitschr. f. physiol. Chem.* **25**. 411 (1898).

[10] O. Cohnheim, *ibid.* **35**. 134 (1902).

[11] W. Falta, *Verh. der Naturforsch. Ges. in Basel*, **15**. Heft 2 (1903); *Korre-*

large amounts of lysin and glutaminic acid. Its paranucleic acid (not quite free from albumin) contains, according to Willdenow[1] and Salkowski,[2] 3 to 4 per cent of phosphorus.

The iodine-caseinogens are discussed on p. 231; the chlorine-caseinogens of Habermann and Panzer and the nitro-substitution-product of v. Fürth on p. 236.

Free caseinogen is quite insoluble in water, while its salts are very readily soluble. Osborne[3] has shown that caseinogen being an acid substance, forms two distinct groups of salts. The first group includes the salts of Ca, Mg, Ba, and Sr, and the salts of strong organic bases such as caffein and strychnin; all these produce markedly opalescent solutions, are unable to pass through the pores of a clay filter, and are precipitated from their solutions by the addition of insoluble finely divided substances. They are acted upon by rennin, and on heating form a haptogen-membrane. On warming their solutions a turbidity occurs between 35° and 45°, which disappears on cooling. This phenomenon is explained as due to hydrolysis occurring as the result of heating, according to the equation:

$$\text{Calcium caseinogenate} + 2H_2O = Ca(OH)_2 + \text{caseinogen}.$$

The second group contains the salts of K, Na, and NH_4. These form comparatively clear solutions; pass through clay-filters; are not precipitated by the addition of finely divided substances; are not visibly altered on heating; do not form a haptogen-membrane; and are not acted upon by rennin.

Caseinogen, being an albuminous substance, resembles the other albumins in also being able to form salts with acids, and is therefore readily soluble in the presence of an excess of an acid, but notwithstanding this it is essentially an acid substance. In its salts with bases it has an equivalent weight of 1135, and is at least 4 to 6 basic according to Laqueur and Sackur.[4] The much higher equivalent weights of 5000 to 6000 calculated from the figures of Salkowski,[5] Hammarsten,[6] Lehmann and Hempel,[7] and Söldner,[8] are partly due to hydrolysis and partly to acid salts having been investigated.

spondenzblatt f. Schweizer Ärzte, 1903, No. 22; L. Blum, *Zeitschr. f. physiol. Chem.* **30**. 15 (1900); E. Bendix, *Archiv f. (Anat. und) Physiol.* 1900, Suppl. p. 309.

[1] C. Willdenow, *Dissertation*, Bern, 1893.

[2] E. Salkowski, *Zeitschr. f. physiol. Chem.* **32**. 245 (1901).

[3] W. A. Osborne, *Journ. of Physiol.* **27**. 398 (1901).

[4] E. Laqueur and O. Sackur, *Hofmeister's Beiträge*, **3**. 193 (1903).

[5] E. Salkowski, *Zeitschr. f. Biol.* **37**. 401 (1899).

[6] O. Hammarsten, *Königl. Gesellschaft der Wissenschaften zu Upsala*, 1877.

[7] W. Hempel, *Pflüger's Archiv f. d. ges. Physiol.* **56**. 558 (1894).

[8] F. Söldner, Dissertation, Erlangen (1888).

Söldner[1] distinguishes two series of salts, while Courant[2] describes three series.

"Eucasein" is ammonium caseinogenate,[3] while "nutrose" and "plasmon"[4] are the sodium caseinogenates.

In addition to being precipitated by chemical agencies, calcium caseinogenate, or the caseinogen + calcium phosphate compound occurring normally in milk, is also precipitated by relatively feeble physical factors as alluded to above. According to Hermann[5] calcium caseinogenate is precipitated if one add to its solution large amounts of calcined clay or animal charcoal. According to Salkowski[6] the caseinogen-lime compound is precipitated on letting milk stand with chloroform. Zahn[7] states that the lime salt is precipitated whenever it comes into contact with porous clay, and therefore if milk be sucked through a Chamberland filter the caseinogen remains behind, while the other albumins pass into the filtrate.[8] Sodium caseinogenate, on the other hand, is filtrable. On this property of caseinogen, namely, to be precipitated by clay, Hempel[9] has based a method of estimating its molecular weight, but according to Simon[10] this is not permissible.

In milk, caseinogen is present as calcium caseinogenate, and, according to Courant,[2] not as a neutral salt, but as calcium di-caseinogenate and in combination with calcium phosphate. How this latter is brought about is as yet unknown. The calcium caseinogenate, as such, may possess the power of keeping somehow the neutral calcium phosphate, which is also present in milk, in solution or in a finely subdivided state, or there may be formed in the milk a true double salt of calcium caseinogenate and calcium phosphate, as is believed by Lehmann and Hempel.[11] At any rate, when caseinogen is precipitated calcium phosphate is thrown down simultaneously, and so is the whole of the fat, the emulsion of which is also due to the presence of calcium caseinogenate. For this reason it is extremely difficult to purify caseinogen from fat and from calcium phosphate.[12]

[1] F. Söldner, Dissertation, Erlangen, 1888.

[2] G. Courant, *Pflüger's Arch.* **50**. 109 (1891).

[3] E. Salkowski, *Zeitschr. f. Biol.* **37**. 401 (1899).

[4] W. Praussnitz and H. Poda, *ibid.* **39**. 277 (1900).

[5] L. Hermann, *Pflüger's Arch.* **26**. 442 (1881).

[6] E. Salkowski, *Zeitschr. f. physiol. Chem.* **31**. 329 (1900).

[7] W. Zahn, *Pflüger's Archiv*, **2**. 598 (1870).

[8] D. F. Harris, *Journ. of Physiol.* **25**. 207 (1900).

[9] W. Hempel, *Pflüger's Archiv*, **56**. 558 (1894).

[10] G. Simon, *Zeitschr. f. physiol. Chem.* **33**. 466 (1901).

[11] W. Hempel, "J. Lehmann's Milchuntersuchungen," *Pflüger's Archiv f. d. ges. Physiol.* **56**. 558 (1894).

[12] R. Cohn, *Zeitschr. f. physiol. Chem.* **22**. 156 (1896); W. Hempel, "J. Lehmann's Milchuntersuchungen," *Pflüger's Archiv f. d. ges. Physiol.* **56**. 558 (1894).

Caseinogen is precipitated from a solution of its salts, and also from milk by small quantities of mineral acids and by larger amounts of acetic acid, but it dissolves on adding an excess of acid. Caseinogen is most readily prepared by Hoppe-Seyler's method as modified by Hammarsten.[1]

Precipitate milk with acetic acid, dissolve the precipitate in dilute ammonia or in sodium carbonate, taking care to avoid the solution becoming alkaline, and repeat the process several times. Free the caseinogen thoroughly of fat by means of alcohol and ether, and then treat it again with acetic acid and soda-solution. There is no danger of denaturalisation if any marked alkaline reaction be avoided. The de-fatting is rendered very much more easy if, instead of the pure milk, we employ centrifugalised milk from which the cream has been removed.

Caseinogen and its salts are salted out by sodium chloride,[2] magnesium sulphate,[3] and sodium sulphate[4] in saturated solutions. The limits for ammonium sulphate[5] are for the main bulk of the caseinogen between 2·2 and 3·6, but a slight turbidity manifests itself already at 1·2.

The other precipitation-reactions are the same as in the case of ordinary albumins, but Schlossmann[6] has found potash-alum in suitable concentrations to precipitate the caseinogen of milk without also precipitating the other albumins; excess of potash-alum redissolves the precipitate, however.

A true heat-coagulation is not shown by caseinogen, for the solutions of its salts may be boiled without undergoing a change. In a dry state, however, caseinogen, according to Laqueur and Sackur,[7] becomes partly insoluble on being heated from 94° to 100°, while according to the older statements of Hammarsten,[8] it requires a temperature of from 120° to 130°. Halliburton[9] noticed a change at 75° on heating caseinogen suspended in water.

What really happens to the caseinogen when milk is boiled is not yet known;[10] pure calcium-caseinogenate undergoes hydrolysis,

[1] O. Hammarsten, auto-abstract in *Maly's Jahresber. f. Tierchemie*, **4**. 135 (1874); *Zeitschr. f. physiol. Chem.* **7**. 227 (1883). [2] J. Sebelien, *ibid.* **9**. 445 (1885).

[3] Tolmatscheff, Hoppe-Seyler's *Medizin.-chem. Untersuchungen*, p. 272 (1867).

[4] K. Storch, *Monatsh. f. Chem.* **18**. 244 (1897).

[5] Fr. Alexander, *Zeitschr. f. physiol. Chem.* **25**. 411 (1898).

[6] A. Schlossmann, *ibid.* **22**. 197 (1896); compare also G. Simon, *ibid.* **33**. 466 (1901). [7] E. Laqueur and O. Sackur, *Hofmeister's Beiträge*, **3**. 193 (1902).

[8] O. Hammarsten, *Maly's Jahresber.* **4**. 135 (1874).

[9] W. D. Halliburton, *Journ. of Physiol.* **11**. 448 (1890).

[10] W. Cronheim and E. Müller, *Jahrbuch f. Kinderheilkunde*, N.F. **47**. 45 (1902); H. Conradi, *Münchener medizin. Wochenschr.* 1901, p. 175.

according to Osborne as stated on p. 274, while the haptogen-membrane has been studied by Jamison and Hertz, see p. 276.

Casein

Milk when acted upon by rennet is coagulated owing to the rennet-ferment converting caseinogen into casein, or, to use the terminology of Hammarsten[1] changing 'Kasein' into 'Parakasein.' Casein resembles the unaltered caseinogen in being readily soluble in alkalies, but its lime salt is insoluble. If, therefore, a soluble lime salt is present in solution along with caseinogen, then under the influence of rennet-ferment the soluble calcium caseinogenate is changed into the insoluble calcium caseinate and the milk curdles.[2]

The process of coagulation takes place in two stages, and these may be separated from one another temporarily.[3] The first stage consists in the fermentative change whereby caseinogen is converted into casein, and depends solely on the presence of the rennet-ferment; the second stage is characterised by the curdling of the milk or caseinogen-solution containing the altered casein. This curdling can only take place, however, in the presence of lime salts; for if the lime salts of the milk be removed by an oxalate, then the casein does not separate out.[4] Halliburton[2] reserves the expression casein for the coagulated caseinogen, and uses the term caseinogen for the native albumin, so as to express the analogy which exists between the coagulation of the mother-substance of casein and the mother-substances of fibrin and myosin, which he terms fibrinogen and myosinogen.

In all its other properties casein completely agrees with caseinogen except that it is more readily precipitated by sodium chloride, and therefore casein may undergo a kind of coagulation[5] if large amounts of sodium chloride be present. Want of knowledge of this fact seems to have led to many of the contradictory statements found in the literature.

The process of true coagulation is according to Hammarsten[1] an

[1] O. Hammarsten, *Maly's Jahresber. f. Tierchemie*, **2**. 118 (1872); *Sitzungsber. der Königl. Gesellschaft d. Wissenschaften zu Upsala*, 1877.

[2] W. D. Halliburton, *Journ. of Physiol.* **11**. 448 (1890); O. Hammarsten, *Maly's Jahresber. f. Tierchemie*, **2**. 118 (1872); *Sitzungsber. der Königl. Gesellschaft d. Wissenschaften zu Upsala*, 1877.

[3] S. Ringer, *ibid.* **12**. 164 (1891).

[4] M. Arthus, *Arch. de Physiol. norm. et pathol.* 1893, p. 673; 1894, p. 257; S. Ringer, *ibid.* **12**. 164 (1891); O. Hammarsten, *Maly's Jahresber. f. Tierchemie*, **2**. 118 (1872); *Sitzungsber. der Königl. Gesellschaft d. Wissenschaften zu Upsala*, 1877.

[5] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **22**. 103 (1896).

irreversible one. Söldner[1] and Courant[2] have stated that to bring about coagulation a soluble lime salt is essential in addition to the calcium caseinogenate, and although Hammarsten[3] contradicts this view, there cannot be any doubt that an additional soluble lime salt facilitates coagulation greatly. Rennet causes coagulation whatever the reaction of the caseinogen-solution may be. Coagulation is hastened by small amounts of acid and is slowed by alkalies.[4] This is due, as Söldner has shown, to acidification of the milk leading to larger amounts of soluble lime salts being formed at the cost of the calcium phosphate, and *vice versa*. The precipitation of caseinogen by means of salt is an altogether different process from the coagulation caused by acids, although both processes may be at work simultaneously, as, for example, when milk reaches the stomach. According to Lindemann[5] no difference exists as regards digestibility between the precipitated calcium caseinogenate and the coagulated calcium caseinate, or, if there be a difference, it is one due to the size of the flocculi.[6]

The rennification of milk by the gastric mucous membrane of the calf and also by certain plants was known to the ancients, but gradually it has also become known that the gastric juice of non-mammals,[7] the pancreatic juice,[8] the digesting secretions of many invertebrates,[9] and also many extracts of organs[10] contain a rennet-like ferment, that, in short, the rennet-ferment seems to occur where proteolytic ferments are met with. Pawlow[11] has therefore expressed the hypothesis that rennet is not a special ferment at all, but that all proteolytic ferments possess the power of coagulating milk. Space forbids to enter more fully into this question.

So far caseinogen has been represented as an individual substance, and this view of Hammarsten's is the one generally accepted, but the following points against this view must be noted (Cohnheim).

[1] F. Söldner, *Dissertation*, Erlangen, 1888.

[2] G. Courant, *Pflüger's Archiv*, **50**. 109 (1891).

[3] O. Hammarsten, *Maly's Jahresber.* **4**. 135 (1874).

[4] A. Weitzel, *Arbeiten a. d. Kaiserl. Gesundheitsamt*, **19**. 126 (1902).

[5] W. Lindemann, *Virchow's Archiv*, **149**. 51 (1897).

[6] E. v. Dungern, *Münchener medizin. Wochenschr.* 1900, II. p. 1661.

[7] R. Neumeister, *Lehrbuch der physiol. Chem.*, 2. Aufl., Jena, 1897, p. 242.

[8] W. Kühne, *Heidelberger naturh.-med. Verein*, N.F. I. Heft 4, 1876; W. D. Halliburton and F. G. Brodie, *Journ. of Physiol.* **20**. 97 (1896); A. Löb, *Zentralbl. für Bakteriol.* 1. Abteil, **32**. 471 (1902).

[9] O. Cohnheim, *Zeitschr. f. physiol. Chem.* **35**. 396 (1902); R. Kobert, *Pflüger's Arch.* **99**. 116 (1903).

[10] A. Edmunds, *Journ. of Physiol.* **19**. 466 (1896).

[11] J. P. Pawlow and S. Parastschouk, *Verh. d. Sektion f. Anat., Phys. u. med. Chem. der Vers. nordischer Naturforscher und Ärzte in Helsingfors*, 1902, p. 28.

1. Hammarsten himself has found that it is impossible to completely remove the caseinogen by means of precipitation with acids and by rennet-coagulation, for there always remains an albumose-like body which he calls whey-albumin ('Molkeneiweiss').

2. Alexander[1] has shown that a slight turbidity occurs on adding 1·2 of a saturated ammonium-sulphate solution, although the main bulk of the caseinogen is precipitated between the points 2·2 and 3·6 ammonium sulphate. Alexander and Hofmeister[2] further hold that the feeble but distinct Molisch-reaction which is obtained with caseinogen also points to its not being a uniform substance.

3. Wroblewski[3] has observed an albumin which is not precipitated by acetic acid but simply rendered opalescent, and which therefore he calls opalisin. This substance occurs in very small amounts in cow's milk, and somewhat more abundantly in horse and human milk. Opalisin may be salted out with sodium chloride or magnesium sulphate; it is soluble in water and non-coagulable; it gives the colour-tests of albumins, also those not given by casein, and is further remarkable for its unusually low C- and high (4·7 per cent) S-content.

4. Storch[4] precipitated the main bulk of the caseinogen with either sodium or magnesium sulphate, and then saturated the filtrate with the other salt. There is separated hereby a substance of low C- and N-content and high S- and P-percentage (2·09 per cent); it does not curdle with rennet, and because of its behaviour towards hydrochloric acid is said to be a nucleo-proteid.

5. Laqueur and Sackur,[5] after drying caseinogen between 94° and 100,° found one portion, the 'sodium caseid,' to become insoluble in alkalies, while another portion, the 'iso-casein,' was still soluble. There existed also differences between these two substances as regards composition, reaction, and equivalent weights.

Against all these statements the objection may be raised that the substances just enumerated are identical with lacto-globulin, which also is precipitated from milk by means of acids, or with some derivative of this lacto-globulin. It is true that the composition of the preparations of Wroblewski and Storch speaks against this hypothesis, but as yet it is uncertain as to whether we are dealing with a pre-existing albumin or whether caseinogen dissociates very readily (Cohnheim).

[1] F. Alexander, *Zeitschr. f. physiol. Chem.* **25**. 411 (1898).
[2] F. Hofmeister, *Ergebnisse d. Physiol.* I. **1**. 759 (1902).
[3] A. Wroblewski, *Zeitschr. f. physiol. Chem.* **26**. 308 (1898).
[4] K. Storch, *Monatshefte f. Chemie*, **18**. 244 (1897); **20**. 837 (1899).
[5] E. Laqueur and O. Sackur, *Hofmeister's Beiträge*, **3**. 193 (1902).

In addition to caseinogen, lacto-globulin and the still hypothetical lactalbumin, no other albumins are met with in milk.[1] Albumoses are completely absent; but milk contains a whole number of nitrogenous extractives, such as Siegfried's phospho-carnic acid, or phospho-sarctic acid,[2] provided that this substance be not formed from the albumin during its manipulation.

The Caseinogens of other Milks

The caseinogen of human milk has been especially investigated by Wroblewski,[3] Kobrak,[4] and Röhmann.[5] It is certainly different from cow-caseinogen. Formerly great stress was laid on the point that cow-caseinogen in curdling forms coarse flakes or a firm coagulum, while human caseinogen gives rise to fine jelly-like flocculi, but Courant[6] and Kobrak have proved that this difference depends simply on the varying amounts of phosphorus found in the milk of the cow and that of woman, and also on differences in the reactions of these two milks. On the other hand, Kobrak[4] and Haffner[7] have found that caseinogen is not precipitated directly by acids from human milk, but only after the latter has been dialysed. According to Kobrak, human milk possesses also quite a different acidity from that found in cow's milk; but after repeated precipitation and re-solution human milk becomes more and more like cow's milk. Kobrak explains this phenomenon as due to the admixture of a second, alkaline albumin. The most important difference has been discovered by Röhmann: human caseinogen gives a strong Molisch-reaction, while cow-caseinogen gives a just perceptible reaction. Therefore these two caseinogens must differ from one another.

Donkey's milk has been studied by Ellenberger[8] and Storch.[9] In its composition and properties it closely resembles human milk; the caseinogen shows no peculiar features; Storch succeeded in decomposing the caseinogen into the same constituents as he obtained with cow's milk.

[1] W. D. Halliburton, *Journ. of Physiol.* **11**. 448 (1890).

[2] M. Siegfried, *Zeitschr. f. physiol. Chem.* **21**. 360 (1895); Martin Müller, *ibid.* **22**. 561 (1897); K. Wittmaack, *ibid.* **22**. 567 (1897); M. Siegfried, *ibid.* **22**. 575 (1897); R. Krüger, *ibid.* **28**. 530 (1899).

[3] A. Wroblewski, *Dissertation*, Bern, 1894.

[4] E. Kobrak, *Pflüger's Archiv*, **80**. 69 (1900).

[5] B. Röhmann, *Verh. des fünften intern. Physiol.-Kongresses zu Turin*, 1901.

[6] G. Courant, *Pflüger's Arch.* **50**. 109 (1891).

[7] E. Haffner, *Dissertation*, Tübingen, 1901.

[8] Ellenberger, *Arch. f.* (*Anat. u.*) *Physiol.* 1899, p. 33; 1902, Suppl. p. 313.

[9] C. Storch, *Monatsh. f. Chem.* **23**. 712 (1902).

Goat- and horse-caseinogen dissociate when dried into two substances, as does cow-caseinogen, according to Laqueur and Sackur. Horse-milk contains, according to Wroblewski, much opalisin.

2. Vitellin

From the yolk of hens' eggs may be prepared a phosphorus-containing albuminous substance, which was investigated by Hoppe-Seyler,[1] and called by him 'vitellin.' According to Zadik[2] it contains 12 per cent nitrogen and 1·31 per cent phosphorus. Its coagulation-temperature is given by Weyl[3] as 75°; it is not salted out by sodium chloride, according to Weyl. Till now vitellin has not been obtained free from lecithin; Hoppe-Seyler assumed vitellin to be a compound with lecithin, in short, a 'lecith-albumin.' The paranucleic acid derivable from vitellin has been carefully investigated by Bunge,[4] who calls it 'hæmatogen,' while Levene and Alsberg[5] simply call it 'paranucleic acid.' Both preparations were, however, not free from albumin, and the analyses differ for this reason greatly from one another. Levene and Alsberg found a phosphorus-content of 9·88 per cent. This substance contains masked iron (see p. 447, under the nucleo-proteids).

Neuberg[6] has obtained glucosamin from an albumin occurring in the yolk, and also a radical which by oxidation is converted into *d*-saccharic acid. That this albumin, which was first prepared by Mayer,[7] is identical with vitellin is probable, judging by the account given of its solubility. If this be so, then vitellin resembles ichthulin even more closely, for the latter also contains a carbohydrate radical. The similarity between vitellin and ichthulin was first pointed out by Hoppe-Seyler.[8]

3. Ichthulin

Substances closely resembling the vitellin of hens' eggs are also found in the eggs of fish; they have been known for a long time, and attracted attention because they occur in a crystalline form as the

[1] F. Hoppe-Seyler, *Medizin-chem. Untersuchungen*, p. 215 (1868); J. L. Parke, *ibid.* p. 209; Diakonow, *ibid.* p. 221 (1868).

[2] H. Zadik, *Pflüger's Archiv*, **77**. 1 (1899).

[3] Th. Weyl, *Zeitschr. f. physiol. Chem.* **1**. 72 (1877).

[4] G. Bunge, *ibid.* **9**. 49 (1884).

[5] P. A. Levene and C. Alsberg, *ibid.* **31**. 543 (1900).

[6] C. Neuberg, *Ber. d. deutsch. chem. Ges.* **34**. III. 3963 (1901).

[7] P. Mayer, *Deutsch. med. Wochenschr.* 1899, p. 95.

[8] M. Gobley, *Journ. de Pharm. et de Chim.* 3rd ser. **17**. 401 (1850); A. Valenciennes and E. Frémy, *Compt. rend.* **38**. 471 (1854); F. Hoppe-Seyler, *Medizin-chem. Unters.* pp. 215, 221 (1868); F. N. Schulz, *Kristallis. von Eiweiss.* Jena, 1901.

so-called 'yolk-platelets.'[1] It has cost much labour to prepare ichthulin in a pure form. Hoppe-Seyler believes ichthulin to be a lecith-albumin. Walter[2] has examined the eggs of the carp and Levene[3] those of the cod.

	C	H	N	S	P	Fe
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Carp .	53·52	7·71	15·64	0·41	0·43	0·1
Cod . .	52·44	7·45	15·96	0·92	0·65	...

The carp-ichthulin contains a reducing substance, but, like all nucleo-albumins, no xanthin-bases; it only dissolves into a clear solution in the presence of alkalies, while to salt-solutions it imparts an opalescence. From such salt-solutions it is precipitated by diluting the salt-solution or by passing carbon dioxide through the solution.

4. Cell-Nucleo-Albumins

In the plasma forming the body of the cell, or the cytoplasma, are found, in addition to the globulins, and substances belonging to the myosin-group, constantly also iron-containing nucleo-albumins. These latter have been investigated by Halliburton,[4] Lilienfeld,[5] Hammarsten,[6] and Lönnberg,[7] a pupil of Hammarsten.

The cell-nucleo-albumins give the ordinary reactions of the nucleo-albumins, *i.e.* their salts are readily soluble, while they themselves are only slightly, or not at all, soluble in pure water; in dilute salt-solutions they are more readily soluble. Some of the substances isolated formerly by Halliburton may be nucleo-proteids derived from the cell-nuclei. From the nucleo-proteid of the liver of the snail Hammarsten isolated a carbohydrate. The nucleo-albumin

[1] M. Gobley, *Journ. de Pharm. et de Chim.* 3rd ser. **17.** 401 (1850); A. Valenciennes and E. Frémy, *Compt. rend.* **38.** 471 (1854); F. Hoppe-Seyler, *Medizin-chem. Unters.* pp. 215, 221 (1868); F. N. Schulz, *Kristallis. von Eiweiss.* Jena, 1891.

[2] G. Walter, *Zeitschr. f. physiol. Chem.* **15.** 477 (1891).

[3] P. A. Levene, *ibid.* **32.** 281 (1901).

[4] W. D. Halliburton, 'The Proteids of Kidney and Liver Cells,' *Journ. of Physiol.* **13.** 896 (1892); **9.** 229 (1888); 'Proteids of Nervous Tissues,' *ibid.* **15.** 90 (1894); Halliburton and Gregor Brodie, 'Nucleo-Albumins and Intravasc. Coagul.' *ibid.* **17.** 135 (1894); Forrest, 'Red Marrow,' *ibid.* **17.** 174 (1894); F. Gourlay, 'Thyroid and Spleen,' *ibid.* **16.** 23 (1894).

[5] L. Lilienfeld, *Zeitschr. f. physiol. Chem.* **18.** 473 (1893).

[6] O. Hammarsten, 'Studies on Mucin,' etc., *Pflüger's Arch.* **36.** 373 (1885).

[7] Ingolf Lönnberg, *Skandiv. Arch. f. Physiol.* **3.** 1 (1890).

of the kidney, that of the lymph-corpuscles and of the liver of the snail, form with concentrated salt-solutions a mucilaginous jelly, while according to Halliburton the nucleo-albumin prepared from mammalian liver does not show this peculiarity. The colour-reactions are those common to other albumins; the biuret-test gives a violet colour. They are only completely precipitated by sodium chloride sulphate if the solutions are saturated; ammonium and magnesium sulphate precipitates in half-saturated solutions, but the exact precipitation-limits have not yet been worked out.

The coagulation-temperatures for the nucleo-albumins of the kidney Halliburton found to be 63°, and from 56° to 60° for those of the liver and many other organs.

The nucleo-albumins of the snail's liver and of the leucocytes have been completely analysed, and those of the kidney and mammalian liver partially.

5. Nucleo-Albumins resembling Mucin

It is difficult to draw a line of demarcation between these substances and those described above. As regards their physical properties they behave like the typical mucins and mucoids, for with neutral ammonium or alkali-salts they form viscous solutions which may be drawn out into threads; they are precipitated by acids. When they are denaturalised by too strong or too prolonged action of alkalies, by prolonged boiling, or treatment with alcohol, they lose their mucinoid character. It is not possible to coagulate them.

Our knowledge regarding these substances we owe to Hammarsten [1] and to his pupils Paijkull [2] and Lönnberg,[3] for they showed that the mucinoid substance which is excreted by the kidney, the gall-bladder, and the synovial membranes of the ox is not a mucin but a nucleo-albumin. The urin of the ox also contains only a mucinoid nucleo-albumin, while the bile of man and dog [4] contains a true mucin. Salkowski [5] has found in the fluid which accumulates during coxitis in the human hip-joint, in addition to the nucleo-albumin also a mucin or mucoid.

All these substances contain a fairly high percentage of sulphur,

[1] O. Hammarsten, 'Chemistry of the Synovia,' auto-abstract in *Maly's Jahresber. f. Tierchem.* **12**. 480 (1882).

[2] L. Paijkull, 'The Mucus of the Bile,' *Zeitschr. f. physiol. Chem.* **12**. 196 (1887).

[3] J. Lönnberg, 'Albumins of the Kidney and Urinary Bladder,' *Skandinav. Arch. f. Physiol.* **3**. 1 (1890).

[4] L. Brauer, *Zeitschr. f. physiol. Chem.* **40**. 182 (1903).

[5] E. Salkowski, *Virchow's Arch.* **132**. 304 (1893).

but otherwise they differ in their composition. They contain no carbohydrate; on digestion with pepsin and hydrochloric acid they yield a pseudo-nuclein; the pseudo-nuclein of the synovial nucleo-albumin amounts to about 4 per cent of the albumin, and contains 5 per cent of phosphorus.

VII. Histone

Histones are albuminous substances which, possessing a relatively high percentage of basic radicals, are therefore preponderatingly basic.[1] For this reason they are precipitated by alkalies—which constitutes their most remarkable property,—although most histones redissolve on adding an excess of alkali. They are very soluble in acids, and therefore are in every respect the opposite of such acid-albumins as the globulins and caseinogens. Histones do not occur in the free state, but always coupled with what Kossel calls a 'prosthetic group.' Histones in such combinations give rise to some of the most important cell-constituents known, namely, to hæmoglobin, nucleo-proteids, etc.

The first histone was isolated by Kossel[2] from the red blood-corpuscles of the goose. Another histone was extracted by Lilienfeld from the leucocytes of the thymus; and to this group belong also certain albumins occurring in combination with nucleic acid in the spermatozoa of fishes. Finally, the albuminous radical of hæmoglobin, namely, the 'globin,' is also held to be a histone, although it differs somewhat in its reactions from the other histones. Globin contains less arginin than a typical histone, but, on the other hand, it contains a higher percentage of histidin than does any other albumin.

Bang[3] gives five reactions as typical of histones (see below); but it is necessary to point out that the distinctly basic character of the histones is of more importance than is the presence or the absence of some of these reactions, which are not common to all the members of the histone-group. Attention must also be drawn to the fact that, as in the case of the globulins so here, the acid-albumin radical of any given albumin has occasionally been mistaken for a histone, for in acid-albumins the albumin plays the part of a base.

[1] A. Kossel, *Deutsche med. Wochenschr.* 1894, p. 146; *Ber. d. deutsch. chem. Ges.* **34**. III. 3214 (1901); *Bull. de la Soc. chim. de Paris*, 3rd ser. vol. xxix. No. 14, July 20, 1903; A. Kossel and F. Kutscher, *Zeitschr. f. physiol. Chem.* **31**. 165 (1900).

[2] A. Kossel, *ibid.* **8**. 511 (1884).

[3] J. Bang *ibid.* **27**. 463 (1897).

Reactions of Histones (Nos. 1 to 5 after Bang)

1. Histones are precipitated from their watery solutions by ammonia. This reaction is the most important, for it led originally to the discovery of the histones.[1] According to v. Fürth[2] myogen (Halliburton's myosinogen) is also precipitated by ammonia, and the acid-albumins behave similarly (Bang), but the precipitates of these substances redissolve in the slightest excess of ammonia, and the precipitation is never complete, while in histone-solutions ammonia produces an abundant heavy precipitate. In excess of ammonia the histones are soluble, and they are even more readily dissolved by an excess of free alkali. The amount of ammonia required to precipitate histones and to redissolve the precipitate differs for the individual histones. The thymus-histone and that prepared from the mackerel is only completely precipitated by ammonia, according to Bang, if salts are also present. Bang's statements regarding the other histones are not correct and are also not quite accurate regarding the thymus-histone (Cohnheim).

2. Histones are coagulated by boiling only in the presence of salts, and even then not completely, for if the precipitates be dissolved in acids and be then neutralised they remain in solution, showing that they were not converted into acid-albumin, and for the same reason they are again precipitated on being heated. It is at present impossible to say in how far histones differ in this respect from other albumins and what part is played by the amount of acid or base present.

3. With nitric acid histones give a precipitate in the cold, which dissolves on heating and reappears on cooling. Therefore they give the reaction which is generally said to be typical of albumoses, and Kossel for this reason classed histones originally amongst the albumoses.

4. While the other albumins are only precipitated by the so-called alkaloidal reagents if the reaction be acid, histones are precipitated from neutral solutions.[3] They are, therefore, precipitated by sodium phosphotungstate or molybdate, sodium picrate, or potassium ferrocyanide; globin is dissolved by an excess of the reagent, probably because the reaction of the solution becomes alkaline. The histone

[1] A. Kossel, *ibid.* **8**. 511 (1884); **32**. 81 (1901).

[2] O. v. Fürth, *Arch. f. experim. Path. u. Pharmak.* **36**. 231 (1895).

[3] A. Kossel, *Zeitschr. f. physiol. Chem.* **25**. 165 (1898); Kossel and F. Kutscher, *ibid.* **31**. 165 (1900); A. Mathews, *ibid.* **23**. 399 (1897); J. Bang, *ibid.* **27**. 463 (1899); **32**. 79 (1900).

prepared from the mackerel resembles the protamins in being precipitated from feebly alkaline solutions. This characteristic reaction of the histones depends on their basic character, which allows them to become less strongly hydrolysed than other albumins.

5. Neutral solutions of histone give a precipitate with solutions of ov-albumin, casein, and serum-albumin, if these are poor in salts, and also with egg-albumin and blood-serum. The precipitate contains for one part of histone two parts of casein and serum-globulin, but only one part of ovalbumin. The precipitate is soluble in acids and alkalies, and is not precipitated by ammonia or other alkalies in the presence of salts.

The two reactions just mentioned, namely, the precipitation of histones by alcaloidal reagents from neutral or alkaline solutions, and the power of combining with albumins to form insoluble compounds, are common to both the histones and the protamins, and also to some albumoses, set free by peptic digestion from fibrin and certain other albumins, according to the statements of Kutscher[1] and Bang.

Histones have also the following reactions in common :

6. They resemble acid-albumins by being precipitated from alkaline, neutral and acid solutions by the addition of small amounts of salts (Bang, Huiskamp).[2]

7. Their sulphur content is low, while in accordance with their basic nature their nitrogen-percentage is high.

In other respects histones give, like other albumins, the usual precipitation-tests. Ether, even when added in small amounts, leads to the histone separating out as a buoyant mass, swimming on the surface of the layer of ether.[3] The salting-out limits, the colour-reactions, the dissociation-products, and the readiness with which dissociation is brought about differ greatly with each individual histone. As already mentioned, the sulphur is low in amount, and the lead-sulphide reaction may give negative results. Finally it is of great interest that Kossel,[4] by combining protamin and albumin, obtained precipitates which possessed all the properties of the histones.

1. The Thymus-histone

Lilienfeld[5] first prepared a nucleo-albumin, which he called 'nucleo-histone,' by adding acetic acid to a watery extract of the thymus

[1] F. Kutscher, *Zeitschr. f. physiol. Chem.* **23**. 115 (1897).

[2] W. Huiskamp, *ibid.* **32**. 145 (1901) ; **34**. 32 (1901).

[3] R. Ehrström, *ibid.* **32**. 350 (1901).

[4] A. Kossel, *Deutsche med. Wochenschr.* 1894, p. 146 ; *Zeitschr. f. physiol. Chem.* **22**. 176 (1896).

[5] L. Lilienfeld, *ibid.* **18**. 473 (1893).

gland. The precipitate obtained in this way, on being shaken up with 0·8 per cent hydrochloric acid, was supposed to break up into histone (which remained in solution and which could be precipitated by ammonia), and into leuco-nuclein, which was held to be an albumin + nucleic acid compound. Since Lilienfeld's time the readily accessible thymus-histone has been investigated by Kossel, Kossel and Kutscher,[1] Fleroff,[2] Bang,[3] Malengreau,[4] and Huiskamp.[5]

Lilienfeld's 'nucleo-histone' consists, according to Huiskamp, Malengreau, and Bang, of two distinct nucleo-albumins. Huiskamp distinguishes between a nucleo-albumin free from histone and a nucleo-histone; Malengreau between a nucleo-histone (A-nucleo-albumin) and a histone-nucleinate (B-nucleo-albumin), and Bang between a nucleo-albumin free from histone, a histone-nucleinate, and Fleroff's para-histone.

Before entering into these divergent views the author has given a short account of the methods which have been employed by Malengreau, Huiskamp, and Bang for the separation of nucleo-albumins, especially as these substances act as very strong oxydases, in which connection they have been investigated by the author.

Methods for separating 'Nucleo-albumins' and 'Histone'

Malengreau's Method.—A watery extract of minced thymus is repeatedly precipitated with acetic acid, and the precipitate redissolved in sodium carbonate or caustic soda solution. From the final sodium carbonate solution the 'nucleo-proteid' or A-nucleo-albumin separates out on being saturated to between 30 and 45 per cent with ammonium sulphate, while the nucleo-histone or B-nucleo-albumin is thrown down by 56 to 72 per cent saturated ammonium sulphate solution. By the addition of 1 per cent HCl to the 'nucleo-proteid' or A-nucleo-albumin, Malengreau obtained a 'histone' which was precipitated by 45 per cent saturated ammonium sulphate, while the 'histone' obtained by 1 per cent HCl from the nucleo-histone or B-nucleo-albumin required at least 55 per cent ammonium sulphate. Malengreau found both the nucleo-histone and the nucleo-albumin to contain adenin and guanin.

[1] A. Kossel, *Zeitschr. f. Chem.* **30**. 520 (1900); **31**. 410 (1900); A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900).

[2] A. Fleroff, *ibid.* **28**. 307 (1899).

[3] J. Bang, *ibid.* **27**. 463 (1899); **30**. 508 (1900); **31**. 407 (1900); *Hofmeister's Beiträge*, **4**. 115 and 331 (1903); and 362; **5**. 317 (1904).

[4] F. Malengreau, *La Cellule*, **17**. 339 (1900); and *ibid.* **19**. 285 (1902).

[5] W. Huiskamp, *Zeitschr. f. physiol. Chem.* **32**. 145 (1901); **34**. 32 (1901); **39**. 55 (1903).

The A-nucleo-albumin is soluble in 0·9 per cent NaCl and in 0·1 per cent CaCl.

Huiskamp.—Extract 200 grammes of finely minced calf-thymus free from fat at a low temperature (which is better than the addition of chloroform) with 600 ccm. of water; filter and centrifugalise the extract to separate the 'nucleo-proteid,' which is soluble in calcium chloride solution, from the 'nucleo-histone' which, within certain limits, is not soluble; add to each 100 ccm. of the clear solution, 1 ccm. of 10 per cent calcium chloride solution, so that the solution contains ± 0·1 per cent of calcium chloride. [The author has repeated these experiments with equivalent amounts of barium chloride and finds it answer even better.] Calcium chloride may be added up to 0·5 per cent without dissolving much of the precipitate, but the latter completely dissolves in 2 per cent $CaCl_2$ solution. Centrifugalise off the calcium chloride-precipitate and dissolve it in water with the help of a few drops of dilute ammonia; filter; reprecipitate with $CaCl_2$; centrifugalise; treat precipitate of nucleo-histone for some hours with 100 ccm. of a 0·8 per cent watery solution of HCl, shaking repeatedly, and the 'nuclein' will remain in the precipitate, while the histone goes into solution. [The insoluble nuclein gives the proteid reactions except the glyoxylic one; it contains large amounts of the purin-bases, especially adenin, and contains phosphorus. It is soluble in dilute ammonia and is reprecipitated by acetic or other acids.] To obtain histone, dialyse the hydrochloric acid extract to remove all traces of HCl and $CaCl_2$, and other salts, as these interfere with the precipitation of the histone by means of ammonia. When the solution is almost neutral precipitate the histone with ammonia. Histone is insoluble in excess of ammonia, but is soluble in excess of NaOH. As histone combines with HCl it must be a base, and the nuclein-radical to which it is joined in the nucleo-histone must therefore be acid.

Huiskamp asks himself the question whether the nucleo-histone precipitated by the calcium chloride is a compound analogous to the casein of milk. Are we dealing with (1) $CaCl_2$ + nucleo-histone; or (2) with Ca + nucleo-histone; or (3) does the calcium chloride solution simply render the nucleo-histone insoluble? His answer to these questions is that calcium plays the part of the base, while nucleo-histone represents the acid radical. From this 'calcium nucleo-histonate' the calcium may be removed by 5 per cent acetic acid. That the nucleo-histone is acid he believes is proved by the fact that the reaction of the solution in which it is suspended remains neutral on the addition of ammonia till the whole of the precipitate is dissolved. As free nucleo-histone

is insoluble in water, and as its compounds with earthly alkalies are slightly soluble, it must be present in the watery thymus extract probably as an alkali-salt, and therefore the following interaction must take place on adding calcium chloride to a watery extract of the thymus:

$$\text{Soluble sodium – nucleo-histonate} + CaCl_2$$
$$= \text{Insoluble calcium – nucleo-histonate} + NaCl.$$

The dissolving action of ions with stronger electro-affinities is exceedingly interesting, and has been fully discussed by the author, as far as corrosive sublimate and sodium chloride are concerned, in his *Physiological Histology*. See this book, pp. 308-313.

The solubilities of the salts of nucleo-histone are, according to Huiskamp, as follows:—

Alkali salts: soluble in water; more or less insoluble in very dilute alkali salt solutions, ± 0·9 per cent NaCl; soluble in excess of salts. The sodium nucleo-histone is a viscous precipitate readily soluble in water without the addition of ammonia.

Magnesium salt: fairly soluble in water; insoluble in 0·1–0·3 per cent $MgSO_4$; soluble in excess of salt.

Calcium salt: slightly soluble in water; insoluble in 0·1–0·5 per cent $CaCl_2$; soluble in 2 per cent $CaCl_2$.

Barium salt: slightly soluble in water; insoluble in 0·1–1·8 per cent $BaCl_2$; soluble in excess.

Heavy metals: insoluble in water and insoluble in salt solutions.

Ammonium sulphate, potassium and sodium nitrate, potassium oxalate and tartrate, first precipitate and in excess dissolve the precipitate.

To obtain the nucleo-proteid from the watery extract of the thymus, Huiskamp first removes the nucleo-histone with calcium chloride, as described above, and then adds acetic acid to the filtrate.

Bang[1] minces perfectly fresh thymus-glands (500–1000 grammes), and extracts for 24-48 hours with 0·9 per cent NaCl (1½–2 litres), a few drops of chloroform are added if necessary. The extract so obtained, after having been centrifugalised and filtered, is milky in appearance, and distinctly amphoteric in reaction, although more basic than acid; the gland apparently had undergone no change.

Bang obtains, apart from globulin, from the thymus a nucleo-proteid, a nucleo-histone, and Fleroff's paratone; the nucleo-proteid is prepared from the extract by first precipitating the nucleo-histone by the addition of $CaCl_2$ to the extent of 0·2 to 0·3 per cent, and then

[1] Ivar Bang, *Hofmeister's Beiträge*, 4. 115 (1903).

adding either lime water or, after the extract had been rendered slightly alkaline by the addition of an alkali, by adding $CaCl_2$. The most abundant precipitate is obtained, however, by the addition of very small amounts of acetic acid, for in 1 per cent acetic or in 0·2 per cent HCl the precipitate dissolves again completely. [This agrees with the behaviour of Malengreau's A-nucleo-albumin, and, like the latter, Bang's nucleo-albumin is soluble in 0·9 NaCl and in 0·1 $CaCl_2$ solutions; like Huiskamp's nucleo-albumin and Malengreau's A-nucleo-albumin, it is precipitated by half saturating the thymus extract with ammonium sulphate. The precipitate so obtained does not, however, completely redissolve in distilled water.

The nucleo-albumins of Huiskamp and Bang have the following percentage composition :—

	C	H	N	P	Ash
Huiskamp .	50·09	7·18	16·11	0·97	3·11.
Bang . .	49·50	6·35	16·15	1·12	2·36.

This nucleo-albumin is very readily decomposed by treatment with acetic acid or by alkali into two components, one of which is very readily soluble in dilute alkalies, while the other is not. Acting on the acetic acid precipitate with 0·3 per cent HCl, the nucleo-albumin is dissociated (agreement between Malengreau and Bang), the substance extracted by the HCl is, however, not a histone, as held by Malengreau, but an acid-albumin, for "all histones form with acids soluble salts possessing a neutral reaction," while the substance extracted by HCl is precipitated by ammonia even before complete neutralisation is reached. This acid-albumin is precipitated by 20 per cent saturated ammonium sulphate, its nitrogen content is 16·59 per cent and it contains no phosphorus. The residue which remains after extracting the acetic acid precipitate of the nucleo-albumin with 0·3 per cent HCl is a nuclein, containing phosphorus and a pentose. The precipitation limits for ammonium sulphate are for this nuclein also 20.

The nucleo-histone is prepared from the original 0·9 per cent NaCl extract of the thymus gland, or the latter may be extracted simply with distilled water. The nucleo-histone is precipitated by Huiskamp's method of adding 10 per cent $CaCl_2$ solution till the amount of this salt equals 0·2, or in very saturated nucleo-histone solutions 0·3 per cent. The precipitate obtained in this way may be extracted directly with 2 per cent NaCl solution,[1] but this extract is not readily filtrated and is therefore apt to putrefy. To facilitate filtration Bang centrifugalises the $CaCl_2$ precipitate, shakes the latter well up with

[1] Not rendered soluble with ammonia, according to the method of Huiskamp, as ammonia decomposes the nucleo-histone.

96. per cent alcohol and centrifugalises at once. The precipitate is now mixed with distilled water, allowed to stand for some hours, and the water filtered off; the residue, thoroughly rubbed up with 50 to 150 ccm. of 2 per cent NaCl, filters readily after 24 hours. The filtrate containing the histone-sodium nucleinate is a perfectly clear somewhat bluish, fluorescent solution. To precipitate the histone-sodium nucleinate, the 2 per cent salt solution is mixed with an equal bulk of water, as the nucleinate is not soluble in 1 per cent NaCl; by still further dilution the precipitate passes, however, into solution again and may then be precipitated by 0·2 per cent $CaCl_2$.

On saturating a solution of histone-nucleinate with solid powdered NaCl the histone moiety is thrown down, while the nucleic acid remains in solution. The latter may now be precipitated with alcohol. To purify the nucleic acid either lead or copper salts may be used, with which it forms insoluble precipitates, or silver or mercury salts with which water-soluble but alcohol-insoluble salts are formed. Dilute acetic acid does not, while 25 per cent acid does, precipitate nucleic acid, but acetic acid quickly decomposes nucleic acid into purin-bases and phosphoric acid. For analysis Bang used the sodium nucleinate.

When a histone-nucleinate solution is saturated with sodium chloride to obtain the histone, and when in the filtrate the nucleic acid is precipitated with alcohol, there still remains a substance in solution which is Fleroff's[1] para-histone. It gives the biuret reaction.

The histone-calcium-nucleinate has the following percentage composition :—

C	H	N	S	P	Ca	Ash
43·69	5·60	16·87	0·47	5·23	1·71	8·57.

The nucleinate of histone is thus characterised by a very high phosphorus content. By calculating the empirical formula from Ca, we obtain

$$C_{85}\ H_{130}\ N_{28}\ S_{0\cdot34}\ P_4\ CaO_{38},$$

trebling this formula because of the sulphur the minimal molecular weight of 6974 is got from

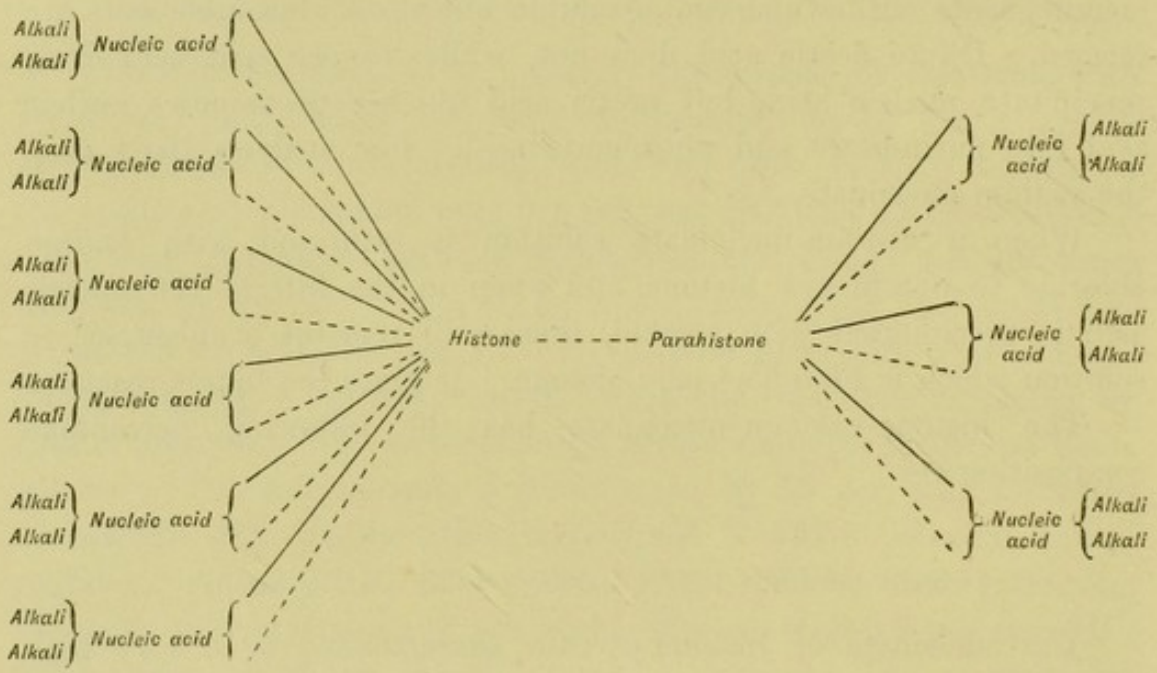
$$C_{255}\ H_{390}\ N_{84}\ SP_{12}\ Ca_3\ O_{114}.$$

The para-histone, according to Fleroff, has the percentage composition :—

	C 51·84	H 7·93	N 17·84	S 1·99.
Bang found :	—	—	N 17·72	S 2·23.

[1] F. A. Fleroff, *Zeitschr. f. physiol. Chem.* **28**. 307 (1899).

According to Bang, thymus-histone forms with HCl two kinds of salts, namely, a neutral salt containing 6 Cl, and an acid salt with 13 Cl. The 6 valencies of the histone, satisfied by the 6 Cl in the neutral salt, are called the chief valencies, while the additional 7 valencies, seen in the acid salt, are called the accessory valencies. Each molecule of histone is linked, normally, to 6 molecules of nucleic acid, each of which has 4 valencies. In each nucleic acid 2 valencies are occupied by alkali-atoms (potassium, calcium), 1 valency is firmly linked to the histone-molecule, while the remaining fourth valency in each of the 6 nucleic acids is linked to 6 out of the 7 accessory valencies of histone above referred to. The remaining seventh valency of histone serves to link it to para-histone, which is supposed to possess 4 valencies, one of which is united to the histone, while the remaining three are joined to 3 molecules of nucleic acid.



[The word alkali means K or Ca. The dotted lines indicate the 7 accessory valencies of the histone and 4 accessory valencies of the para-histone. Bang in his schematic representation, of which the above is a modification, did not represent the accessory valencies of the para-histone, as the neutral para-histone sulphate precipitates albumin much less than does the neutral histone-salt.—The Author.]

As soon as any substance which is a stronger base than histone is brought into contact with histone-alkali-nucleonate, those valencies in the nucleic acid molecules which are satisfied by the 6 accessory valencies of the histone will link up with the stronger base and the histone radical be converted from a 13 into a 7 valent base.

The following analysis of thymus-histone and para-histone have been made :—

[TABLE

	C	H	N	S	
	Per cent.	Per cent.	Per cent.	Per cent.	
Histone . .	52·34	7·31	...	...	Lilienfeld.
,, . .	52·37	7·7	18·35	0·62	Fleroff.
,, . .	52·35	7·50	18·10	0·62	Bang.[1]
Para-histone .	51·84	7·93	17·73	2·11	,,

The thymus-histone differs widely from other histones as regards its properties, its high sulphur-content, and its dissociation-products, which have been investigated by Kossel and Kutscher, and are given on p. 74 No. 37. The high arginin- and tyrosin-contents are especially interesting. Abderhalden and Rona,[2] employing Fischer's ester-method, found amongst the dissociation-products of thymo-histone: glycocoll, alanin, α-pyrrolidin-carboxylic acid, phenylalanin, glutaminic acid, tyrosin, and probably also aspartic acid and cystin. Thymus-histone is readily digested, being even attacked by erepsin.[3]

2. Globin

Globin is the only, or, at any rate, the most essential albumin-radical of hæmoglobin. It has been known for some time and was given its name by Preyer,[4] but it was first prepared in a pure state by Schulz,[5] and identified by him as a histone.

Globin differs from other histones in being precipitated by a specially small amount of ammonia or alkali, and also by being equally readily dissolved in the slightest excess of these reagents, and if the excess be at all great even in the presence of ammonia salts. Schulz gives the following percentage composition:—

C 54·97 H 7·2 N 16·89 S 0·42 O 20·52.

Its dissociation-products are given on p. 70, No. 1. Its high histidin- and leucin-content are worthy of notice.

As globin is readily obtainable from hæmoglobin, and as the latter amongst all albumins is the one which can be prepared in the purest form, globin has often been used for experiments on digestion[6] and dissociation, and excepting some of the protamins, it is the most thoroughly analysed substance. According to Schulz[7] it is unusually

[1] J. Bang, *Zeitschr. f. physiol. Chem.* **27**. 463 (1899); **30**. 508 (1900); **31**. 407 (1900); *Hofmeister's Beiträge*, **4**. 115 and 331 (1903); and 362; **5**. 317 (1904).

[2] E. Abderhalden and P. Rona, *Zeitschr. f. physiol. Chem.* **41**. 278 (1904).

[3] O. Cohnheim, *ibid.* **35**. 134 (1902).

[4] W. Preyer, *Pflüger's Archiv.* **1**. 395 (1868); and *Die Blutkristalle*, Jena, 1871.

[5] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **24**. 449 (1898).

[6] S. Salaskin and K. Kowalewsky, *ibid.* **38**. 571 (1903).

[7] F. N. Schulz, *ibid.* **24**. 449 (1898).

readily digested by pepsin and by trypsin, which is difficult to understand because it contains much phenylalanin and but little tyrosin. It is not attacked by erepsin.[1]

3. The Histone from the Red Blood Corpuscles of the Goose

This histone was the first to be discovered. Kossel[2] found and described it, and also prepared it by dissociating its nucleic acid salt. The percentage composition of Kossel's preparations varied, but the N-content was high, being over 18 per cent, while the amount of sulphur only amounted to 0·5 per cent.

This histone is quite distinct from globin, as it is contained in the nuclei, while hæmoglobin is only present in the cell-plasm.

4. The Histones from the Testes of Fishes and of other Animals

Miescher[3] found in the unripe testes of the salmon in combination with nucleic acid a body which he regarded as an albumose, but which probably belongs to the histones. Bang[4] obtained from the immature testes of the mackerel a compound which greatly resembles Miescher's albumose and which he calls scombron. But the affix *-on* had better be reserved for the peptone-like decomposition products of the protamins (Cohnheim). Both histones by maturing are changed into the protamins: salmin and scombrin. But the spermatozoa of other fish, such as the cod (Gadus morrhua[5]) and Lota vulgaris,[6] as well as those of the sea-urchin (Arbacia pustulosa[7]), even when mature, contain not protamins but histones.

The following analyses are available:—

C	H	N	S		
Per cent.	Per cent.	Per cent.	Per cent.		
51·21	7·6	17·64	...	Salmo-histone	Miescher.
49·86	7·23	19·79	0·79	Scomber-histone	Bang.
...	...	16·48	...	Lota-histone	Ehrström.
...	...	18·65	..	Gadus-histone	Kossel and Kutscher.
...	...	(15·91	...	Arbacin-sulphate	Mathews).

The dissociation-products of the Lota- and Gadus-histones are given on p. 74, Nos. 38 and 39. Here also the high arginin-

[1] O. Cohnheim, *ibid.* **35**. 134 (1902).

[2] A. Kossel, *ibid.* **8**. 511 (1884).

[3] F. Miescher and O. Schmiedeberg, *Arch. f. experiment. Path. u. Pharmak.* **37**. 1 (1896).

[4] J. Bang, *Zeitschr. f. physiol. Chem.* **27**. 463 (1899).

[5] A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900).

[6] R. Ehrström, *ibid.* **32**. 350 (1900). [7] A. Mathews, *ibid.* **23**. 399 (1897).

content is noteworthy. The Lota-histone gives a strong reaction of Molisch, while the Scomber-histone reacts especially to Millon's test.

The Scomber-histone and Miescher's albumose differ from other histones in not being soluble in an excess of ammonia even if salts are absent; in not being completely precipitated by ammonia, and in not being thrown down by corrosive sublimate. The precipitation-limits of ammonium sulphate lie for Lota-histone between 4·1 and 4·9.

VIII. The Protamins

Miescher[1] found in 1874 in the ripe spermatozoa of the salmon a base which he called protamin. Miescher's investigations were continued by Kossel,[2] who has made the protamins into one of the best known groups of albumins. By him and his pupils protamins have been discovered in the spermatozoa of several kinds of fish. These protamins resemble one another strongly and have been called by Kossel after the animal they are derived from: salmin, from the salmon; sturin, from the sturgeon; clupein, from the herring; scombrin, from the mackerel.

The protamins form a well-defined group, which differs considerably from most of the other albuminous groups. They contain no sulphur and less carbon but a great deal more nitrogen than do the other albumins because protamins are composed essentially of basic dissociation-products, in particular arginin, which may rise from 58 to 84 per cent of the total dissociation-products (Kossel). The mono-amino acids, on the other hand, are very scanty.

[1] F. Miescher, 'Die Spermatozoen einiger Wirbeltiere,' *Verh. d. naturf. Ges. zu Basel*, VI. 138 (1874); J. Piccard, *Ber. d. deutsch. chem. Ges.* **7**. II. 1714 (1874); posthumous papers of Miescher, edited by O. Schmiedeberg, *Arch. f. experiment. Path. u. Pharm.* **37**. 1 (1896).

[2] A. Kossel, *Zeitschr. f. physiol. Chem.* **22**. 176 (1896); **25**. 165 (1898); **26**. 588 (1899); *Bull. Soc. chim. de Paris*, 3. Sér. t. 29, Nr. 14; 20 Juli 1903; *Ber. d. deutsch. chem. Ges.* **34**. III. 3214 (1901); *Zeitschr. f. physiol. Chem.* **40**. 311 (1903); A. Kossel and A. Mathews, *ibid.* **25**. 190 (1898); A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900); D. Kurajeff, *ibid.* **26**. 524 (1899); **32**. 197 (1901); N. Morkowin, *ibid.* **28**. 313 (1899); W. H. Thompson, **29**. 1 (1899); M. Goto, **37**. 84 (1902); A. Kossel and H. D. Dakin, **40**. 565 (1903).

[Table

The composition of the protamins Kossel has put into tabular form: [1]—

	Scombrin.	Salmin.	Clupein.	Sturin.	Cyclopterin.	α-Cyprinin.	β-Cyprinin.
Alanin	+	0	+	+	?	?	?
Serin	0	+	+	0	?	?	?
Amino-valerianic acid	0	+	+	0	?	+	+
Leucin	0	0	0	+	?	?	?
Di-amino-valerianic acid (ornithin)	+	+	+	+	+	+	+
Di-amino-caproic acid (lysin)	0	0	0	+	0	+	+
Histidin	0	0	0	+	0	0	0
α-Pyrrolidin-carboxylic acid	+	+	+	0	?	?	?
Tyrosin	0	0	0	0	+	0	+
Urea	+	+	+	+	+	+	+
Tryptophane	0	0	0	0	+	0	0

The radicals of which protamins are built up may be as numerous as they are in other albumins, but there is less variety and each kind is repeated with great regularity in the different protamins. For this reason it is possible to dissociate the protamins much more readily into their dissociation-products than to dissociate other albumins. Kossel believes the protamins to be the simplest albumins, and he has placed them in the centre of his system.

Protamins in their free state are difficult to prepare, but the sulphates are comparatively easily obtained. Protamins have been analysed directly and also after conversion into chlorides, which are then precipitated from methyl-alcoholic solutions by means of platinum chloride (Goto). Miescher was, however, the first to prepare the platinum chloride double salt of salmin.

The most recent analyses of Goto give these figures:—

C	H	N	Pt	Cl	O		
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.		
22·96	4·22	14·83	24·73	26·56	6·7	Salmin	Salmon (*Salmo salar*).
22·81	4·30	12·59	24·64	26·57	9·09	Clupein	Herring (*Clupea harengus*).
23·49	4·75	13·57	24·09	25·99	8·11	Scombrin	Mackerel (*Scomber scombrus*).
24·32	4·49	14·20	23·10	25·42	8·47	Sturin	Sturgeon (*Acipenser sturio*).

[1] A. Kossel, *Berliner klinische Wochenschrift*, No. 41, Oct. 1904, p. 1065.

Clupein has this percentage-composition :—

C 47·28, H 8·14, N 25·72, O 18·9.

From these figures Goto calculates the formulæ :—

$C_{30}H_{57}N_{17}O_6$	Salmin.
$C_{30}H_{62}N_{14}O_9$	Clupein.
$C_{32}H_{72}N_{16}O_8$	Scombrin.
$C_{34}H_{71}N_{17}O_9$	Sturin.

These formulæ serve, of course, only for a preliminary orientation ; the older analyses of Miescher, Piccard, and Kossel differ more or less. It would appear that several similar but not identical substances occur together in the spermatozoa of one and the same species of fish.

The dissociation-products are given on pp. 74-75, Nos. 40-46. The prevalence of bases has already been pointed out, but in other respects the individual protamins differ greatly from one another. Salmin, sturin, and clupein have been more completely analysed than any of the other albumins.

The analysis of salmin by Kossel and Dakin[1] has yielded the following figures :—

	Grammes.	Percentage.	
Total nitrogen . . .	5·369	100	
Arginin nitrogen . . .	4·787	89·2	
Alcohol insoluble fraction . .	0·265	4·9	
Of this serin . . .			3·25
Of this amino-valerianic acid .			1·65
Alcohol soluble fraction . .	0·230	4·3	
Demonstrable loss . .	0·087	1·6	

The ratios of the weights of the dissociation-products are :—

	Grammes.	Percentage.
Amount of salmin decomposed .	17·03	100
Arginin . . .	14·87	87·4
Serin	1·33	7·8
Amino-valerianic acid . .	0·74	4·3
Pyrrolidin-carboxylic acid .	1·88	11·0

Salmin may be composed of 10 molecules of arginin, 2 molecules of serin, 1 molecule of amino-valerianic acid, and 2 molecules of pyrrolidin carboxylic acid, which would give the formula $C_{81}H_{155}N_{45}O_{18}$, or 12 mol. arginin, 2 mol. serin, 1 mol. amino-valerianic acid, and 3 mol. pyrrolidin-carboxylic acid, which would yield $C_{98}H_{186}N_{54}O_{21}$. Which of these two formulæ is the correct one has not yet been settled.

[1] A. Kossel and H. D. Dakin, *Zeitschr. f. physiol. Chem.* **41**. 407 (1904).

Kossel and Dakin [1] deny the presence of alanin and of leucin, which two mono-amino acids were obtained by Abderhalden,[2] who examined salmin by Emil Fischer's ester-method. Abderhalden thought to have obtained, in all probability, also phenylalanin and aspartic acid. It is suggested by Kossel and Dakin that Abderhalden probably worked with immature testicles.

The protamin of the Californian salmon is, according to Taylor,[3] identical with that of the European variety. He found salmin-sulphate to diffuse very slowly through parchment paper; to be markedly dissociated in watery solutions, a half per cent solution giving $60 \times 10^{\circ}$. The lævo-rotation produced by a saturated solution is -6°. The watery solution undergoes 'spontaneously' a hydrolytic change, and it is suggested that salmin should be used for experiments on such ferments as trypsin.

As will be seen from Kossel's table on p. 420, protamins contain a certain amount of mono-amino acids, and therefore a sharp line of demarcation between the protamins and the other albumins no longer exists, especially as the histones form both genetically and chemically the transition to the true albumins. Protamins must therefore be classed amongst the albumins. They form a special group which, owing to the absence of certain radicals, does not give the reactions common to most of the albumins, but in this respect they are analogous to gelatine or hetero-albumose, which contain neither tyrosin nor tryptophane, or to casein or globin in which glycocoll is absent. Salmin, with its rich store of di-amino-acids, forms one end of a chain, the other end of which is represented by serin, which contains hardly any di-amino-acids (Cohnheim).

Protamins differ from ordinary albumins in not being coagulated by heat. On standing they undergo a change, which points to a kind of denaturalisation. The protamins resemble the histones in being precipitated by alkaloidal reagents not only from acid, but also from neutral solutions, but as the protamins are stronger bases than are the histones, they are even precipitated from alkaline solutions, the serial arrangement, albumin → histone → protamin, in this connection being very evident. Protamins are therefore precipitated by the alkali-tungstates and phospho-tungstates, by ferro- and ferri-cyanide, iodine potassium iodide, iodide of mercury + iodide of potash, alkali picrates, corrosive sublimate, mercuric nitrate, platinum chloride, gold chloride,

[1] A. Kossel and H. D. Dakin, *Zeitschr. f. physiol. Chem.* **41**. 407 (1904).

[2] Emil Abderhalden, *Zeitschr. f. physiol. Chem.* **41**. 55 (1904).

[3] A. E. Taylor, *Univ. of California public. Path.* **1**. p. 49. Abstract by Alsberg (Boston) in *Zentralbl. f. Physiol.* **18**. 631 (1904).

and other salts of the heavy metals. Salmin is also precipitated by chromic acid, potassium bichromate, and tannic acid, but not by osmium tetroxide (Mann).[1]

Protamins are salted out by ammonium sulphate and sodium chloride, but the exact precipitation-limits are not known.

They also give with albumin and with the primary albumoses precipitates which resemble histones very closely (Kossel).

Of the salts, in addition to the picrate mentioned above, the chromate of scombrin is also known.[2] Clupein sulphate is readily soluble in hot water; 100 parts of cold water dissolve 1·62 parts; when cooled to + 2°, or on the addition of ether, protamin sulphate separates out as a dark oil. The refractive index and the specific rotatory power of clupein sulphate is given by Kossel,[3] and that of scombrin sulphate by Kurajeff.[2]

Peptic digestion leaves protamin unaltered,[4] while trypsin[4] and erepsin[5] break it up into crystalline dissociation-products. As intermediate products are formed peptone-like bodies, the 'protones,' which differ from the protamins in giving a pure red biuret-reaction and in not being able to precipitate albumin.[6] As regards percentage-composition and dissociation-products Goto found no distinct difference between protamins and protones. Goto found, on splitting up clupein with hydrochloric acid, an increase in alkalinity, while with sulphuric acid a diminution was noticed. How protones are related to kyrin is mentioned on p. 201.

Thompson[7] has shown that protamins in their toxic properties resemble the albumoses formed by digestive enzymes. The toxicity is great, for 15 to 18 milligrammes of salmin, clupein, and scombrin, and 20-25 mg. of sturin per kilo. of dog, is sufficient to cause death. The protones are much less toxic than are the protamins. Whether protamins are toxic in themselves, or whether the toxicity depends on admixtures, is not known for certain.

In addition to salmin, clupein, scombrin, and sturin, the protamin cyclopterin has been prepared from the spermatozoa of the sea-hare (*Cyclopterus lumpus*) by Morkowin;[8] the acipenserin, from *Acipenser stellatus*, by Kurajeff;[9] two protamins, namely, cyprinin α and β, from

[1] Mann, *Physiological Histology*, 1902, p. 102.
[2] D. Kurajeff, *Zeitschr. f. physiol. Chem.* **26**. 524 (1899).
[3] A. Kossel, *ibid.* **22**. 176 (1896).
[4] A. Kossel and A. Mathews, *ibid.* **25**. 190 (1898).
[5] O. Cohnheim, *ibid.* **35**. 134 (1902).
[6] M. Goto, *ibid.* **37**. 94 (1902).
[7] W. H. Thompson, *ibid.* **29**. 1 (1899).
[8] N. Morkowin, *ibid.* **28**. 313 (1899).
[9] D. Kurajeff, *ibid.* **32**. 197 (1901).

the spermatozoa of the carp, by Kossel and Dakin.[1] Protamins have further been found in the spermatozoa of the trout[2] (*Salmo fario*), the pike[3] (*Esox lucius*), and of *Coregonus oxyrhynchus*[2] and *Silurus glanis*.[3]

The tuberculosamin which Ruppel[4] has isolated from the tubercle bacillus seems to possess the properties of a protamin. It occurs in the bacilli in combination with a nucleic acid. Whether the sulphur-free albumins which Nencki found in putrefactive[5] bacteria and in anthrax bacilli[6] belong to the protamins is not known.

Protamin nucleinate has been investigated from the micro-chemical and micro-structural points of view by Berg.[7]

[1] A. Kossel and H. D. Dakin, *Zeitschr. f. physiol. Chem.* **40**. 565 (1903).
[2] A. Kossel, *ibid.* **22**. 176 (1896).
[3] D. Kurajeff, *ibid.* **32**. 197 (1901).
[4] W. G. Ruppel, *ibid.* **26**. 218 (1898).
[5] M. Nencki and F. Schaffer, *Journ. f. prakt. Chem.* (2) **20**. 443 (1879).
[6] M. Nencki, *Ber. d. deutsch. chem. Ges.* **17**. 2605 (1884).
[7] Walther Berg, *Arch. f. mik. Anat.* **65**. 298 (1904).

CHAPTER X

THE PROTEIDS

PROTEIDS are composed of one or of several albumins in combination with a radical which is not an albumin, and which Kossel has called the 'prosthetic group.'[1] As the peculiar properties of the proteids are due to the prosthetic group, the latter is used for the classification of the proteids.

I. NUCLEO-PROTEIDS

The nucleo-proteids were discovered simultaneously by Miescher[2] and Plósz[3] as constituents of the cell-nucleus. They are built up of albumin and nucleic acid and always contain iron.[4]

(a) Nucleic Acid

The nucleic acids contain phosphorus and nitrogen, but no sulphur. They are organic acids of unknown constitution, although their dissociation-products are fairly well known. In the year 1874 Miescher[5] discovered in the spermatozoa of the salmon an acid substance containing phosphorus, which he called nuclein. Altmann[6] in 1889 prepared from all nucleo-proteids the 'nucleic' acid, and proved its identity with the nuclein of Miescher. The composition of nucleic acid has been cleared up to a certain extent by Kossel[7] and his school.

[1] A. Kossel, *Arch. f.* (*Anat. u.*) *Phys.* 1891, p. 181; 1893, p. 157.

[2] F. Miescher, 'Chemische Zusammensetzung der Eiterzelle,' *Hoppe-Seyler's Med.-chem. Untersuch.* p. 441 (1871).

[3] P. Plósz, 'Kerne der Vogel- und Schlangenblutkörperchen,' *ibid.* p. 461 (1871).

[4] See index under iron.

[5] F. Miescher, *Verh. d. naturforsch. Ges. in Basel*, **6**. Heft 1, 138 (1874).

[6] R. Altmann, *Arch. f.* (*Anat. u.*) *Physiol.* 1889, p. 524.

[7] A. Kossel, *Zeitschr. f. physiol. Chem.* **7**. 7 (1882); *Arch. f.* (*Anat. u.*) *Physiol.* 1893, p. 157, and *Liebreich's Enzyklopädie*, III. Bd. 'Nucleinstoffe' (in both works are reviews); A. Kossel and A. Neumann, *Ber. d. deutsch. chem. Ges.* **27**. II. 2215 (1894). The other papers are indicated in the following pages at their proper places.

Alsberg[1] finds the nucleic acid of the spermatic fluid of *Lota vulgaris* to be identical with the nucleic acid prepared from salmon-melt:—

$$C_{40}H_{56}N_{14}O_{16} + 2P_2O_5 + H_2O.$$

By the action of dilute mineral acids one-half of the purin-bases may be removed from the salmon-nucleic acid, while the other half remains firmly united. Alsberg has given the name of hemi-nucleic acid to a compound possessing only one molecule of purin-bases for two molecules of P_2O_5:—

$$C_{35}H_{51}N_9O_{15} + 2P_2O_5 + 3H_2O.$$

The alkali-salt of hemi-nucleic acid differs from that of nucleic acid in not being precipitated from dilute acetic-acid solutions by chloride of copper or by hydrochloric acid.

Stronger mineral acids, by their action on nucleic acid, give rise to a mixture of hemi-nucleic acid, nucleotin-phosphoric acid, nucleotin and lævulinic acid. 'Nukleotin' is a name which was first given by Schmiedeberg to the nucleic-acid compound minus the phosphorus and minus the nuclein-bases. Nucleotin, according to Alsberg, has the composition $C_{30}H_{42}N_{40}O_{13}$, and contains 14 per cent of water, held in a 'cement-like' manner. Nucleic acid, when acted upon by barium hydrate, gives rise to a saccharic acid, this latter being formed probably from the same radical in nucleic acid which gives rise to lævulinic acid when nucleic acid is acted upon by mineral acids.

Nucleic acid gives rise to the following dissociation-products:—

1. *Phosphoric Acid*

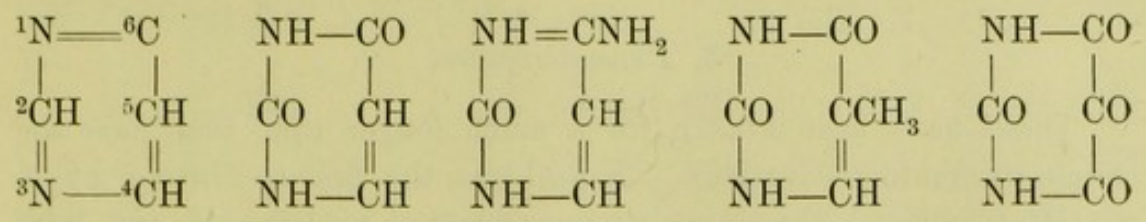
It is a constant and characteristic dissociation-product of nucleic acids. As to the probable manner of its linking, see p. 431; whether 'plasminic acid,' a metaphosphoric acid found in yeast, is related to nucleic acid is uncertain.[2]

2. *Pyrimidin-derivatives*

Three simple pyrimidin-derivatives of nucleic acid are known:—

1. Uracil or 2·6 dioxy-pyrimidin.
2. Cytosin or 2-oxy-6-amino-pyrimidin.
3. Thymin or 2·6-dioxy-5-methyl-pyrimidin.

[1] C. L. Alsberg, *Arch. f. exper. Path.* **51**. 239 (1904).
[2] A. Ascoli, *Zeitschr. f. physiol. Chem.* **28**. 426 (1899).



Uracil was first prepared by Ascoli [1] from yeast; its constitution determined by Steudel,[2] and confirmed by the synthesis of E. Fischer.[3] It crystallises in needles arranged in a rosette-like manner; does not sublime without undergoing decomposition; is readily soluble in hot water, slightly soluble in cold, and hardly soluble in alcohol and ether; it is readily soluble in ether. It is precipitated by phosphotungstic acid and mercuric sulphate.

Cytosin was discovered by Kossel and Neumann; [4] its constitution found out by Kossel and Steudel,[5] and confirmed by the synthesis of Wheeler and Johnson.[6] It resembles thymin as regards solubility; to isolate it, recourse is had to precipitation with silver nitrate and barium hydrate; [7] for analysis the double salt with platinum-chloride is used.

Thymin was discovered by Kossel,[8] and described by his pupils Jones [9] and Gulewitsch.[10] Its constitution was cleared up by Steudel,[11] and confirmed by the synthesis of E. Fischer.[12] It crystallises in small needles or dendritic platelets belonging to the rhombic system. Thymin is not precipitated by silver nitrate, hydrochloric acid, and nitric acid, but by silver nitrate and barium-hydrate; ammonia precipitates, but redissolves, if used in excess. Thymin being slightly soluble in cold water, and readily soluble in hot water, may therefore be recrystallised from water. When carefully heated it sublimes; when heated more strongly it melts at 290°. Thymin is identical with what Miescher and Schmiedeberg have described as nucleosin.

[1] A. Ascoli, *Zeitschr. f. physiol. Chem.* **31**. 161 (1900).

[2] H. Steudel, *ibid.* **30**. 539 (1900); **32**. 241 (1901).

[3] E. Fischer and G. Roeder, *Ber. d. deutsch. chem. Ges.* **34**. III. 3751 (1901).

[4] A. Kossel and A. Neumann, *ibid.* **27**. 2215 (1894).

[5] A. Kossel and H. Steudel, *Zeitschr. f. physiol. Chem.* **37**. 177 (1902); **37**. 377 (1903); **38**. 49 (1903). (Here is given a full description.)

[6] H. L. Wheeler and T. B. Johnson, *Amer. Chem. Journ.* **29**. 492 (1903), and *ibid.* **32**. 342 (1904). [7] F. Kutscher, *Zeitschr. f. physiol. Chem.* **38**. 170 (1903).

[8] A. Kossel and A. Neumann, *Ber. d. deutsch. chem. Ges.* **26**. 2753 (1893); *Zeitschr. f. physiol. Chem.* **22**. 188 (1896); A. Kossel and H. Steudel, *ibid.* **29**. 303 (1900).

[9] W. Jones, *ibid.* **29**. 20 (1899); **29**. 461 (1900).

[10] W. Gulewitsch, *ibid.* **27**. 292 (1899).

[11] H. Steudel, *Zeitschr. f. physiol. Chem.* **30**. 539 (1900); **32**. 241 (1901).

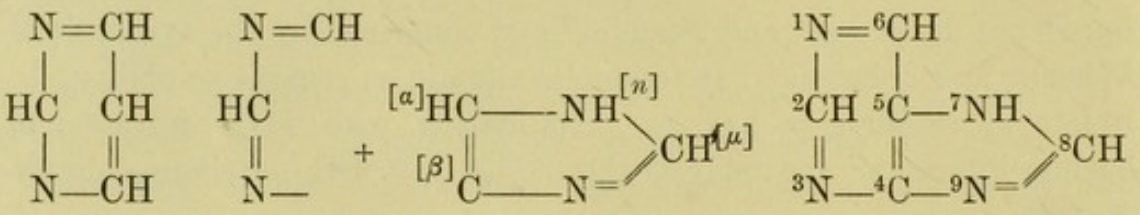
[12] E. Fischer and G. Roeder, *Ber. d. deutsch. chem. Ges.* **34**. III. 3751 (1901).

3. *Purin-derivatives*

These have been known for a much longer time than have the simple pyrimidin-derivatives. Kossel was the first to discover hypoxanthin[1] amongst the dissociation-products of nucleic acid, and later found also xanthin, guanin, and adenin.[2]

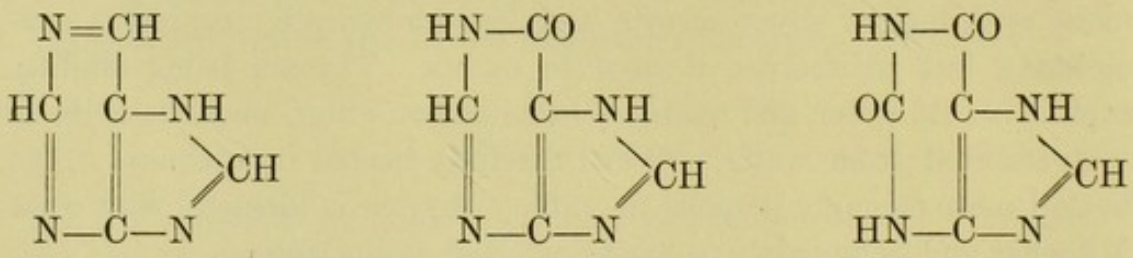
Hypoxanthin,	$C_5H_4N_4O$	= oxy-purin.
Xanthin,	$C_5H_4N_4O_2$	= 2·6-dioxy-purin.
Adenin,	$C_5H_5N_5$	= 6-amino-purin.
Guanin,	$C_5H_5N_5O$	= 2-amino-6-oxy-purin.

Purin, the mother-substance of these 'xanthin-bases,' contains a pyrimidin remainder: the meta-di-azin and the imido-azol radical.

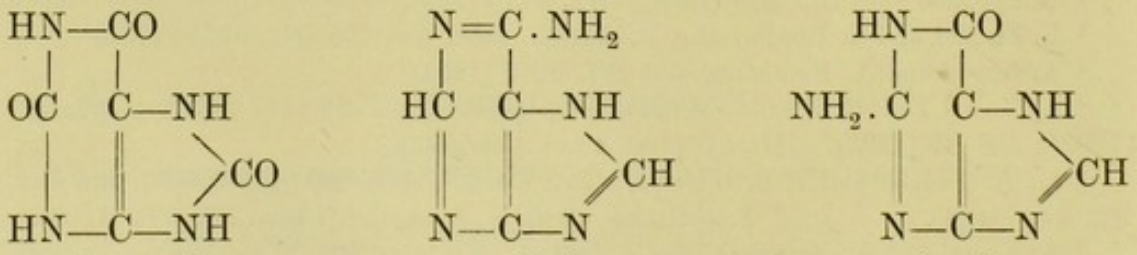


Pyrimidin. Meta-di-azin + Imido-azol (glyoxalin). Purin.

The following formulæ are arranged by the author in this order: purin → oxy-purins → amino-purin → amino-oxy-purin → oxy-methyl-purins :—



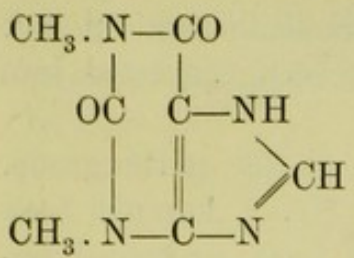
Purin. Hypoxanthin (6 oxy-purin). Xanthin (2·6 oxy-purin).



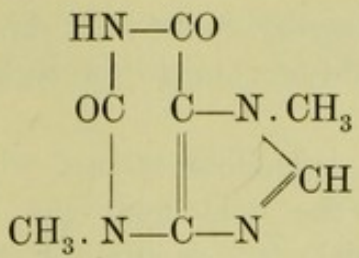
Uric acid (2,6,8 trioxy-purin). Adenin (6-amino-purin). Guanin (2-amino, 6-oxy-purin).

[1] A. Kossel, *ibid.* **4**. 290 (1880).

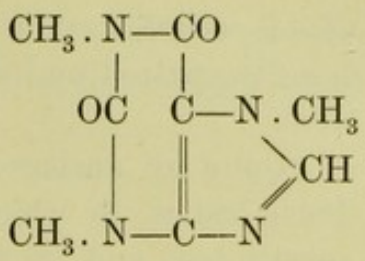
[2] A. Kossel, *ibid.* **7**. 7 (1882); **10**. 248 (1886); *Arch. f.* (*Anat. u.*) *Physiol.* 1893, p. 157; Kossel and A. Neumann, *Ber. d. deutsch. chem. Ges.* **27**. II. 2215 (1894).



Theophyllin or Theocin (2,6-dioxy-1,3-dimethyl-purin).



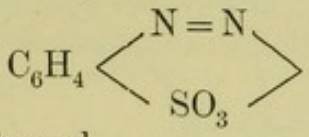
Theobromine (2,6-dioxy-3,7-dimethyl-purin).



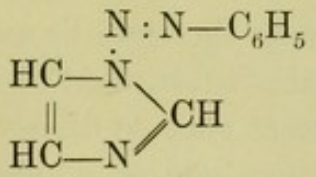
Caffeine (2,6-dioxy-1,3,7-trimethyl-purin).

The synthesis of guanin and similar compounds has been studied specially by Behrend and Rosen[1] (uric acid), Stollé and Hofmann[2] (diamino-guanidin), Traube,[3] Pellizzari and Cantoni[4] (diamino-guanidin).

The four purin-derivatives—hypoxanthin, xanthin, adenin, and guanin—occur in the body only in the nucleic acids and their dissociation-products, and hence are called 'nuclein-bases,' 'alloxur-bases,' or 'xanthin-bases.' The manner in which the purins are linked to the nucleic acids has been investigated by Burian,[5] who points out that purin, being composed of pyrimidin and imido-azol (see p. 428), gives for this reason both the alloxan reaction of pyrimidin and the silver reaction of imido-azol. Imido-azol with diazobenzene-sulphonic acid



forms diazo-benzene-imido-azol



Diazo-benzene-imido-azol.

Analogously with other azotising mixtures, yellow or red, stable substances are formed which, on the addition of ammoniacal silver nitrate, give red, generally gelatinous precipitates, and these, like the silver compounds of imido-azol and of the purin bodies, are almost insoluble in an excess of ammonia.

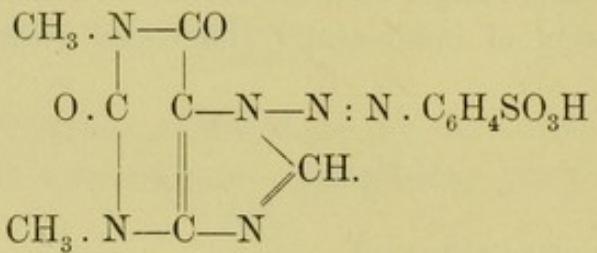
The same reaction is obtained with α, β, and μ substitution products of imido-azol (see formula on p. 428), but it does not occur—(1) if place n (= No. 7 of the purin group) is already occupied by a substi-

[1] Behrend and Rosen, *Liebig's Annalen*, **161**. 235 (1872).
[2] R. Stollé and K. Hofmann, *Ber. d. deutsch. chem. Ges.* **37**. 4524 (1904).
[3] Wilhelm Traube, *ibid.* **37**. 4544 (1904).
[4] G. Pellizzari and C. Cantoni, **38**. 283 (1905).
[5] R. Burian, *Ber. d. deutsch. chem. Ges.* **37**. 696 (1904).

tuted radical, *e.g.* in *n*-methylimido-azol; or (2) if the amidin link has disappeared by hydration; or (3) if it has been converted into urea.

Quite an analogous behaviour is met with in the purin group. Purin-bodies, in which the imide-hydrogen in No. 7 has not been substituted, and in which the amidin-link has been preserved unchanged (xanthin, hypoxanthin, guanin, adenin), yield with diazo-bodies intensely coloured compounds, which closely resemble the diazo-amino-compounds of the imido-azols. Substitution in the pyrimidin-ring does not hinder the reaction, for it is given by theophyllin (1·3- dimethylxanthin), but the reaction is prevented if the No. 7 imid-hydrogen is replaced by methyl, as it is, for example, in theobromine (3·7- dimethylxanthin), or in caffeine (1 3·7- trimethylxanthin); or if, as in uric acid, the imido-azol ring does not possess the structure of a cyclic amidin, but that of a cyclic urea.

It follows that the diazo-compound remainder must link on to the purin-nucleus in No. 7, and therefore purin-bases + diazo-compounds = diazo-amino-compounds, which are quite analogous to the imido-azols, thus :—



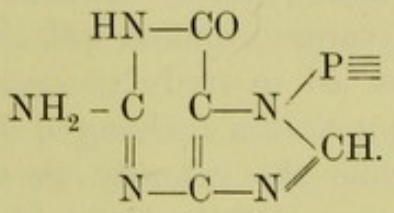
The diazo-reaction may therefore be used for determining whether No. 7 in the purin radical is substituted or not; if it is not substituted a red colour is obtained at once on dissolving the purin-derivative in alkali, cooling it, and then adding a diazo-substance or diazotising mixture.

Burian[1] also points out that purin-bases must be preformed in the nucleic acid molecule, as they are very readily separated from the nucleic acid remainder. They are liberated partially by heating nucleic acid to 60°, and completely by boiling the same for ten minutes in water or by dilute acids; this fact, along with the observation that nucleic acids do not give the diazo-reaction described above, led Burian to assume that the purin-bases are linked to the remainder of the nucleic acid molecule by the No. 7 nitrogen.

As nucleic acids are further very resistant to caustic potash, and in this they resemble other organic phosphoric-acid amides, there exists probably in nucleic acids a direct union between the phosphorus of the

[1] R. Burian, *Ber. d. deutsch. chem. Ges.* **37**. 708 (1904).

nucleic acid remainder and the No. 7 nitrogen of the purin-base. The union of guanin in nucleic acid would therefore be represented by the formula

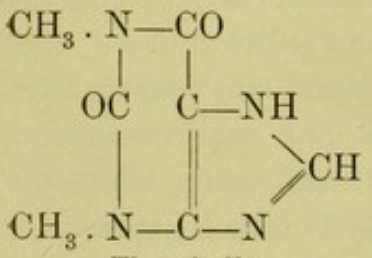


These results of Burian have been criticised by Steudel,[1] who found that, if sodium hydrate was used, thymin gave colour-reactions although no nitrogen is present in the No. 7 position; but Burian points out that the diazo-reaction is so very sensitive that Steudel ought to have isolated and analysed the diazo-benzene sulphonic acid derivative of thymin to make sure that the thymin did not contain traces of guanin; and, even if thymin does give a colour reaction, then the diazo-remainder may perhaps link on to the No. 4 carbon, giving rise to an azo-dye.

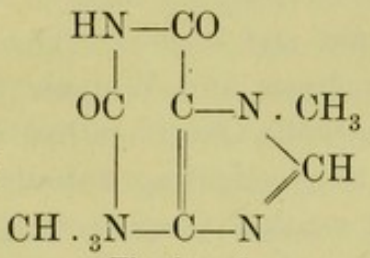
The essential points in favour of Burian's[2] view are:—

(1) Uric acid does not give the colour reaction, although it only differs from xanthin, which does give the reaction, in the structure of its imido-azol ring.

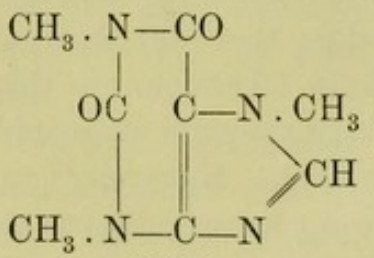
(2) Theophyllin gives the 'diazo-reaction,' while theobromine and caffeine do not.



Theophyllin.



Theobromine.



Caffeine.

Therefore substitution in the pyrimidin ring does not matter, while substitution of the imide-hyrogen-atom in No. 7 prevents the reaction.

The liberation of Purin-compounds by the Autodigestion of Nucleo-proteids.

The autodigestion of nucleo-proteids has been investigated by many observers, and the historical account of it given here is based on the recent article by Walter Jones.[3]

[1] H. Steudel, *Zeit. f. physiol. Chem.* **42**. 165 (1904).

[2] R. Burian, *ibid.* **42**. 297 (1904).

[3] Walter Jones, *ibid.* **42**. 35 (1904).

Schützenberger[1] found, in 1874, amongst the products of the autodigestion of yeast, in addition to other substances, also xanthin, hypoxanthin, guanin, and carnin. Salomon,[2] in 1878, observing xanthin in the blood of corpses, while it was absent in freshly drawn arterial blood, was thus led to study the autodigestion of different organs.[3] He especially noticed a doubling in the amount of the hypoxanthin in muscle, while the quantity in the pancreas or liver remained the same, or was slightly increased. Lehmann,[4] working under Rossel, arrived in 1885 at the conclusion that yeast kept for twenty-four hours in water showed a diminution of hypoxanthin, but an increase in the xanthin + guanin fraction when it was compared with yeast boiled directly with mineral acids. In 1888 Salkowski[5] introduced the method of studying the autodigestion of yeast in chloroform water, and showed that watery extract of yeast which had not been sterilised gave a considerable amount of xanthin-bases, and in a second paper[5] confirmed the results previously obtained by Salomon. Schwiening[6] in 1894 showed autodigestion to go on in extracts of organs containing no cellular elements, and that the presence of alkalies was injurious to enzyme action during autolysis, and Biondi[7] in 1896 proved that the enzyme was not trypsin. Okerblom[8] stated to have found amongst the autodigestion-products of the suprarenal: xanthin, 1-methyl xanthin, hypoxanthin, epiguanin, and a trace of adenin; but Jones and Whipple,[9] on repeating Okerblom's researches, failed to get 1-methyl xanthin and epiguanin, while they did find guanin and adenin. Their results differed also from Okerblom's in this: Jones and Whipple found no xanthin but large amounts of guanin; while Okerblom had found xanthin but no guanin. Kutscher,[10] on reinvestigating autodigested yeast, found no base corresponding to the xanthin fraction, while in the hypoxanthin fraction he found guanin and adenin. He expresses the view that xanthin must have disintegrated during the digestion, and in support of this view[11] he refers to Lehmann, who showed that preformed

[1] Schützenberger, *Bull. de la Soc. chim. de Paris* (1874), p. 194.

[2] Salomon, *Zeit. f. physiol. Chem.* **2**. 65 (1878).

[3] Salomon, *Arch. f. Physiol.* 1881, p. 361.

[4] Lehmann, *Zeit. f. physiol. Chem.* **9**. 563 (1885).

[5] Salkowski, *Deutsch. med. Wochensch.* **16**. (1888); *Zeit. f. physiol. Chem.* **13**. 506 (1889); and *Zeit. f. klin. Med.* 1890, Suppl. p. 77.

[6] Schwiening, *Virchow's Arch.* **136**. 444 (1894).

[7] Biondi, *ibid.* **144**. 373 (1896).

[8] J. Okerblom, *Zeit. f. physiol. Chem.* **28**. 60 (1898).

[9] Walter Jones and Whipple, *Amer. Journ. of Physiol.* **7**. 423 (1902).

[10] Kutscher, *Zeit. f. physiol. Chem.* **32**. 59 (1901).

[11] Kutscher, *Sitzb. d. Ges. z. Bef. d. gesammten Naturwiss.* (1900).

xanthin-bases disappeared. Jones and Whipple finding guanin and adenin, but no xanthin or hypoxanthin amongst the hydrolytic dissociation-products of Hammarsten's α-nucleo-proteid prepared from the pancreas, reasoned that autolysis must give rise to the same products as does hydrolysis.

Kutscher[1] in 1901 prepared from the thymus gland a product which he called thymin, and this he believed to be contaminated by traces of uracil (the constitutional formulæ are given on p. 427). Levene[2] in 1903 obtained uracil from autodigested pancreas, while by the action of mineral acids on the pancreas there is produced thymin. As Levene failed to find thymin amongst the products of autolysis, he concluded that uracil is formed in place of thymin, but did not say whether this result was due to the presence of trypsin or to some special enzyme.

Araki[3] showed in 1903 that an enzyme is contained in the thymus, which converts α-thymo-nucleic acid into the β-variety. This, however, is a purely hydrolytic process, for the same result may be obtained with trypsin or with hot alkalies. Reh[4] believes also in some causal connection between the presence of thymin and uracil amongst the products of autodigested lymph-glands and the absence of xanthin-bases.

Iwanoff[5] discovered in 1903, in the moulds Aspergillus niger and Penicillium glaucum, an enzyme capable of disintegrating the sodium salt of thymo-nucleic acid into phosphoric acid and into xanthin-bases, and called the enzyme 'nuclease.' He believes it to play an important part during ordinary cell metabolism.

Schittenhelm and Schröter[6] confirmed the view that putrefactive bacteria in general, and the coli-bacillus in particular, are able to convert one kind of xanthin-base into another kind; and Walter Jones[7] then showed that in autodigested thymus xanthin is formed at the expense of guanin and adenin, *i.e.* that the amino-group of guanin must become replaced by a hydroxyl group, while the conversion of adenin into xanthin is brought about by the substitution of oxygen for the amino-group and the replacement of a hydrogen-atom by a hydroxyl-group. In both these cases ammonia is split off, and in the case of

[1] Kutscher, *Zeit. f. physiol. Chem.* **34**. 114 (1901).

[2] Levene, *ibid.* **37**. 527 (1903); and the autodigestion of the spleen, *ibid.* **38**. 404 (1903), and **39**. 135 (1903).

[3] Araki, *ibid.* **38**. 84 (1903).

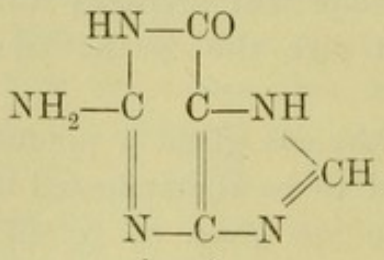
[4] Reh, *Hofmeister's Beiträge*, **3**. 569 (1903).

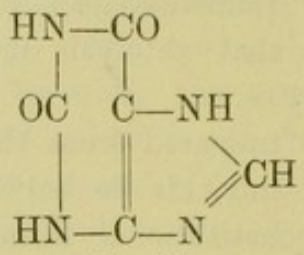
[5] Iwanoff, *Zeit. f. physiol. Chem.* **39**. 31 (1903).

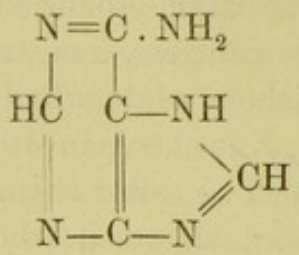
[6] Schittenhelm and Schröter, *ibid.* **39**. 203 (1903).

[7] Walter Jones, *ibid.* **41**. 101 (1904).

adenin, hypoxanthin is formed as a by-product, provided the amino-group is replaced primarily.


Guanin.


Xanthin.


Adenin.

The following table shows the effects of enzyme-action and of hydrolysis by means of boiling acids on the nucleo-proteids of the thymus, the suprarenal, and the spleen, according to Walter Jones :—

	Thymus.	Thymo-nucleic Acid	Suprarenal.		Spleen.	
	Auto-digested.	Hydrolysed by Mineral Acids.	Auto-digested.	Hydrolysed by Acids.	Auto-digested.	Hydrolysed.
Xanthin . .	+	...	+	...	...	...
Hypoxanthin .	+ (traces)	...	+	...	+	...
Guanin . .	−	+	...	+	+	+
Adenin . .	−	+	...	+	...	...
Thymin . .	−	+	...	...	...	+
Uracil . .	+	...	...	...	+	...
Cytosin . .	...	...	...	...	...	+

These results might be explained by assuming that the enzymes and the mineral acids attack the nucleic acids at different points, and thereby give rise to different products; but Walter Jones is of the opinion that the enzymes act in exactly the same way as do boiling mineral acids, and that, in addition, they have the power of removing amino-groups, and of oxidising and of splitting up CO_2. "It is interesting that these three effects are produced by the ordinary putrefactive bacteria, as in the formation of paroxyphenyl-propionic acid, paracresol and phenol from tyrosin, and in the formation of putrescin from α-δ-diamino-valerianic acid."

Jones and Partridge[1] then studied the differences between the purin-derivatives formed during autodigestion and those formed by hydrolysis of the corresponding nucleo-proteids by means of boiling mineral acids. They found the pancreas to contain an enzyme capable of converting guanin into xanthine, and they called the

[1] W. Jones and C. L. Partridge, *Zeit. f. physiol. Chem.* **42**. 343 (1904).

enzyme 'guanase.' This body is also present in the thymus and suprarenal, but not in the spleen. As in autodigested spleen adenin is converted into hypoxanthin in the absence of guanase, there must be present a second ferment (adenase), which also occurs in the thymus, the suprarenal, and the pancreas.

To show the difference between the hydrolysing action of boiling mineral acids, resulting always in the production of guanin and adenin, and that produced by the autodigestion of nucleo-proteids, Jones and Partridge have put the products of autolysis into tabular form:

	Xanthin.	Hypoxanthin.	Guanin.	Adenin.
Thymus . .	In large amounts	Traces	Absent	Absent.
Suprarenal . .	In large amounts	Traces	Absent	Absent.
Spleen . . .	Absent	Present	Present	Absent.

Jones and Partridge assume that both autodigestion and hydrolysis originally liberate the same bases, but that subsequently the bases undergo a further change as the result of special ferments which are present in the autodigesting organs.

Schittenhelm[1] was the first to establish the quantitative transition of the amino-purins: adenin and guanin into uric acid under the influence of tissue ferments, and also to show that the purin-compounds of thymus-nucleic acid were convertible into uric acid, and that the uric-acid-forming oxydase of the spleen can be salted out with ammonium sulphate.

In a second paper, Schittenhelm[2] has thrown still further light on the ferments of nuclear metabolism. By subjecting spleen-extract to fractional precipitation, according to Jakoby's plan,[3] he found the oxidising ferment to be thrown down in largest amounts by 66 per cent saturated ammonium sulphate solutions. The precipitate obtained in this way was suspended in water; the mixture shaken for a half to one hour and then dialysed in running water for six to eight days till ammonia was no longer demonstrable. The filtered solution, of a slightly yellowish brown colour, was very active, as at incubation temperature it converted guanin quantitatively into uric acid, provided air was passed through the solution containing the ferment and the guanin, while if no air was passed through, guanin was converted

[1] A. Schittenhelm, *Zeitsch. f. physiol. Chem.* **42**. 251 (1904) (here the older literature).
[2] A. Schittenhelm, *ibid.* **43**. 228 (1904).
[3] Jakoby, *ibid.* **30**. 135 (1900).

into xanthin. Therefore guanin is converted through the xanthin stage into uric acid. The 2-amino,—6, 8-dioxy-purin, which might have given rise to uric acid, was absent. The spleen is thus capable of reacting, contrary to the statement of Jones, in the same way as the thymus, the pancreas, and the suprarenal, and also the liver, lung, and muscle, while apparently no uric acid formation out of guanin occurs in the thymus, intestine, blood, and kidney.

As guanin is converted into xanthin in the thymus and the kidney, there must be present two ferments, one of which is a desaminating one, converting guanin into xanthin and adenin into hypoxanthin, while the second ferment is an oxidising one, changing hypoxanthin into xanthin and xanthin into uric acid.

While the desaminating ferment is distributed widely over the body, the oxidising ferment seems to be more restricted in its distribution.

As the desaminating ferment acts on both guanin and adenin, Schittenhelm believes the terms 'guanase' and 'adenase' to be superfluous. But as the spleen ferment, which converts guanin into xanthin and xanthin into uric acid, is unable to dissociate α-nucleic acid, and thus to liberate the purin-bases contained in it and then to change them into uric acid, there must be present a third ferment, the 'nuclease,' which can split up nucleic acid.

A fourth ferment capable of disintegrating uric acid, suggested by the destruction of uric acid in the tissues (Wiener[1] and Ascoli[2]), Schittenhelm has also isolated. It is most abundant in the kidney, but is also present in the liver, muscle, and perhaps bone-marrow.

Walter Jones in his last paper[3] has pointed out, in collaboration with Winternitz, against Schittenhelm, that watery infusions of spleen do not alter guanin, and that therefore they do not contain guanase, and that spleen infusion by autodigestion quickly gives rise to hypoxanthin, which by prolonged digestion is gradually converted into xanthin. As further, guanin is not altered by an infusion of spleen, while adenin added to spleen infusion is quickly changed into hypoxanthin, it follows that an oxydase must be present which converts adenin into xanthin. On autodigesting liver they found guanin, considerable quantities of xanthin, and traces of hypoxanthin. The presence of guanin in autodigested liver, as well as the fact that guanin added to autodigesting liver remains unaltered, shows again the absence of guanase. Adenin is, however, quickly changed into xanthin

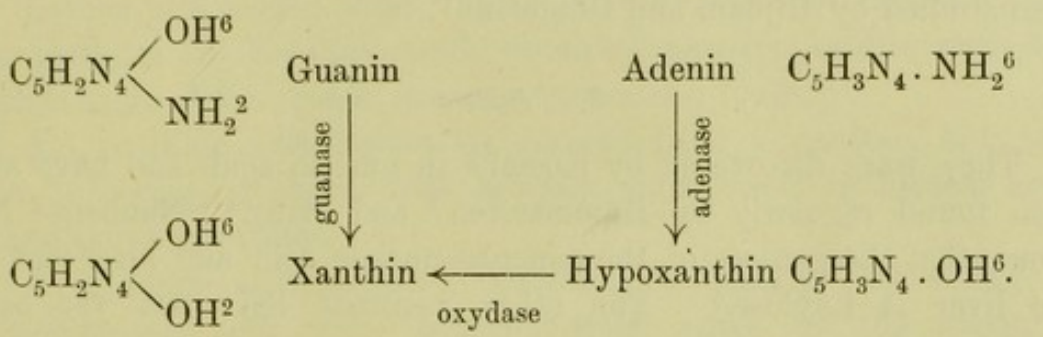
[1] H. Wiener, *Arch. f. exp. Pathol. u. Pharmak.* **42**. 375 (1899).

[2] G. Ascoli, *Pflüger's Arch.* **72**. 340 (1898).

[3] W. Jones and M. C. Winternitz, *Zeitschr. f. physiol. Chem.* **44**. 1 (1905).

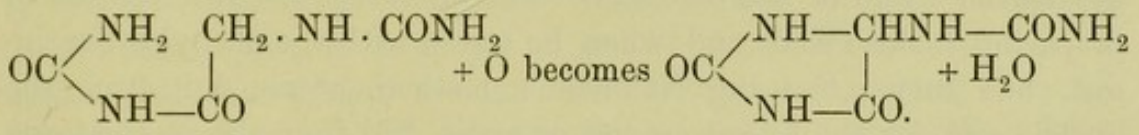
by autodigesting liver. Spleen and liver agree in having the same ferments, but the spleen contains much more oxidase.

The changes which purin-bases undergo are expressed diagrammatically by Jones and Winternitz in this manner:



Schittenhelm and Bendix[1] have found that in rabbits the intravenous injection of guanin increases the uric acid output.

The allantoin in the body is another product of the oxidative destruction of purin-bases, and in particular of uric acid (Salkowski, Minkowski, Cohn.). Eppinger[2] has synthetised allantoin from glycolyl-di-urea, which latter becomes oxidized and converted into a ring-compound, both in the test-tube and when administered to dogs in the food.



The purin-bodies of the urine are not all derived from decomposing nucleo-proteids according to Burian.[3] He believes them to be formed mostly synthetically, for violent muscular exercise during hunger leads firstly to the production of purin-bases and only secondarily to that of uric acid. Hypoxanthin and kreatinin are supposed to be synthetised by one and the same fundamental process during muscular work. The author from his histological researches knows that all cell-activity is accompanied during the stages of restitution by an increase in the size of the nuclear chromatin-segments, and an over-production of purin-bases during violent muscular exercise can be readily accounted for in this way, particularly as during starvation the nucleic-acid radicals will be poorly supplied by the necessary albumin-complements.

Burian[4] has described a xanthin-oxydase as occurring in extracts of ox-livers which in the presence of a stream of oxygen oxidizes the

[1] A. Schittenhelm and E. Bendix, *Zeitschr. f. physiol. Chem.* **43**. 365 (1905).
[2] H. Eppinger, *Hofmeister's Beiträge*, **6**. 287 (1905).
[3] R. Burian, *Zeitschr. f. physiol. Chem.* **43**. 532 (1904).
[4] R. Burian, *ibid.* **43**. 497 (1904).

purin-bases into uric acid, and which then decomposes the uric acid, while in the absence of oxygen neither a formation nor a decomposition of uric acid takes place.

How urea is split up by the Bacillus acidi urici (Ulpiani, 1903) has been studied by Ulpiani and Cingolani.[1]

4. *Pentoses*

They were discovered by Kossel [2] in nucleic acid, and have also been found regularly by Hammarsten [3] and Bang.[4] Neuberg [5] has shown that the pentose of the pancreas-nucleic acid and also that of the liver is l-xylose.[6] The other pentoses have not yet been investigated.

5. *Lævulinic Acid*

Lævulinic acid, $CH_3 . CO . CH_2 . CH_2 . COOH$, was discovered by Kossel [7] in the nucleic acid of the thymus, and more thoroughly investigated by Kossel and Neumann.[8] Noll,[9] working under Kossel, found it in the nucleic acid of the spermatozoa of the sturgeon, and Araki [10] in the nucleic acid of the intestinal mucous membrane. Levene failed at first (see below) to get it from the nucleic acids of the spleen [11] and of the ox-testis [12] when he used his own method of preparing nucleic acids and when he tested with the phenyl-hydrazin-test, but Inouye,[13] using Neumann's method, obtained it from the nucleic acids of the ox-spleen and ox-testis, and from the spermatozoa of Murænoesox cinereus, for he got—

1. A yellow precipitate on adding iodine-potassium iodide and caustic soda.
2. A red colour with sodium-nitro-prusside and caustic soda, and a conversion of the red into a violet colour on adding acetic acid.

[1] F. C. Ulpiani and M. Cingolani, *Gazetta chimica italiana*, **34**. 377 (1904). Abstract in *Zentralbl. f. Physiol.* **19**. 166 (1905).

[2] A. Kossel, *Arch. f.* (*Anat. u.*) *Physiol.* 1891, p. 181 ; 1893, p. 157 (*Verhandlungen der physiol. Gesellschaft*).

[3] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **19**. 19 (1893).

[4] J. Bang, *ibid.* **26**. 133 (1898) ; **31**. 411 (1900).

[5] C. Neuberg, *Ber. d. deutsch. chem. Ges.* **35**. II. 1467 (1902).

[6] J. Wohlgemuth, *Zeitschr. f. physiol. Chem.* **37**. 475 (1903).

[7] A. Kossel, *Arch. f.* (*Anat. u.*) *Physiol.* 1893, p. 157.

[8] A. Kossel and A. Neumann, *Ber. d. deutsch. chem. Ges.* **27**. II. 2215 (1894).

[9] Noll, *Zeitschr. f. physiol. Chem.* **25**. 430 (1898).

[10] Araki, *ibid.* **38**. 98 (1903).

[11] P. A. Levene, *ibid.* **37**. 402 (1903).

[12] Levene, *ibid.* **39**. 479 (1903).

[13] P. A. Katsuji Inouye, *ibid.* **42**. 116 (1904).

3. A phenyl-hydrazon on treatment with phenyl-hydrazin acetate.

The watery solution of lævulinic acid, after neutralising with ammonia, was converted into a silver salt $AgC_5H_7O_3$.

Levene in a second paper,[1] using Inouye's methods of testing for the presence of lævulinic acid, obtained positive results with the nucleic acids of the spleen, pancreas, testis, and brain.

As lævulinic acid cannot be derived from a pentose, but only from a hexose, it shows that hexoses must occur in nucleic acids in addition to pentoses.

A saccharic acid has been obtained by Alsberg[2] by acting on salmon-nucleic acid with barium-hydrate, while by the action of mineral acids he obtained lævulinic acid. That a hexose in the form of a very stable polysaccharid occurs in nucleic acids seems to be shown by the investigations of Levene.[3] By acting for four hours with 2 per cent H_2SO_4 on thymo-nucleic acid in an autoclave at a temperature of 100° to 125,° Levene obtained an acid resembling Neumann's nucleo-thyminic acid, for it is soluble in dilute mineral acids and contains 12·33 per cent N and 11·33 per cent P. This acid is more readily soluble in alkalies than is the original acid, and it does not gelatinise. When this acid was still more dissociated by 10 per cent H_2SO_4, most of the purin and pyrimidin radicals and the mother-substance giving the furfurol reaction were destroyed, but a hexose radical must still have been present, for on boiling equal amounts of the substance obtained by the action of 10 per cent H_2SO_4 and of the original nucleic acid with 25 per cent H_2SO_4, the original nucleic acid yielded less lævulinic acid.

Ammonia has been found by Kossel and Neumann,[4] Noll,[5] Bang,[6] and Bottazzi;[7] formic acid by Kossel and Neumann,[4] and Neumann,[8] but both ammonia and formic acid are probably formed secondarily.[9] The simple pyrimidin derivatives are, on the other hand, certainly primary dissociation-products, and are not derived from the purins. Bang's[9] statement that the pancreas-nucleic acid contains glycerine has not yet been confirmed.

[1] P. A. Levene, *Zeitschr. f. physiol. Chem.* **43**. 199 (1904).
[2] C. L. Alsberg, *Arch. f. experim. Pathol.* **51**. 239 (1904).
[3] P. A. Levene, *Amer. Journ. of Physiol.* **12**. 213 (1904).
[4] A. Kossel and A. Neumann, *Ber. d. deutsch. chem. Ges.* **27**. II. 2215 (1894).
[5] Noll, *Zeitschr. f. physiol. Chem.* **25**. 430 (1898).
[6] J. Bang, *Zeitschr. f. physiol. Chem.* **26**. 133 (1898).
[7] F. Bottazzi, *Zentralbl. f. Physiol.* **18**. 98 (1904).
[8] A. Neumann, *Arch. f. (Anat. u.) Physiol.* 1898, p. 374.
[9] J. Bang, *Zeitschr. f. physiol. Chem.* **31**. 411 (1900).

General Properties of Nucleic Acids

The number of purin-bases in one molecule of nucleic acid is not known. It is possible that four nucleic acids occur naturally, and that each contains only one base, and that these four acids occur mixed in different proportions[1] in different tissues, but it is also possible that a nucleic acid may contain, for example, adenin and guanin.[2] The same holds good of the pyrimidin-derivatives. The transformation of purin-bases into one another is dealt with on p. 446. At present it is only possible to enumerate the occurrence of the dissociation-products of each individual organ. The phosphoric acid, which is always found, and the pentose, which as a rule is present, are not given in the table below.

The substances not indicated in the following tables need not therefore be absent in the nucleic acids mentioned; in the most cases the substances not tabulated have not been specially looked for.

	Uracil.	Thymin.	Cytosin.	Guanin.	Adenin.	Xanthin.	Hypo-xanthin.	Lævulinic acid.
Spermatic fluid, salmon	...	23	...	6	6	6	6	...
,, ,, sturgeon	...	8	19	...	...	...	...	3
,, ,, herring	18	7	20	...	...	...	...	...
,, ,, ox and boar	...	...	...	6	6	6		...
Thymus	18	4 5	5 19 20	17	5 6	22	...	5
Suprarenal	...	...	...	16	16	...	...	...
Pancreas	...	...	...	14	6	6	6	...
Wheat-embryo	24	...	25	24	24	...	...	...
Yeast	9	5	21	10	10	13	12	11

[1] A. Kossel and A. Neumann, *Zeitschr. f. physiol. Chem.* **22**. 74 (1896).

[2] O. Schmiedeberg, *Schmiedeberg's Archiv f. experiment. Path. u. Pharmak.* **43**. 57 (1899). [3] A. Noll, *Zeitschr. f. physiol. Chem.* **25**. 430 (1898).

[4] A. Kossel and A. Neumann, *Ber. d. deutsch. chem. Ges.* **26**. III. 2753 (1893).

[5] Kossel and Neumann, *ibid.* **27**. II. 2215 (1894).

[6] Yoshito Inoko, *Zeitschr. f. physiol. Chem.* **18**. 540 (1893).

[7] W. Jones, *ibid.* **29**. 20 (1900).

[8] A. Kossel, *ibid.* **22**. 188 (1896). [9] A. Ascoli, *ibid.* **31**. 161 (1900).

[10] A. Kossel, *Arch. f.* (*Anat. u.*) *Physiol.* 1891, p. 181.

[11] A. Kossel, *ibid.* 1893, p. 157.

[12] A. Kossel, *Zeitschr. f. physiol. Chem.* **4**. 290 (1879).

[13] A. Kossel, *ibid.* **4**. 290 (1880).

[14] J. Bang, *ibid.* **26**. 133 (1898). [15] J. Bang, *ibid.* **31**. 411 (1900).

[16] W. Jones and G. H. Whipple, *Amer. Journ. of Physiol.* **7**. 423 (1902) (*Maly's Jahresber.* 1902, p. 45).

[17] A. Kossel and A. Neumann, *Zeitschr. f. physiol. Chem.* **22**. 74 (1896).

[18] A. Kossel and H. Steudel, *ibid.* **37**. 245 (1903).

[19] A. Kossel and H. Steudel, *ibid.* **37**. 177 (1903).

Steudel[26] has decomposed thymus-nucleic acid by means of hydriodic acid in the presence of phosphoric acid, and has succeeded in accounting for 75 per cent of the total nitrogen. He gives, with all reserve, the following tabular statement:—

Thymin and Uracil	15·88
Adenin	13·45
Humin-nitrogen	11·54
Cytosin	11·45
Ammonia	7·00
Xanthin	6·74
Hypoxanthin	5·20
Guanin	3·61
	74·87 per cent.

Some of the dissociation-products, especially purin-bases, have also been found in a large number of organs; Kossel[27] found all the bases which he by then (1882) had discovered in the spleen, kidney, liver, testes, brain, blood, and muscle, and especially large quantities in leucæmic blood and in embryonic muscle. Pekelharing[28] found xanthin in the nucleo-proteid of the gastric juice; Inoko,[29] working under Kossel, adenin in the red blood-corpuscles of geese; Kossel and Neumann,[30] thymin in the milk; Grund,[31] pentoses in the pancreas, liver, thymus, thyroid, salivary gland, spleen, brain, and muscle; Araki,[32] lævulinic acid in a nucleic acid prepared from the intestinal mucous membrane; Hammarsten,[33] a carbohydrate in the mammary gland.

A large number of nucleic acids and their dissociation-products have been described by Levene.[34]

A definite picture of the constitution of nucleic acids we cannot as yet draw from the known data, especially because quantitative

[20] A. Kossel and H. Steudel, *Zeitschr. f. physiol. Chem.* **37**. 377 (1903).

[21] A. Kossel and H. Steudel, *ibid.* **38**. 49 (1903).

[22] A. Neumann, *Arch. f.* (*Anat. u.*) *Physiol.* 1899, Suppl. p. 552.

[23] F. Miescher and O. Schmiedeberg, *Arch. f. experiment. Path. und Pharm.* **37**. 1 (1896).

[24] T. B. Osborne and J. F. Harris, *Zeitschr. f. physiol. Chem.* **36**. 85 (1902).

[25] H. L. Wheeler and T. B. Johnson, *Amer. Chem. Journ.* **29**. 505 (from the abstract in the *Chem. Centr.* 1903, I. p. 1311).

[26] H. Steudel, *Zeitschr. f. physiol. Chem.* **42**. 165 (1904).

[27] A. Kossel, *ibid.* **7**. 7 (1882).

[28] C. A. Pekelharing, *ibid.* **35**. 8 (1902).

[29] Y. Inoko, *ibid.* **18**. 57 (1893).

[30] See footnote 5, p. 440.

[31] G. Grund, **35**. 111 (1902).

[32] T. Araki, *ibid.* **38**. 98 (1903).

[33] O. Hammarsten, *ibid.* **19**. 19 (1893).

[34] P. A. Levene, *ibid.* **32**. 541 (1901); **37**. 402 (1903); **38**. 80 (1903); **39**. 4 and 133 (1903).

estimations of the dissociation-products are in most cases wanting. According to Bang the pancreas-nucleic acid contains about 30 per cent of pentose, and at least as much guanin. The pyrimidin-derivatives seem to occur in smaller amounts. The constitutional formulæ of Bang [1] and Osborne and Harris [2] are, according to Cohnheim, premature, but the author has thought it right to give Bang's views on guanylic acid on p. 459.

What is known regarding the percentage-composition of nucleic acids is given in the following table of analysis :—

C	H	N	P	O		
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.		
37·32	4·21	15·24	9·62	33·59	Sperma, salmon.	Miescher.[3]
...	...	...	9·33	...	,, sturgeon.	Noll.[4]
...	...	15·34	9·59	...	,, sea-urchin.	Mathews.[5]
34·07	4·31	16·03	9·04	...	Yeast.	Miescher.[3]
34·17	4·39	18·2	7·67	35·56	Pancreas.	Bang.[6]
...	...	...	9·94	...	Thymus.	Lilienfeld.[7]
38·91	5·54	15·55	9·25	...	,,	Kostytschew.[8]
35·85	4·23	15·26	9·3	...	,,	Bang.[9]
34·65	4·30	15·88	8·70	35·05	Wheat-embryo.	Osborne and Harris.[10]
...	...	...	9·6	...	Intestinal mucous membrane.	Araki.[11]
...	...	...	9·5	...	Nucleic acid.	Altmann.[12]
32·88	3·73	16·0	8·60	...	Inosinic-acid, muscle.	Haiser.[13]

The authors calculate from these analyses the following minimal formulæ :—

$C_{40}H_{52}N_{14}O_{25}P_4$ (milt of salmon, Schmiedeberg [14]).
$C_{40}H_{56}N_{14}O_{26}P_4$ (milt of salmon, Herlant [15]).
$C_{40}H_{54}N_{14}O_{27}P_4$ (milt of herring, Mathews [5]).
$C_{36}H_{48}N_{14}O_{30}P_4$ (yeast, Herlant [15]).
$C_{40}H_{54}(OH)_5N_{14}O_{27}P_4$ (yeast, Miescher and Schmiedeberg [3]).

[1] J. Bang, *Zeitschr. f. physiol. Chem.* **31**. 411 (1900).
[2] T. B. Osborne and J. F. Harris, *ibid.* **36**. 85 (1902).
[3] F. Miescher, "Milt of Salmon," in the posthumous papers edited by O. Schmiedeberg, *Arch. f. experiment. Pathol. u. Pharm.* **37**. 1 (1896).
[4] A. Noll, *Zeitschr. f. physiol. Chem.* **25**. 430 (1898).
[5] A. Mathews, *ibid.* **23**. 399 (1897).
[6] J. Bang, *ibid.* **26**. 133 (1898) ; **31**. 411 (1900).
[7] L. Lilienfeld, *ibid.* **18**. 473 (1893). [8] S. Kostytschew, *ibid.* **39**. 545 (1903).
[9] J. Bang, *Hofmeister's Beiträge*, **4**. 331 (1903).
[10] T. B. Osborne and J. F. Harris, *Zeitschr. f. physiol. Chem.* **36**. 85 (1902).
[11] T. Araki, *ibid.* **38**. 98 (1903).
[12] R. Altmann, *Arch. f.* (*Anat. u.*) *Physiol.* 1889, p. 524.
[13] F. Haiser, *Monatsh. f. Chem.* **16**. 190 (1895).
[14] O. Schmiedeberg, *Arch. f. experim. Path. u. Pharm.* **43**. 57 (1899).
[15] L. Herlant, *ibid.* **44**. 148 (1900).

$C_{44}H_{66}N_{20}O_{34}P_4$ (pancreas, Bang [1]).
$C_{41}H_{74}N_{14}O_{26}P_4$ (thymus, Kostytschew [2]).
$C_{41}H_{61}N_{16}O_{31}P_4$ (wheat-embryo, Osborne and Harris [3]).
$C_{40}H_{56}N_{14}O_{26}P_4$ (thymus, Bang [4]).

It will be seen that the differences are not inconsiderable, yet a fair agreement is shown in the nucleic acids obtained from the spermatic fluids of fish, the yeast, and the thymus, in respect of the nitrogen- and phosphorus-contents. The guanylic acid differs more widely, a fact which Bang explains by the high amount of guanin which it contains (see p. 459). The inosinic acid which Liebig and Haiser prepared from meat-extract gives rise, according to Haiser, to dissociation-products which differ from those usually obtained, for there are present, besides hypoxanthin and phosphoric acid, a trioxy-valerianic acid, but further investigations into this matter are needed.

Altmann and Neumann [5] and others describe nucleic acids in their dry state as white, loose, dust-like, and non-hygroscopic powders. They are slightly soluble in cold water—(0·3 parts of pancreas-nucleic acid in 100 parts of water); in hot water they are much more soluble, and very soluble in alkalies, and also in potassium acetate; they are precipitated by mineral acids, and dissolved by an excess of acid; acetic acid precipitates only the pancreas-nucleic acid and not the others. By the addition of an equal bulk of ethyl-alcohol they are precipitated, but most readily by 50 per cent alcohol containing hydrochloric acid, especially if ether be also added, and this method has been employed by Altmann and Neumann for preparing the nucleic acids in a pure state. With most of the salts of the heavy metals nucleic acids give insoluble salts, and are therefore precipitated by copper-, silver-, zinc-, lead-, and iron-salts. Barium-, calcium-, and strontium-salts produce gelatinous precipitates, according to Neumann and Kostytschew.[6] As nucleic acids are also precipitated by tannic, picric, and phospho-molybdic acids, they must possess basic radicals like all the other purin derivatives. The colour-reactions of the albumins are, of course, absent.

The nucleic acids are pluribasic; Miescher has examined the ammonia and baryta salts of the nucleic acid of the salmon, and Schmiedeberg the copper salt. Other investigators have employed, as

[1] J. Bang, *Zeitschr. f. physiol. Chem.* **26**. 133 (1898); **31**. 411 (1900).
[2] S. Kostytschew, *ibid.* **39**. 545 (1903).
[3] T. B. Osborne and J. F. Harris, *Zeitschr. f. physiol. Chem.* **36**. 85 (1902).
[4] J. Bang, *Hofmeister's Beiträge*, **4**. 331 (1903).
[5] A. Neumann, *Arch. f. (Anat. u.) Physiol.* 1899, Suppl. p. 552.
[6] S. Kostytschew, *Zeitschr. f. physiol. Chem.* **39**. 545 (1903).

a rule, the barium salt. Bang has investigated the silver salt of guanylic acid. The equivalent weight of the nucleic acids is between 300 and 600, while the molecular weight must be much higher.

Kossel[1] has pointed out that the salts of the nucleic acids, especially that prepared from the leucocytes of the thymus, possess the remarkable physical property of forming jellies or mucilaginous solutions. According to Plenge,[2] a 5 per cent solution of sodium nucleate on being cooled to 42° solidifies into a glassy, perfectly clear, firm, gelatinous jelly, and 2·5 per cent solutions solidify also if they contain sodium chloride or bouillon made by extracting meat with water and then adding peptone. Plenge has made use of this sodium nucleate for preparing culture media which remain solid at 37°.

If the solution contains still less nucleic acid it does not set into a firm jelly; but, especially in the presence of albumin, it possesses a marked viscous consistency reminding one of mucin-solutions. The blood of birds solidifies on the addition of caustic-soda solution into a jelly, owing to the nucleic acid contained in the nuclei of the red corpuscles; and even human blood[3] has a tendency towards jelly formation, especially if the number of leucocytes be excessive. The same holds good for urine[4] rich in leucocytes.

The nucleic acids and their derivatives, the nucleins and nucleoproteids, are dextro-rotatory, as Gamgee and Jones[5] have found. The albumin-moiety is lævo-rotatory, but the dextro-rotatory power of the nucleic acid is greater. The salts of nucleic acids with albumin are the most important, for they occur normally in the heads of the spermatozoa of fish, and perhaps also in other situations. The albumin salts are insoluble; but they behave analogously to the salts which albumins form with the alcoloidal reagents, *i.e.* they dissociate hydrolytically if an excess of acid be not present. Nucleic acid, therefore, precipitates albumin only if the reaction be acid, and not if the reaction be either alkaline or neutral.

From the experimental-histological point of view, protamins, nucleins, and nucleic acids have been very thoroughly investigated by Berg.[6] See also Wetzel.[7]

[1] A. Kossel, *Arch. f.* (*Anat. u.*) *Physiol.* 1891, p. 181; A. Neumann, *ibid.* 1899, Suppl. p. 552. [2] H. Plenge, *Zeitschr. f. physiol. Chem.* **39**. 190 (1903).

[3] C. Hirsch and E. Stadler, *Zeitschr. f. physiol. Chem.* **41**. 125 (1904).

[4] Joh. Müller, *Münchener medizin. Wochenschr.* 1903, p. 1360.

[5] Arthur Gamgee and Croft Hill, *Ber. d. deutsch. chem. Ges.* **36**. 913 and 914 (1903); A. Gamgee and W. Jones, *Hofmeister's Beiträge*, **4**. 10 (1903); see also T. B. Osborne, *Amer. Journ. of Physiol.* **9**. 69 (1903).

[6] Walter Berg, *Arch. f. unk. Anat.* **62**. 367 (1903), and **65**. 298 (1904).

[7] J. Wetzel, *Arch. f.* (*Anat. u.*) *Physiol.* 1904, p. 544; and in *Verh. d. physiol. Ges. zu Berlin*, **10**. p. 71.

Many toxins are also precipitated by nucleic acids according to Tichomiroff,[1] and the strong disinfecting action of free nucleic acid observed by Kossel[2] depends, perhaps, also on its albumin-precipitating power. Introduced into the blood, thymus-nucleic acid causes a marked hyperleucocytosis, while its salts are inactive, according to Neumann;[3] in doses of 10 grammes it is indifferent to man.

Goto[4] has noticed a very important property of nucleic acid, namely, that of keeping purin-bases and uric acid in solution. Whether uric acid is kept by this means in solution in the body is as yet uncertain, but the property is of the very greatest importance in physiological investigations, for it is impossible to determine purin-bases and uric acid quantitatively in the presence of nucleic acids.

Nucleic acids are, as Neumann has shown, very readily decomposed by boiling with acids, or even with pure water; but they are very resistant towards the action of alkalies, especially if sodium acetate be added. Alkalies have, therefore, been used for isolating nucleic acids.

When nucleic acids disintegrate, they do not at once give rise to the above-mentioned dissociation-products, as a number of intermediate products are formed in the first instance. Neumann[5] and Kostytschew[6] state that the nucleic acid 'a,' which can be isolated from such tissues as the thymus, is converted when it is boiled for two hours with alkalies into nucleic acid 'b.' This latter no longer gelatinises; with barium and calcium it does not give a gelatinous precipitate; it contains only a portion of the purin-bases originally present, and for this reason less nitrogen than the a-nucleic acid. When b-nucleic acid is still further hydrolysed it gives rise to the thyminic acid, which contains no purin-bases, and to which Kossel and Neumann[7] have given the formula $C_{16}H_{25}N_3P_2O_{12}$. The thyminic acid also precipitates albumin in an acid solution, and keeps purin-bases in solution. As an intermediate product between b-nucleic acid and the thyminic acid Neumann[5] has described the nucleo-thyminic acid.

The dissociation of the nucleic acids into the just-mentioned intermediate and into the final products does not only occur when

[1] M. Tichomiroff, *Zeitschr. f. physiol. Chem.* **21**. 90 (1895).

[2] A. Kossel, *Arch. f. (Anat. u.) Physiol.*, Physiol. Abtl. 1893, p. 157; A. and H. Kossel, *ibid.* 1894, p. 200.

[3] A. Neumann, *ibid.* 1898, p. 374 (*Verhandlungen der Berliner physiol. Gesellschaft*).

[4] M. Goto, *Zeitschr. f. physiol. Chem.* **30**. 473 (1900).

[5] A. Neumann, *Arch. f. (Anat. u.) Physiol.* 1899, Suppl. p. 552.

[6] S. Kostytschew, *Zeitschr. f. physiol. Chem.* **39**. 545 (1903).

[7] A. Kossel and A. Neumann, *ibid.* **22**. 74 (1896).

they are boiled with water or with acids, but is also induced by ferments, the so-called 'nucleases.'

Gumlich,[1] working under Kossel, has shown that nucleic acid dissolves readily in the alkaline intestinal and pancreatic juices. This solution depends, according to Araki,[2] in the conversion of the a-nucleic acid into the b-variety, and is brought about by ferments contained in the trypsin- and erepsin-preparations, and also in the thymus. Iwanoff[3] and Plenge,[4] and Schittenhelm and Schröter[5] found similar ferments in bacteria, for Plenge could show their presence in a clear manner, for his solid culture-medium consisting of a sodium-nucleate compound became soluble. This liquefaction is for diagnostic purposes as valuable as is the liquefaction of gelatine. According to Kutscher[6] and Araki the dissociating action of the nucleases of such organs as the thymus, and of bacteria, yeast, etc., does not simply stop at the liquefaction of sodium nucleate, but results in a complete dissociation of the latter. Nucleases play an important part in the autolysis of the meat of fish, according to Schmidt-Nielsen,[7] as they convert the nucleic acid into purin-bases, etc. During metabolism nucleic acid is, without doubt, completely disintegrated, its phosphorus being excreted as phosphoric acid.[8] The fate of the pyrimidin- and of the purin-derivatives has not yet been cleared up.[9] Lehmann[10] found that if yeast be kept for some time that all its purin-radicals are converted into xanthin.

According to Bang,[11] the pancreas-nucleic acid possesses definite poisonous properties, such as that of preventing coagulation, etc. The nucleic acid of wheat-embryos behaves analogously, according to Mendel, Underhill, and White.[12]

[1] Gumlich, *Zeitschr. f. physiol. Chem.* **18**. 508 (1893).
[2] T. Araki, *ibid.* **38**. 84 (1903), (here the older literature).
[3] L. Iwanoff, *ibid.* **39**. 31 (1903).
[4] H. Plenge, *ibid.* **39**. 190 (1903).
[5] A. Schittenhelm and F. Schröter, *ibid.* **39**. 203 (1903).
[6] F. Kutscher, *ibid.* **32**. 50 (1900).
[7] S. Schmidt-Nielsen, *Hofmeister's Beiträge*, **3**. 266 (1902).
[8] P. M. Popoff, *Zeitschr. f physiol. Chem.* **18**. 533 (1893).
[9] H. Steudel, *ibid.* **32**. 285 (1901); **39**. 136 (1903).
[10] V. Lehmann, *ibid.* **9**. 563 (1885).
[11] J. Bang, *ibid.* **32**. 201 (1901).
[12] L. B. Mendel, F. P. Underhill, and B. White, *Amer. Journ. of Physiol.* **8**. 377 (1903).

Plasminic Acid and Iron

Here may be mentioned the plasminic acid, which Kossel[1] and Ascoli[2] prepared from yeast. It contains much more phosphorus than do the nucleic acids. Ascoli found up to 27 per cent of phosphorus, and the substance he analysed was probably a mixture of plasminic acid and of yeast-nucleic acid, so that in pure plasminic acid the phosphorus-content will be even higher.

Ascoli believes the plasminic acid to be a metaphosphoric acid, or the salt of such an acid with an organic base. Plasminic acid is readily soluble in water and in dilute hydrochloric acid, and, therefore, it is easy to separate it from the unchanged nucleic acid. Plasminic acid precipitates albumins and albumoses, and gives, with silver nitrate, a precipitate which is readily soluble in ammonia and partly soluble in hydrochloric acid; it is insoluble in acetic acid. Its most important property is that it renders iron 'masked.' If one add to a solution of metaphosphoric acid as much ferric chloride as can be kept in solution by the excess of acid, and if one then add ammonia to neutralisation and precipitate with alcohol and ether, a substance is obtained which gives the following reactions: It is soluble in water, hydrochloric acid, and ammonia; its iron does not react to small amounts of ammonium sulphide, and not immediately to larger amounts, and it does not give up its iron to hydrochloric-acid-alcohol except under certain conditions. Plasmin behaves exactly as does this metaphosphoric acid: it too contains iron, and also in a non-ionic form, as its presence cannot be demonstrated by either the Prussian blue reaction or by other direct tests. See also p. 450 and the index for other iron-containing compounds.

Ascoli[3] was unable to show the presence of metaphosphoric acid in yeast-nucleic acids.

(b) The Nucleo-Proteids

According to the unanimous statements of Miescher,[4] Kossel,[5] and Schmiedeberg,[6] nucleic acid occurs in the spermatozoa of several

[1] A. Kossel, *Arch. f. (Anat. u.) Physiol.* 1893, p. 157.

[2] A. Ascoli, *Zeitschr. f. physiol. Chem.* **28**. 426 (1899).

[3] A. Ascoli, *ibid.* **31**. 156 (1900).

[4] F. Miescher, *Verhandl. d. naturforsch. Gesellschaft in Basel VI.*, Heft I. p. 138 (1874). F. Miescher, "Milt of Salmon" in posthumous papers, edited by O. Schmiedeberg, *Schmiedeberg's Archiv für experiment. Path. u. Pharmak.* **37**. 1 (1896).

[5] A. Kossel, *Zeitschr. f. physiol. Chem.* **22**. 176 (1896); A. Mathews, *ibid.* **23**. 399 (1897); A. Kossel, *ibid.* **25**. 165 (1898); *Bull. de la Soc. chim. de Paris*, 1903, Juli.

[6] O. Schmiedeberg, *Arch. f. experiment. Path. u. Pharm.* **43**. 57 (1899).

fish in the form of a salt, namely, as protamin nucleate or histone nucleate, but in the tissues of mammals nucleic acid occurs in some other still unexplained state. Bang[1] and Osborne[2] have even denied the existence of nucleo-proteids as special compounds for these reasons: Nucleic acid precipitates albumin only if the reaction be acid (see p. 444). If one extracts an organ with a neutral or an alkaline fluid, the sodium nucleate may pass into solution along with the albumin, and therefore albumin nucleate will be precipitated as soon as the extract, which we have made, is acidified.

If, therefore, a 'nucleo-proteid' is precipitated by the addition of acetic acid to a watery extract of the thymus, the precipitate might in reality be an artefact, and not being preformed in the cell, the albumin nucleates would therefore be comparable to albumin phospho-tungstates or tauro-cholates. Kossel[3] points out, however, that Huiskamp[4] has shown that some of the nucleo-proteids are not salts at all, and Malengreau[5] and Huiskamp[6] have further succeeded in obtaining nucleo-proteids by 'salting-out' and by other means, without having had to acidify the extracts. The strongest positive proof for the existence of 'nucleo-proteids' is, however, the histological study of the distribution of iron and phosphorus by microchemical tests.

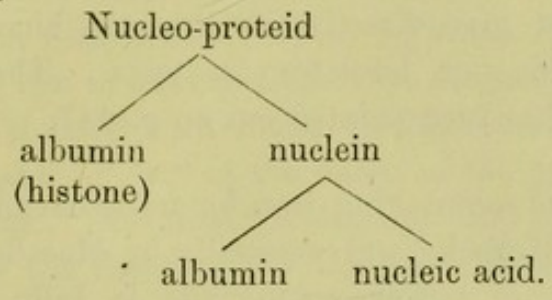
It is therefore imperative to regard nucleo-proteids as chemical individuals which form a special group of albumins. We have to admit, on the other hand, that the precipitates which are formed by bringing albumins and nucleic acids together, according to Kossel,[7] fairly closely resemble the naturally-occurring nucleo-proteids, and that the property of nucleic acids to form insoluble precipitates with albumins, and also otherwise to combine with albumins, makes the isolation and investigation of nucleo-proteids a very difficult task. For this reason the nucleo-proteids are even less known than are the simple albumins of the cell-plasm.

The albuminous radicals to which the nucleic acid is united in the testes of fish are the protamins and histones. In the leucocytes of the thymus, and in the nucleated red blood corpuscles, nucleic acid is also linked to histone and parahistone (see pp. 408, 411). In all the other organs the albumin-moiety has not yet been investigated.

[1] J. Bang, *Zeitschr. f. physiol. Chem.* **30**. 508 (1900).
[2] T. B. Osborne, *ibid.* **36**. 85 (1902).
[3] A. Kossel, *ibid.* **30**. 520 (1900).
[4] W. Huiskamp, *ibid.* **34**. 32 (1901).
[5] F. Malengreau, *La Cellule*, **17**. 339 (1900).
[6] W. Huiskamp, *Zeitschr. f. physiol. Chem.* **32**. 145 (1901); **39**. 55 (1903).
[7] A. Kossel, *Arch. f. (Anat. u.) Physiol.* 1893, p. 157; T. H. Milroy, *Zeitschr. f. physiol. Chem.* **22**. 307 (1896); Y. Inoko, *ibid.* **18**. 57 (1893).

The nucleic acid is linked to the albumin in the following manner: According to Lilienfeld,[1] 0·8 per cent hydrochloric acid liberates the basic histone from the nucleo-histone, which is a nucleo-proteid of the thymus, and therefore nucleo-histone may be regarded simply as a salt; but Huiskamp[2] on electrolysing a solution of sodium nucleo-histone, found that it gave rise to the ions: nucleo-histone and sodium, and not to nucleic acid and histone. Cohnheim believes this dissociation (nucleic) to speak against the existence of acid histone-nucleates. In other nucleo-proteids, as, for example, in that of the pancreas, which has been investigated very fully, a dissociation by acids analogous to that occurring with the nucleo-histone of the thymus cannot be observed, for the pancreas nucleo-proteid[3] when boiled in neutral solutions dissociates into albumin and nuclein, which speaks strongly against a salt-like combination, according to Cohnheim.

On dissociation taking place, nucleic acid is never split off from the nucleo-proteid as a free acid, but as a nucleic-acid-compound containing a certain amount of albumin. This albumin-nucleate is known as nuclein. Nucleic acid would therefore appear to be linked to two albumin-radicals, one of which is readily split off while the other is firmly adherent. Lilienfeld[4] has expressed this by the following scheme:



It must, however, be pointed out that nucleins are still more difficult to prepare in a pure state than are the nucleo-proteids, and that therefore it is still easier to get mixtures of substances and artefacts.[5]

Nucleo-proteids in a pure state resemble other albumins in forming loose, white, non-hygroscopic powders; as they do not occur in solution, but only as cell-constituents, it is difficult to say in how far we are acquainted with their natural properties, and to what extent they have been altered by the process of isolating them. They are all markedly acid, are soluble in water and in salt solutions, and still more soluble in alkalies. They are precipitated by acids, but with

[1] L. Lilienfeld, *Zeitschr. f. physiol. Chem.* **18**. 473 (1893).
[2] W. Huiskamp, *ibid.* **34**. 32 (1901).
[3] F. Umber, *Zeitschr. f. klin. Med.* **40**. Hefte 5 and 6 (1900).
[4] L. Lilienfeld, *Arch. f. (Anat. u.) Physiol.* 1892, p. 128.
[5] F. Umber, *Zeitschr. f. klin. Med.* **43**. Hefte 3 and 4 (1901).

excess of acids, specially mineral acids, they pass into solution again, and then may undergo disintegration. The salting-out limits differ with each nucleo-proteid. The nucleo-proteids are under suitable conditions as readily and as completely coagulated and denaturalised by heat or other agencies as are the native albumins, but the nucleic acid may be recovered from the coagulum in an unaltered state, for only the albumin component becomes coagulated.

Nucleo-proteids give all the colour-reactions of the albumins, and are precipitated by the ordinary precipitating agents. They yield, as far as they have been investigated, the dissociation-products of the ordinary albumins, but in addition also products peculiar to the nucleic-acid radical, namely, the nuclein-bases, etc., and above all phosphoric acid. Their percentage-composition varies greatly and can be given, with a fair amount of assurance, only in the case of a few substances.

Iron is contained in most, if not in all, nucleo-proteids, and if we except the iron present in the hæmoglobin, the main bulk of the remaining iron concerned in metabolism is contained in the nucleo-proteids; nothing is known as to how the iron is linked up, but we know that it is present in a non-ionic or 'masked' state, and that therefore it cannot give directly the Prussian blue reaction, or the ammonium-sulphide- or hæmatoxylin-tests. That plasminic acid behaves similarly has been pointed out on p. 447.

The methods of unmasking iron for micro-chemical research have been studied by Molisch,[1] and especially by Macallum.[2] The micro-chemical reactions of nuclein-compounds is fully discussed in the author's *Physiological Histology*, 1902.

The occurrence of copper in place of iron in certain leucocytes of the oyster has been described by Boyce and Herdman.[3]

On digesting nucleo-proteids with pepsin-hydrochloric acid, the albumin-moiety breaks up into albumoses and peptones, while the nuclein is precipitated. This property of giving a precipitate with pepsin-hydrochloric acid led originally to the discovery of nucleo-

[1] Molisch, 'Bemerk. ü. d. Nachweiss von maskirtem Eisen,' *Ber. d. deutsch. bot. Gesellsch.* **11**. 73 (1893).

[2] A. B. Macallum, 'On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal and Vegetable Cells,' *Quarterly Journ. of Microsc. Science*, **38**. 175 (1895-96), and 'A New Method of distinguishing between Organic and Inorganic Compounds of Iron,' *Journ. of Physiol.* **22**. 92 (1897).

[3] R. Boyce and W. A. Herdman, *Phil. Trans. Lond.* **62**. 34 (1897-98).

proteids by Miescher,[1] and constitutes one of their chief characteristics, the others being their phosphorus and iron content, and the presence of purin-bases. Milroy[2] and Umber[3] have, however, shown that a considerable amount of nucleic acid is liberated by good pepsin-hydrochloric acid. The precipitate, according to Umber, may be pure nucleic acid, but as a rule it still contains albumin, and is termed a 'nuclein' (see p. 449). By trypsin nucleo-proteids are dissolved; peptones, amino-acids and nucleic acids pass into solution (Umber).

That extracts of the liver nucleo-proteids liberate ionic iron as the result of auto-digestion has been pointed out by Bottazzi.[4]

The nucleins are intermediate between the nucleo-proteids and the nucleic acid both genetically and as regards their properties. They are much more strongly acid than are the nucleo-proteids, and only with difficulty soluble in acids, even if acids are used in excess. Judging by their percentage-composition, they are far removed from the albumins, for, as a rule, they contain only about 40 per cent, or a little more, carbon, but 4 to 7 per cent of phosphorus, from which it follows that they are composed of nucleic acid to the extent of at least 150 per cent. They still give the reactions of albuminous substances. In gastric juice they are only soluble with difficulty, and therefore pepsin-hydrochloric acid is employed in preparing them. They are, however, readily dissolved by trypsin. When treated with alkalies they give rise to nucleic acids or to sodium nucleates.

Nucleo-proteids have been found by Miescher[1] in the nuclei of pus-corpuscles, and by Plósz[5] in the nuclei of the red blood corpuscles of birds and snakes. Lilienfeld,[6] Huiskamp[7] and others have pointed out in the case of the thymus, and Miescher,[8] Kossel[8] and Schmiedeberg[8] in the case of spermatozoa, that nucleo-proteids pass always into solution, and only into solution when the nucleus disintegrates. The nucleo-proteids are therefore constituents of the cell nuclei, and in those organs specially rich in cells, such as the thymus and the lymph glands, they exceed in quantity all the other albumins. Lilienfeld could show that 77 per cent of the dried leucocytes of the thymus represent nucleo-histone, and, not taking into account the ether-soluble products, the heads (nuclei) of the ripe spermatozoa of

[1] F. Miescher, *Hoppe-Seyler's Med.-chem. Untersuch.* p. 441 (1871).
[2] T. H. Milroy, *Zeitschr. f. physiol. Chem.* **22**. 307 (1896).
[3] F. Umber, *Zeitschr. f. klin. Medizin*, **43**. Hefte 3 and 4 (1901).
[4] F. Bottazzi, *Zentralbl. f. Physiol.* **18**. 98 (1904).
[5] P. Plósz, *ibid.* p. 461 (1871).
[6] L. Lilienfeld, *Arch. f.* (*Anat. und*) *Physiol.*, Physiol. Abteil. 1892, p. 128.
[7] W. Huiskamp, *Zeitschr. f. physiol. Chem.* **32**. 145 (1901).
[8] See footnotes 1 and 2 on p. 452.

fish contain, according to Miescher and Schmiedeberg,[1] 96 per cent of protamin-nucleate, the other albumins being only present in traces. As the so-called 'chromatin' of the nuclei is basophil, *i.e.* is acid in character, there is no doubt that the nuclear chromatin network consists essentially of acid, nuclein-like substances (Miescher,[2] Ehrlich,[3] Malfatti,[4] Kossel,[5] Zacharias,[6] Lilienfeld,[7] Heine,[8] and the author, who has entered fully into the question of micro-chemical methods in his book on *Physiological Histology*).[9]

The inter-relation between nucleins and nucleic acids on the one hand, and globulin-like bodies on the other hand, during cell-metabolism has been especially investigated by Lily Huie, working under the author's direction.[10]

Nucleo-proteids resemble many ferments as regards their solubilities, and these two classes of compounds are therefore frequently considered together. Hammarsten[11] obtained nucleo-proteid along with trypsin from the pancreas; Pekelharing,[12] Schumow-Simonowsky,[13] Nencki and Sieber[14] a nucleo-proteid besides pepsin from the gastric mucous membrane and from the gastric juice; Pekelharing[15] the fibrin ferment as well as a nucleo-proteid from the blood and from the thymus. This purely chemical result is in full agreement with the histological evidence, for L. H. Huie,[16] working with the author,

[1] F. Miescher and O. Schmiedeberg, *Arch. f. exper. Path. u. Pharm.* **37**. 1 (1896).

[2] Miescher, 'Die Spermatozoen einiger Wirbelthiere,' *Verh. d. naturf. Gesellsch. in Basel*, **6**. 138-208 (1874).

[3] Ehrlich, *Farbenanalytische Untersuchungen z. Hist. u. Klinik d. Blutes, Gesammelte Mitth. Berlin* 1891, Hirschwald.

[4] Malfatti, *Ber. d. naturw. med. Vereins in Innsbruck* (1891-92).

[5] A. Kossel, *Arch. f.* (*Anat. u.*) *Physiol.* 1891, p. 181.

[6] E. Zacharias, *Ber. d. deutsch. botan. Ges.* **11**. 190 and 299 (1893); **14**. 270 (1896); **16**. 185 (1898); **19**. 377 (1901); **20**. 238 (1902). *Verhandl. d. naturwissenschaftl. Vereins Hamburg*, 1900 and 1901. See also L. Heine, *Zeitschr. f. physiol. Chem.* **21**. 494 (1896).

[7] L. Lilienfeld, *ibid.* 1893, p. 391; 'Über d. Wahlverwandtschaft d. Zellelemente z. gewiss. Farbstoffen,' *Arch. f.* (*Anat. u.*) *Physiol.* 1903; 'Über d. Farbenreaction d. Mucins,' *ibid.*; 'Zur Chemie d. Leucocyten,' *Zeitschr. f. physiol. Chem.* **18**. (1894).

[8] Heine, 'Die Mikrochemie d. Mitose, zugleich eine Kritik microchemischer Methoden,' *ibid.*

[9] Gustav Mann, *Methods and Theory of Physiol. Hist.* Clarendon Press, 1902.

[10] L. H. Huie, *Quart. Journ. of Micros. Soc.* **39**. 387 (1896-97), and **42**. 203 (1899).

[11] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **19**. 19 (1893).

[12] C. A. Pekelharing, *ibid.* **22**. 233 (1896); **35**. 8 (1902).

[13] O. O. Schumow-Simonowsky, *Arch. f. exper. Path. u. Pharm.* **33**. 336 (1896).

[14] M. Nencki and N. Sieber, *Zeitschr. f. physiol. Chem.* **32**. 291 (1901).

[15] C. A. Pekelharing, *Zentralbl. f. Physiol.* 1895, p. 102; C. A. Pekelharing and W. Huiskamp, *ibid.* **39**. 22 (1903).

[16] L. H. Huie, *Quart. Journ. of Micr. Soc.* **39**. 387 (1896-97), and **42**. 203 (1899).

has shown for Drosera rotundifolia, Trambusti[1] for Spelerpes, Galeotti[2] for the same amphibian, and the author for mammalian salivary glands and intestinal cells (unpublished observations), that the zymogen-granules which ultimately are converted into ferments are of nuclear origin.

To this group of substances belong also those tissue-albumins which accelerate coagulation,[3] the tissue-fibrinogen of Wooldridge and the cytoglobulin and præglobulin of Alexander Schmidt. The effects produced by the injection of nucleo-proteids, obtained by Halliburton's method from the thymus and from lymph glands, have been carefully studied by Macwilliam, Mackie, and Murray,[4] and Mandel[5] has investigated the part played by alloxur-bases in producing fever during aseptic conditions.

The enterokinase of the intestinal mucous membrane, according to Stassano and Billon,[6] has also the nucleo-proteid attached to it, and Galeotti[2] and Hahn[7] found the nucleo-proteids to be the carriers of the immunising substances of the bacterial cells. Lustig[8] has isolated, by a chemical process not divulged, nucleo-proteids from the cholera-, pest-, and anthrax bacilli, the micrococcus ureæ and micrococcus prodigiosus, etc. He finds that nucleo-proteids react as do living bacteria, as far as intra-cellular toxins are concerned.

Cohnheim says: "That the ordinary nucleo-proteids are the ferments themselves does not follow from what has just been stated, for it has been proved that the digestive ferments are separate bodies, while it is possible that the fibrin ferment and nucleo-proteid may be one and the same substance. The observation of Tichomiroff[9] that nucleic acid precipitates ricine, tetanus, and diphtheria toxins is in this connection very interesting. It is specially pointed out that the very abundant occurrence of nucleo-proteids must not be taken as a proof in itself that they possess an important biological function, for they

[1] Arnaldo Trambusti, *Lo Sperimentale*, anno 49 (Sezione Biologica, fasc. **3**. (1895), (here the older literature).

[2] Gino Galeotti, *Zeitschr. f. physiol. Chem.* **25**. 48 (1898).

[3] A. Neumann, *Arch. f.* (*Anat. u.*) *Physiol.*, Physiol. Abteil. 1898, p. 374 (*Verhandl. der Berliner physiol. Gesellschaft*).

[4] J. A. Macwilliam, A. H. Mackie, and C. Murray, *Journ. of Physiol.* **30**. 381 (1904).

[5] A. R. Mandel, *Amer. Journ. of Physiol.* **10**. 452 (1903).

[6] H. Stassano and F. Billon, *Compt. rend. Soc. de Biologie*, **54**. 623 (1902).

[7] M. Hahn, 'On the Chemical and Immunising Properties of the Plasmins,' *Verhandl. d. 4. internation. physiol. Kongresses zu Cambridge*, *Journ. of Physiol.* **23**. Suppl. p. 45 (1898).

[8] A. Lustig, *Archivio di Fisiologia*, **1**. 336 (1904).

[9] M. Tichomiroff, *Zeitschr. f. physiol. Chem.* **21**. 90 (1895).

may be simply the framework and the protective medium for the living matter proper."

The author is at a complete loss as to Cohnheim's meaning, for what we call life is simply the manifestation of special chemical compounds, and if we see microscopically that every manifestation of metabolism is accompanied by enormous changes in the nucleo-proteids, and that the rapidity with which nucleo-proteids or the nuclear basophil chromatin reacts to food substances is directly proportional to the ease with which the food is absorbed (Lily Huie,[1] working with the author), we cannot arrive at any other conclusion but that the nucleo-proteids are the agencies by which amino-acids are built up into the cell-plasm, as already emphasised in the introduction.

(c) The Individual Nucleo-Proteids and Nucleic Acids

1. *Nucleo-Proteids from the Heads of the Spermatozoa*

The spermatozoa of many fish, after the removal of ether-soluble substances, consist of 96 per cent of protamin-nucleates or of histone-nucleates, the other albumins being only present in traces. A more detailed account has already been given on pp. 440 and 441 whilst discussing the protamins and histones. The composition of the individual nucleic acids is given on p. 442. Miescher and Schmiedeberg[2] found in the spermatic fluid of the salmon—

60·50 per cent nucleic acid
35·56 per cent protamin.

Schmiedeberg calculates from these figures an acid salt built up of ten molecules of protamin and eleven molecules of nucleic acid.

Clupein nucleate, prepared from the spermatozoa of the herring, possesses, according to Mathews,[3] the following percentage composition—

C 41·2 H 5·75 N 21·06 P 6·07 O 25·92

and is, according to him, a neutral salt.

The spermatozoa of the sea-urchin Arbacia pustulosa consist, apart from lecithin, etc., essentially of a nucleic acid in combination with arbacin, which is a histone.[4] The spermatozoa of the ox have been

[1] L. H. Huie, *Quart. Journ. of Micros. Science*, **39** 387 (1896-97), and **42**. 203 (1899).

[2] F. Miescher, 'Milt of Salmon,' in the posthumous papers edited by O. Schmiedeberg, *Arch. f. experiment. Path. und Pharm.* **37**. 1 (1896).

[3] A. Mathews, *Zeitschr. f. physiol. Chem.* **23.** 399 (1897).

[4] A. Mathews, *ibid.* **23**. 399 (1897).

investigated by Miescher[1] and Mathews,[2] according to whom they contain neither protamin nor histone, but another albumin. The proteid contains, according to Miescher, 2·32 per cent P and 1·17 per cent S. Miescher prepared from it a nuclein with 4·73 per cent P and 1·74 per cent S, and, further, a nucleic acid, the composition of which has been given on p. 442. The spermatozoa of the boar are similar to those of the ox.

2. *Nucleo-Proteids of the Thymus and Nucleo-Histone*

Lilienfeld[3] obtained by collation a large number of leucocytes from the thymus of the calf, which he then extracted with water. By precipitating the watery extract with acetic acid he obtained the 'nucleo-histone,' which amounts to 77 per cent of the dried leucocytes. It is soluble in water, alkalies, and alkaline carbonates; it is precipitated by dilute acetic acid. Lilienfeld considers it to be an acid salt of nuclein with histone. On being treated with 0·8 per cent hydrochloric acid it gives rise to histone, which has already been described on p. 410, and to a nuclein, the 'leuco-nuclein.' This nuclein contains 4·702 per cent of P. When it is boiled or digested with pepsin-hydrochloric acid it also gives rise to nucleins, which contain 4·99 per cent of P. Subsequently this question has been rèinvestigated by Malengreau,[4] Huiskamp,[5] and Bang,[6] who have arrived at the conclusion that there are present in the leucocytes of the thymus two different nucleo-proteids, which differ from one another in their solubilities, and particularly in their P contents. The contradictory statements of the older observers[7] may be explained by this fact. According to Huiskamp the thymus contains—

(*a*) *A nucleo-histone having the percentage-composition*—

C 48·8 H 7·03 N 18·37 S 0·51 P 3·7

It is precipitated by 0·1 to 0·5 per cent $CaCl_2$ or 0·9 per cent NaCl, but it remains in solution if these salts are either in lower or in higher concentration than those just mentioned. The precipitation limits for ammonium sulphate are 5·6 and 7·2. Owing to the

[1] F. Miescher, *Verhandl. d. naturforsch. Gesellsch. in Basel*, **6**. 138 (1874).
[2] A. Mathews, *Zeitschr. f. physiol. Chem.* **23**. 399 (1897).
[3] L. Lilienfeld, *ibid.* **18**. 473 (1893).
[4] F. Malengreau, *La Cellule*, **17**. 339 (1900).
[5] W. Huiskamp, *Zeitschr. f. physiol. Chem.* **32**. 145 (1901); **34**. 32 (1901).
[6] J. Bang, *Hofmeister's Beiträge*, **4**. 115 and 331, 362 (1904); **5**. 317 (1904).
[7] J. Bang, *Zeitschr. f. physiol. Chem.* **30**. 508 (1900); **31**. 407 (1900); A. Kossel, *ibid.* **30**. 520 (1900).

existence of this nucleo-histone, leucocytes do not dissolve in 0·9 per cent NaCl solution, while they do so in pure water. This work of Huiskamp is of great interest, because the dissolution of the leucocytes had always been explained on physical grounds, while it depends apparently on chemical grounds (Cohnheim).[1] This nucleo-histone gives a strong Molisch reaction, but not those of Millon or of Adamkiewicz. The descriptions and analyses of Bang differ but little from those of Huiskamp. It is possible that the nucleo-histone may have mixed with it a third substance containing a yet higher amount of phosphorus.

(*b*) *A nucleo-proteid having the percentage-composition*—

C 51·78 H 7·47 N 16·42 S 1·2 P 0·95

It is neither precipitated by $CaCl_2$ nor by other salts, and passes, therefore, into the thymus extract, made with 0·9 per cent salt-solution. It may be a derivative of the nucleus, and need not be derived, as Bang believes, from the intermediate substance. Its precipitation limits for ammonium sulphate are 3·0 and 4·5 according to Malengreau. It gives the reactions of Millon and Adamkiewicz strongly.

Both the nucleo-histone and the nucleo-proteid form calcium salts containing 1·3 per cent of calcium; both are dissociated by hydrochloric acid into a nuclein and a histone; the two histones have the same salting-out limits, as have the corresponding proteids. Both yield a nucleic acid containing adenin and guanin (Malengreau). Both are considered by Huiskamp[2] and Pekelharing to be the precursors of the fibrin-ferment.

The nucleo-histone prepared according to Lilienfeld has been found by Gamgee and Jones[3] to be dextro-rotatory:

$$\alpha_D = +37{\cdot}5$$

The thymus-nucleic acid is one of the best known, as it has been very thoroughly investigated by Kossel.[4] Originally it was also

[1] See also Bang, *Hofmeister's Beiträge*, **5**. 317 (1904).

[2] W. Huiskamp and C. A. Pekelharing, *Zeitschr. f. physiol. Chem.* **39**. 22 (1903); W. Huiskamp, *ibid.* **32**. 145 (1901).

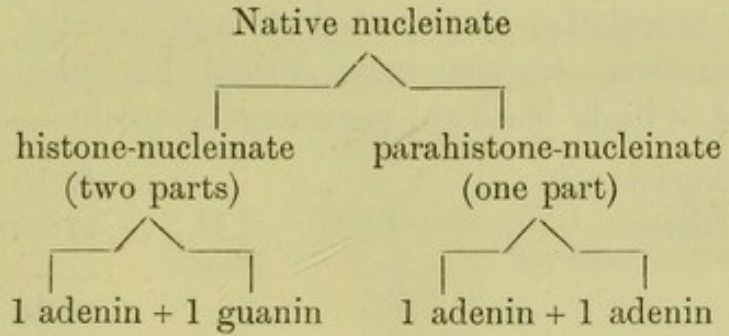
[3] A. Gamgee and W. Jones, *Hofmeister's Beiträge*, **4**. 10 (1903).

[4] A. Kossel, *Arch. f.* (*Anat. u.*) *Physiol.* 1891, p. 181; 1893, p. 157; 1894, p. 194; A. Kossel and A. Neumann, *Ber. d. deutsch. chem. Ges.* **26**. III. 2753 (1893); **27**. II. 2215 (1894); *Zeitschr. f. physiol. Chem.* **22**. 74 (1896); A. Neumann, *Arch. f.* (*Anat. u.*) *Physiol.* 1898, p. 174; 1899, p. 552; S. Kostytschew, *Zeitschr. f. physiol. Chem.* **39**. 545 (1903); H. Plenge, *ibid.* **39**. 190 (1903). Compare also with the account given of the pyrimidin- and purin-derivatives which Kossel and Steudel were the first to prepare, and mostly out of the thymo-nucleinic acid.

called adenylic acid, but it is possible to prepare from the thymus all the four purin and also all the three simple pyrimidin derivatives. Huiskamp[1] states that the fresh thymus contains about 12 per cent of soluble albumin, of which there is contained in

Nucleo-histone	69 per cent.
Nucleo-proteid	19 „
Other albumins	12 „

Bang[2] states that the thymus nucleic acid contains two adenin-molecules for each guanin-molecule; that the nucleic acid contains only two molecules of purin-bases, and that therefore two distinct nucleic acids must exist in the thymus. One of these, the 'normal acid,' contains 1 molecule of adenin + 1 molecule of guanin, and occurs in the true histone-nucleate, while the second acid, built up of 2 molecules of adenin, is the adenylic acid in combination with the parahistone.



Kossel[3] obtained from 10 kilogrammes of thymus 120 grammes of nucleic acid.

The action of dilute mineral acids on the thymo-nucleic acid has been studied by Levene[4] (see under 'Lævulinic Acid,' on p. 438).

From the nuclei of pus-cells, *i.e.* from leucocytes, Miescher[5] prepared the first-known nuclein, and Bang[6] isolated nucleo-proteids from a number of lymphatic organs: lymph glands, bone marrow, spleen, white blood-corpuscles, and also from a sarcoma.

Thymus Nucleic Acid

Kutscher and Seemann,[7] by oxidising thymus nucleic acid with calcium permanganate, obtained urea and imido-urea, no uric acid

[1] H. Huiskamp, *Zeitschr. f. physiol. Chem.* **32**. 145 (1901).

[2] Ivar Bang, *Hofmeister's Beiträge*, **5**. 317 (1904); see also *ibid.* **4**. 115, 331, 362 (1904).

[3] A. Kossel and A. Neumann, *Ber. d. deutsch. chem. Ges.* **27**. II. 2215 (1894).

[4] P. A. Levene, *Amer. Jour. of Physiol.* **12**. 213 (1904).

[5] F. Miescher, *Hoppe-Seyler's Med.-chem. Untersuchungen*, p. 441 (1871).

[6] J. Bang, *Hofmeister's Beiträge*, **4**. 362 (1903).

[7] Kutscher and Seemann, *Ber. d. deutsch. chem. Ges.* **36**. 3023 (1904).

being formed. In addition to urea and imido-urea Kutscher and Schenck[1] have obtained 'martamic acid,' having the formula $C_5H_8N_6O_5$ or $C_5H_{10}N_6O_5$, giving neither the murexide test nor Weidel's reaction, being readily soluble in hot water and alcohol, very slightly soluble in ether, and resembling oxalic acid so closely in its behaviour as to necessitate the use of lime for the removal of the oxalic acid; oxalic and acetic acids; an unknown acid; adenin; guanidin, derived from guanin; urea and a biuret-giving substance.

3. *Nucleo-Proteids from the Nucleated Red Blood-Corpuscles of Birds and Reptiles*

The existence of a nuclein in the nuclei of the red blood-corpuscles of birds and snakes was first demonstrated by Plósz.[2] Later the albumin-radical, or 'histone,' was isolated by Kossel,[3] who acted on the nuclear substances with dilute acids (see p. 408). Araki[4] studied the dissociation-products which are liberated by ferment action. Ackermann,[5] working under Kossel, has calculated for the nucleo-proteid of birds' blood the percentage-composition—

Nucleic acid	42·10
Histone	57·82
	99·92

According to Bang[6] the histone-nucleate of goose's blood consists only of histone, and nucleic acid as parahistone (see p. 415) is absent.

4. *The Nucleo-Proteid of the Pancreas*

Hammarsten[7] extracted from the pancreas of the ox an 'α-nucleo-proteid,' which subsequently was investigated more thoroughly by Umber.[8] This nucleo-proteid is prepared by extracting minced pancreas with ice-cold sodium chloride solution to prevent tryptic digestion, and then precipitating the proteid with acetic acid. One kilogramme pancreas yields 17 grammes of proteid. Analysis gives these percentage figures :—

C 51·35 H 6·81 N 17·82 P 1·67 S 1·29 Fe 0·13

[1] Fr. Kutscher and M. Schenck, *Zeitschr. f. physiol. Chem.* **44**. 309 (1905).
[2] P. Plósz, *Hoppe-Seyler's Med.-chem. Untersuchungen*, p. 461 (1871).
[3] A. Kossel, *Zeitschr. f. physiol. Chem.* **8**. 511 (1884).
[4] T. Araki, *ibid.* **38**. 84 (1903). [5] D. Ackermann, *ibid.* **43**. 299 (1904).
[6] Ivar Bang, *Hofmeister's Beiträge*, **5**. 317 (1904).
[7] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **19**. 19 (1893).
[8] F. Umber, *Zeitschr. f. klin. Med.* **40**. Hefte 5 and 6 (1900).

It gives the reactions of Millon, Adamkiewicz, and Vogel [the tyrosin, tryptophane, and sulphur reactions]. Umber[1] has also investigated the dissociation of this nucleo-proteid by means of pepsin and trypsin. When boiled with water it forms a nuclein having this percentage-composition :—

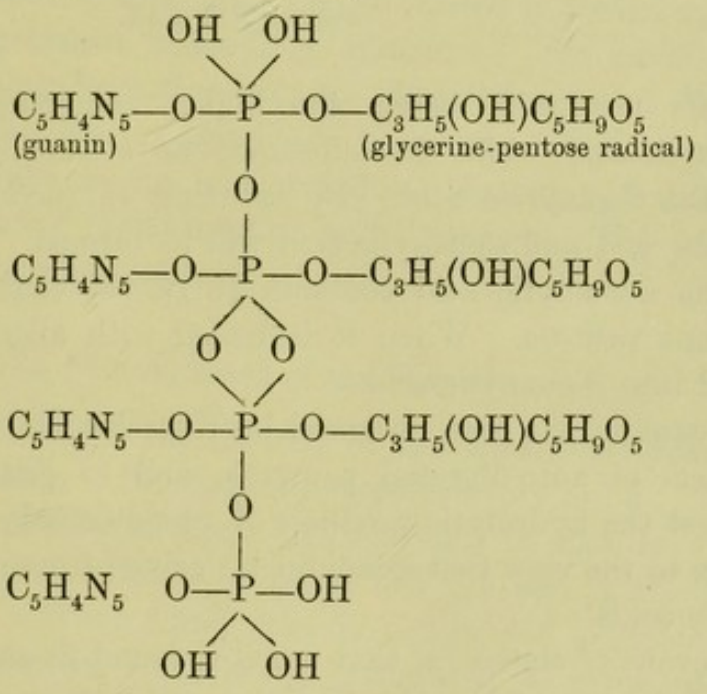
C 43·62 H 5·45 N 17·39 P 4·48 S 0·728 O 28·33

This compound Hammarsten had previously described under the name of 'β-nucleo-proteid.'

According to Gamgee and Jones[2] the polarisation of the nuclein bodies of the pancreas is—

α-nucleo-proteid . . .	$\alpha_D = +37{\cdot}8$
Nuclein	$\alpha_D = +64{\cdot}4$

The pancreas contains all the four purin-bases,[3] but Bang[4] prepared from Hammarsten's pancreas-nucleo-proteid a nucleic acid containing only guanin $C_5H_5N_5O$, and called it, therefore, guanylic acid.[5] To this acid he gave the following structural formula :—



β-guanylic acid.

Quite recently, owing to having used a $\frac{1}{2}$ instead of 2 per cent NaOH for isolating the guanylic acid, Bang and Raaschou[6] obtained a

[1] F. Umber, *Zeitschr. f. klin. Med.* **43**. Hefte 3 and 4 (1901).

[2] A. Gamgee and W. Jones, *Hofmeister's Beiträge*, **4**. 10 (1903).

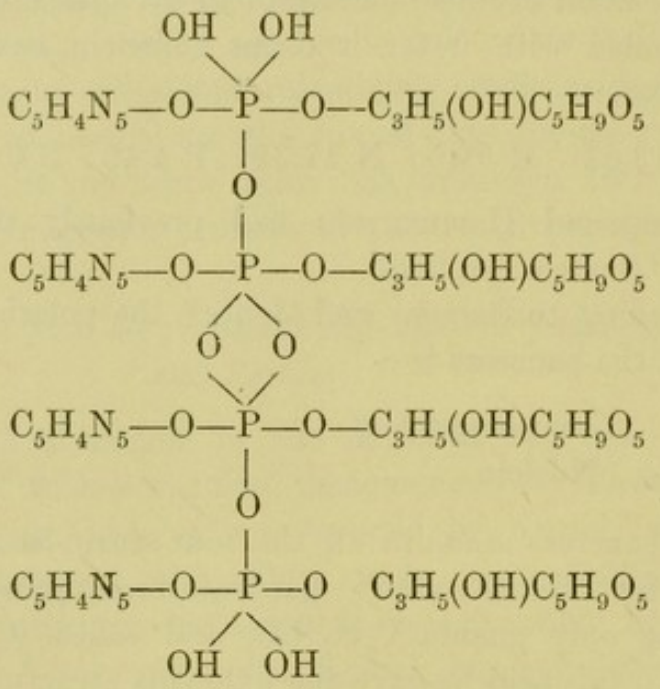
[3] Y. Inoko, *Zeitschr. f. physiol. Chem.* **18**. 541 (1893).

[4] J. Bang, *ibid.* **26**. 133 (1898); **31**. 411 (1900); **32**. 201 (1901).

[5] Ivar Bang, *ibid.* **26**. 133 (1898); and **31**. 411 (1901); 'Mindre Middelelser om Guanylsyren,' *Arch. f. Mathem. og Naturvidenskab.* **24**.

[6] Ivar Bang and C. A. Raaschou, *Hofmeister's Beiträge*, **4**. 175 (1904).

guanylic acid which differs from the one above in containing one additional glycerine-pentose radical :—



α-guanylic acid.

This new acid, being the higher compound, Bang calls the *α*-guanylic acid, while the substance he obtained previously he now calls the *β*-guanylic acid. By splitting off further glycerine-pentose radicals, *γ*, *δ*, and *ϵ*-guanylic acid will be formed.

The *α*-guanylic acid contains 29·46 per cent guanin and 34·07 per cent pentose. When it is boiled with alkalies it becomes converted into *β*-guanylic acid.

Guanidin has been observed by Kutscher and Otori [1] amongst the products of auto-digested pancreas, and as guanidin is also found amongst the hydrolytic products of pseudo-mucin (Otori) the authors incline to the view that guanidin is derived from arginin and not from nucleic acids.

Levene's [2] statement that uracil is found in auto-digested pancreas has not been confirmed by Kutscher and Lohmann.[3]

The pentose occurring in the nucleo-proteid is, according to Neuberg,[4] *l*-xylose.

[1] Fr. Kutscher and Otori, *Zentralbl. f. Physiol.* **18**. 248 (1904).
[2] Levene, *Zeitschr. f. physiol. Chem.* **37**. 527 (1903).
[3] Kutscher and Lohmann, *ibid.* **44**. 385 (1905).
[4] C. Neuberg, *Ber. d. deutsch. chem. Ges.* **35**. II. 1467 (1902).

5. *The Nucleo-Proteid of the Gastric Juice*

Pekelharing,[1] Schumow-Simonowsky,[2] and Nencki and Sieber[3] found a nucleo-proteid not only in extracts of the gastric mucous membrane, but also in pure gastric juice prepared by Pawlow's method. Analysis gave these figures:—

C	H	N	S	P	Fe	
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
51·99	7·07	14·44	1·63	...	..	Pekelharing.
51·26	6·74	14·33	1·5	0·1	0·16	Nencki and Sieber.

The preparations were, however, not quite pure. The proteid is soluble in water and in strong hydrochloric acid, but insoluble in very dilute hydrochloric acid. It is therefore precipitated by dialysing gastric juice, and may be readily obtained pure by this means or by salting out with ammonium sulphate. When heated it breaks up into a nuclein and a substance resembling albumose. On standing it decomposes, there being formed peptones. Pekelharing and Nencki and Sieber believe this nucleo-proteid to be pepsin, but Glässner's[4] work throws some doubt on this interpretation. Amongst its dissociation-products Pekelharing found xanthin and a pentose. The proteid gives the ordinary albumin-tests.

6. *The Nucleo-Proteid of the Thyroid*

Oswald[5] prepared it from the thyroid of the sheep. It contains 0·16 per cent phosphorus. Its coagulation-temperature is 73°. It is insoluble in water, but soluble in salt-solutions and in alkalies. The precipitation-limits for ammonium sulphate are 6·4 and 8·2. It is precipitated by acids. When acted upon by pepsin and hydrochloric acid it splits off a nuclein. On being dissociated it gives rise to xanthin-bases and a carbohydrate which is not a pentose. It does not contain iodine, and has nothing to do with the specific action of the thyroid gland.

[1] C. A. Pekelharing, *Zeitschr. f. physiol. Chem.* **22**. 233 (1896); **38**. 8 (1902).

[2] O. O. Schumow-Simonowsky, *Arch. f. experiment. Pathol. und Pharmak.* **33**. 336 (1896).

[3] M. Nencki and N. Sieber, *Zeitschr. f. physiol. Chem.* **32**. 291 (1901).

[4] K. Glässner, *Hofmeister's Beiträge*, **1**. 1 (1901).

[5] A. Oswald, *Zeitschr. f. physiol. Chem.* **27**. 14 (1899).

7. *The Nucleo-Proteid of the Suprarenal Capsules*

Jones and Whipple[1] prepared and analysed it. It resembles the nucleo-proteid of the pancreas. Gamgee and Jones[2] determined

$$a_D = +48{\cdot}1.$$

Amongst its dissociation-products are guanin and adenin.

8. *The Nucleo-Proteid of the Liver*

This compound has been investigated by Wohlgemuth.[3] Fresh minced ox-liver was repeatedly boiled out with water; the combined extracts treated with acetic acid to precipitate the proteids; the precipitate kept under alcohol, which was frequently changed and extracted with ether to remove the fat; dissolved in dilute sodium carbonate and reprecipitated with dilute acetic acid. On redissolving and reprecipitating, the phosphorus-content was found to be 3·05 per cent. The precipitate was preserved for some weeks under alcohol and ether, and finally dried at 60°. From 50 kilogrammes liver were obtained 140 grammes of nucleo-proteid. Analysis gave the following percentage-composition :—

C 45·22 H 5·72 N 16·67 S 0·637 P 3·06.

Wohlgemuth calculates that 100 grammes of liver-nucleic acid contain—

Xanthin	2·2526	grammes.
Guanin	2·5402	„
Adenin	1·9051	„
Hypoxanthin	1·7875	„

These figures, compared with those given by Steudel (see p. 441) for the xanthin-bases of thymus-nucleic acid, show that the liver contains more guanin and xanthin, but less adenin, while the hypoxanthin is present in about equal amounts in the thymus and in the liver.

From 95 grammes of nucleo-proteid Wohlgemuth obtained, according to his last paper, 0·723 g. histidin (?), 1·08 g. lysin, and 3·38 g. arginin. Other substances obtained were xanthin, hypoxanthin, guanin, adenin, tyrosin, leucin, glycocoll, alanin, phenylalanin, α-prolin, glutamic-, aspartic-, oxyamino-, oxydiamino-, sebacic acids, and *l*-xylose.

[1] W. Jones and G. H. Whipple, *Amer. Journ. of Physiol.* **7**. 423 (1902).

[2] A. Gamgee and W. Jones, *Hofmeister's Beiträge*, **4**. 10 (1903).

[3] J. Wohlgemuth, *Zeitschr. f. physiol. Chem.* **37**. 475 (1903); **42**. 519 (1904); and **44**. 530 (1905).

9. *Nucleo-Proteids of Muscle and of other Organs*

Pekelharing[1] and Kossel[2] found in adult muscle only traces of nucleo-proteid, while in embryonic muscle it is much more abundant.[2] It stands perhaps in some relation to Siegfried's sarctic or carnic acid (see p. 203). The inosinic acid of Haiser,[3] mentioned on p. 442, is a nucleic acid contained in muscle-extracts. As nucleo-proteids are present in all nuclei, they are found, of course, in all the organs of the body. Dissociation-products of nucleic acids have already been mentioned on p. 440. Acid-albumins containing phosphorus and usually also iron have been found, amongst others, by Plósz,[4] Zaleski,[5] and Schmiedeberg ['ferratin'][6] in the liver, by Halliburton[7] and his pupils in many organs, by Hammarsten[8] in the liver of the snail *Helix pomatia*, by Sosnowski[9] in amœbæ, by Petry[10] in cellular tumours, and by Pekelharing[11] in the blood. Pekelharing believes that this nucleo-proteid plays a part in blood-coagulation. Whether all the substances just mentioned are really nucleo-proteids is by no means quite certain, for they may be nucleo-albumins of the cell-plasm (compare p. 406).

10. *The Nucleo-Proteids of Plants*

Yeast.—One of the earliest discovered and best known nucleic acids is that of the yeast, first isolated by Kossel.[12] Its composition- and dissociation-products are given on pp. 440, 442. Kossel also prepared a nuclein. In addition to it there occurs in yeast the plasminic acid (see p. 447).

[1] C. A. Pekelharing, *Zeitschr. f. physiol. Chem.* **22**. 245 (1896).

[2] A. Kossel, *ibid.* **7**. 7 (1882).

[3] F. Haiser, *Monatsh. f. Chem.* **16**. 190 (1895).

[4] P. Plósz, *Pfluger's Archiv f. d. ges. Physiol.* **7**. 371 (1873).

[5] S. Zaleski, *Zeitschr. f. physiol. Chem.* **10**. 453 (1886).

[6] O. Schmiedeberg, 'Ferratin,' *Schmiedeberg's Archiv f. experiment. Path. u. Pharm.* **33**. 1 (1893).

[7] W. D. Halliburton, 'Blood Proteids,' *Journ. of Phys.* **7**. 319 (1886); 'Fibrin-ferment,' *ibid.* **9**. 224 (1888); Halliburton and W. M. Friend, 'Stromata of the Red Corpuscles,' *ibid.* **10**. 532 (1889); Halliburton, 'Nervous Tissues,' *ibid.* **15**. 90 (1894); Fr. Gourlay, 'Thyroid and Spleen,' *ibid.* **16**. 23 (1894); J. R. Forrest, 'Red Marrow,' *ibid.* **17**. 174 (1894); W. D. Halliburton and Gregor Brodie, 'Nucleo-albumins and Intravascular Coagulation,' *ibid.* **17**. 135 (1894); W. D. Halliburton, 'Nucleo-proteids,' *ibid.* **18**. 304 (1895).

[8] O. Hammarsten, *Pflüger's Archiv*, **36**. 373 (1885).

[9] J. Sosnowski, 'Chem. der Zelle,' *Zentralbl. f. Physiol.* **13**. 267 (1899).

[10] E. Petry, *Zeitschr. f. physiol. Chem.* **27**. 398 (1899).

[11] C. A. Pekelharing, *Zentralbl. f. Physiol.* 1895, p. 102.

[12] A. Kossel, *Zeitschr. f. physiol. Chem.* **3**. 284 (1879); **4**. 290 (1880); **7**. 7 (1882). See also under the dissociation-products.

Bacteria.—In bacteria Galeotti[1] found a nucleo-proteid, and, according to Stutzer,[2] 40·75 per cent of the nitrogen in moulds is nuclein-nitrogen.

Higher Plants.—Osborne and Harris[3] prepared a nucleic acid from wheat-embryos, which they called tritico-nucleic acid, and which they investigated very thoroughly. Its composition- and decomposition-products are given on p. 440. It is strongly dextro-rotatory, the rotation varying according to its concentration from + 66 to + 74.[4]

Petit[5] isolated from barley an iron-containing nuclein, which contained no sulphur, and which did not give Millon's reaction.

Klinkenberg[6] obtained from different food-stuffs, poppy-seed- and palm-cakes, nucleo-proteids and nucleins which in their composition resemble those of yeast.

Ordinary parts of plants rich in cells are also rich in nucleic acid. Kovchoff[7] found an increase in the amount of nucleo-proteids whenever plants formed new tissue as the result of injury.

[1] G. Galeotti, *Zeitschr. f. physiol. Chem.* **25**. 48 (1898).

[2] Stutzer, *ibid.* **6**. 572 (1882).

[3] T. B. Osborne and J. F. Harris, *ibid.* **36**. 85 (1902); also in *Journ. Amer. Chem. Soc.* **22**. 379 (1899).

[4] T. B. Osborne, *Amer. Journ. of Physiology*, **9**. 69 (1903).

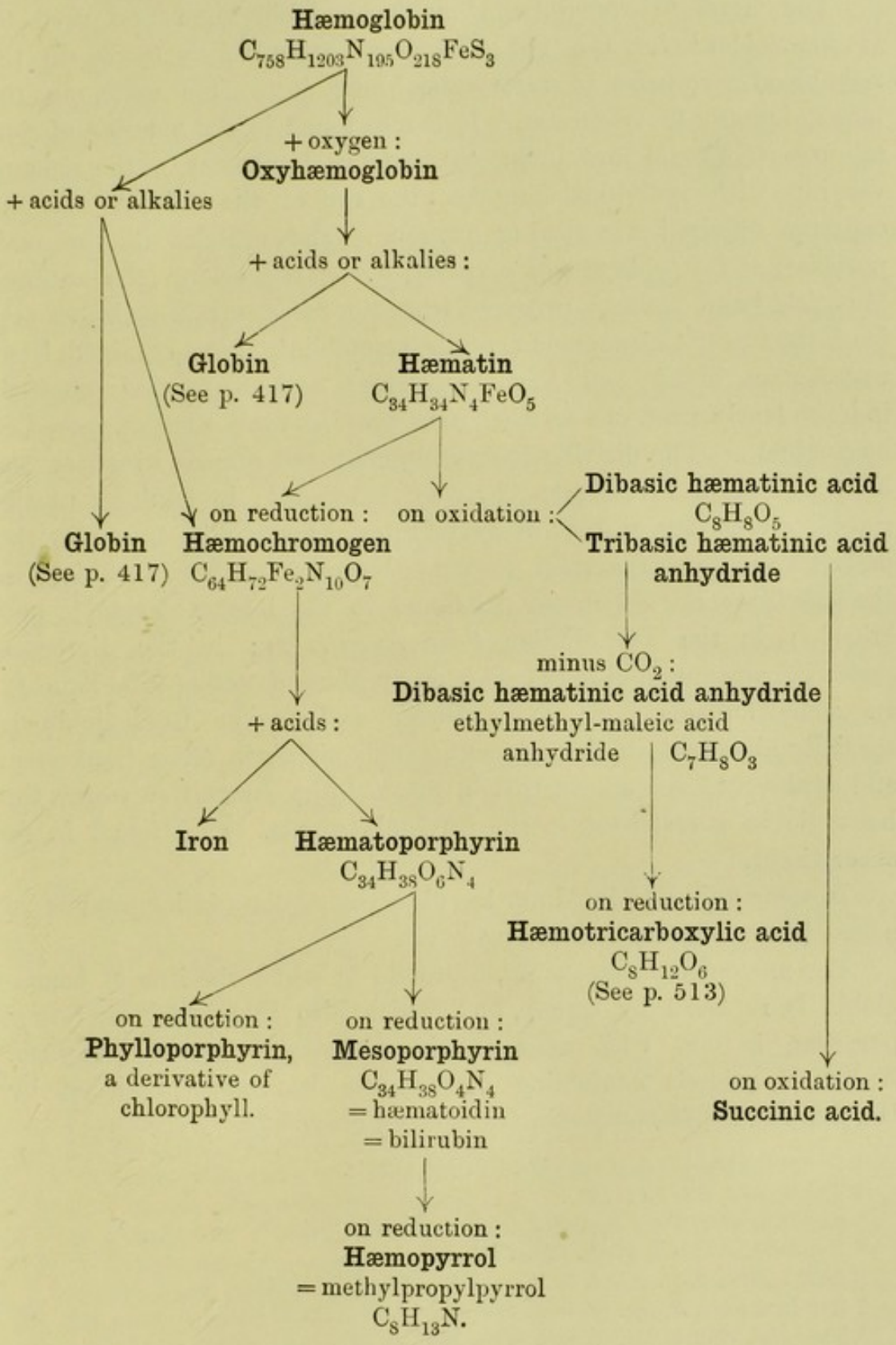
[5] P. Petit, *Compt. rend.* **116**. 995 (1893).

[6] W. Klinkenberg, *Zeitschr. f. physiol. Chem.* **6**. 155, 566 (1882).

[7] J. Kovchoff, *Ber. d. deutsch. botan. Ges.* **21**. 165 (1903).

[TABLE

Hæmoglobin Derivatives



Hæmoglobin

The terms 'Hæmoglobulin' or hæmoglobin were first used by Hoppe-Seyler[1] for the red colouring matter which occurs in the red blood-corpuscles of vertebrates, and, in a free state, in the blood of many invertebrates. It is also found in the muscles of vertebrates (Kühne[2]) and invertebrates (Ray Lankester[3]), the amount varying with individual muscles, the age of the animal, and the capacity required for storing oxygen. The muscles of the seal, porpoise, and dolphin are of a specially deep-red colour (the author). Hunefeld[4] and Rollet[5] prepared from the earthworm Lumbricus and from the insect Chironomus crystals resembling those obtained from mammalian blood, and Ray Lankester[6] and Nawrocki[7] showed, independently from one another, that these crystals were composed of hæmoglobin. The distribution throughout the animal kingdom has been thoroughly investigated by Ray Lankester: in addition to the typical circulatory system of vertebrates, hæmoglobin occurs in the perivisceral fluid of certain worms (Glycera, Capitilla, Phoronis); in the lamellibranchs Solen and Arca, and in the leeches Nephelis and Hirudo; in the Turbellarian *Polia sanguirubra*; in the dipterous insect Chironomus, and in the common fly *Musca domestica* (MacMunn[8]). In some animals, such as *Aphrodita aculeata* the hæmoglobin is specially abundant in the nervous ganglionic chain (Ray Lankester) and Hubrecht[9] has shown that in some of the Nemertian worms the hæmoglobin is restricted to the cerebral ganglia, being found in no other tissue.

In some animals MacMunn has observed a dissociation-product of hæmoglobin, namely, hæmatoporphyrin.

The analogous hæmocyanin, a proteid in which the iron of hæmoglobin is replaced by copper, and which also subserves respiratory purposes, is met with amongst the cephalopods and the crayfish. A list of animals containing hæmocyanin has been published by Halliburton.[10] Hæmocyanin is discussed on p. 529.

[1] Hoppe-Seyler, *Virchow's Archiv*, **29**. 223 (1864).

[2] Kühne, *ibid.* **33**. 79 (1865).

[3] Ray Lankester, *Proc. Roy. Soc. London*, **21**. 70, 1872.

[4] Hunefeld, *Journ. f. prakt. Chem.* **16**. 152 (1839).

[5] Rollet, *Sitzb. d. R. Akad. d. Wiss. Wien.* **44**. 615 (1861).

[6] Ray Lankester, *Journ. of Anat. and Physiol.* 1867, p. 114.

[7] Nawrocki, *Centralbl. f. d. med. Wiss.* 1867, p. 196.

[8] MacMunn, *Proc. Birmingham Phil. Soc.* **3**. 130.

[9] Hubrecht, *Niederl. Arch. f. Zool.* 1876; abstracted in *Jahresb. ü. d. Fortschritte d. Tierchemie*, **6**. 92.

[10] W. D. Halliburton, *Journ. of Physiol.* **6**. 300 (1885).

The red blood-corpuscles of mammals are composed for the greater part of hæmoglobin, for Hoppe-Seyler[1] found that dried red blood-corpuscles contain in man 94·3, in the dog 86·5, in the hedgehog 92·25, in the goose 62·65, and in the grass-snake 46·70 per cent of hæmoglobin. More recently Abderhalden[2] has studied the percentage composition of different species of blood. The remainder is composed of the framework, an envelope, and in non-mammals also of the nuclei.

Peskind[3] and R. du Bois Reymond[4] have studied the conditions under which blood becomes laked. Peskind believes that ether, *e.g.*, acts on the cholesterin- and lecithin-envelopes of the red blood-corpuscles in some such way as to permit of the diffusion outwards of the blood-pigment, even before the cholesterin and lecithin have been extracted from the envelopes by the ether.

For the estimation of hæmoglobin in the blood a number of colorimetric methods have been devised by Gowers, Hoppe-Seyler,[5] Giacosa,[6] Fleischl,[7] Zangemeister,[8] Gärtner,[9] Miescher and Veillon,[10] Oliver and Haldane.[11] Haldane's modification of the instrument of Gowers gives the most accurate results. In this apparatus the standard coloured liquid is a 1 per cent solution of blood of 18·5 per cent oxygen-capacity, saturated with CO and sealed up in a glass tube. The solution is then unalterable. The blood to be examined is saturated with CO and diluted in a graduated tube till the tint of the standard solution is reached. The results are reliable to within 1 per cent. See p. 499.

O. and R. Adler[12] have devised a special method for the recognition of blood, which is based on the oxidising power of the hæmoglobin, for the leucobase of malachite-green and alcoholic benzidin solutions

[1] F. Hoppe-Seyler, *Med.-chem. Unters.* p. 391 (1868).

[2] E. Abderhalden, *Zeitschr. f. physiol. Chem.* **25**. 65 (1898).

[3] S. Peskind, *Amer. Journ. of Physiology*, **12**. 184 (1904).

[4] R. du Bois Reymond, *Zentralbl. f. Physiol.* **19**. 65 (1905).

[5] F. Hoppe-Seyler, *Zeitschr. f. physiol. Chem.* **16**. 504 (1892); G. Hoppe-Seyler, *ibid.* **21**. 461 (1896); H. Winternitz, *ibid.* **21**. 468 (1896).

[6] P. Giacosa, *Maly's Jahresbericht für Tierchemie*, **26**. 140 (1896).

[7] E. v. Fleischl, *Med. Jahrbücher* 1885, p. 425.

[8] W. Zangemeister, *Zeitschr. f. Biologie*, **33**. 72 (1896).

[9] G. Gärtner, *Münchener med. Wochenschr.* 1901, No. 50.

[10] Miescher and Veillon, *Schmiedeberg's Archiv f. experiment. Pathol. u. Pharm.* **39**. 385 (1897); Wolf, *Zeitschr. f. physiol. Chem.* **26**. 452 (1899); R. Magnus, *Schmiedeberg's Archiv f. experiment. Pathol. u. Pharm.* **44**. 68 (1900); Franz Müller, *Archiv f. (Anat. u.) Physiol.* 1901, p. 443. Compare also J. Haldane, *Journ. of Physiology*, **26**. 497 (1901).

[11] J. Haldane, *Journ. of Physiol.* **26**. 497 (1900-1901).

[12] O. and R. Adler, *Zeitschr. f. physiol. Chem.* **41**. 59 (1904).

are converted into coloured compounds by the hæmoglobin. This test in the absence of other indirectly oxidising media is better than is the guaicum test.

For blood pigment Riegler[1] has devised the following hydrazin-reagent:—

Dissolve NaOH . . .	10 grammes
In water. . . .	100 cc.
Add hydrazin-sulphate . .	5 grammes
Alcohol 96-97 per cent . .	100 cc.

Shake vigorously and filter after 2 hours.

0·05 grs. commercial hæmoglobin or ½ cc. of blood + 30 cc. of reagent when shaken and allowed to stand shows the beautiful purple colour of hæmochromogen. When shaken with air the solution turns green owing to the formation of hæmatin; on standing a reconversion into hæmochromogen takes place.

Hæmoglobin is composed of a histone-like, basic, albuminous radical called globin, and a non-albuminous, acid moiety, containing iron, which is termed hæmatin. See chart on p. 465. Hæmoglobin is characterised by combining with oxygen, carbon dioxide, carbon monoxide, and perhaps with other gases, to form the loose, chemical compounds of oxyhæmoglobin, carbonic acid hæmoglobin, carbonic oxide hæmoglobin, etc. Amongst these the most important is oxyhæmoglobin, as respiration depends on it. According to Cohnheim oxyhæmoglobin is called in Germany simply 'hæmoglobin,' while in this country the reduced hæmoglobin is called hæmoglobin. Most of the chemical investigations and most of the analyses have been made with oxyhæmoglobin, as this compound crystallises more readily than does reduced hæmoglobin, and as the latter by absorption of oxygen is constantly changed into the former. Owing to the gigantic size of the hæmoglobin molecule, no difference in the analyses of oxy- and reduced hæmoglobin can be made out. The double method of estimating the molecular weight of hæmoglobin has already been discussed on p. 328. Jaquet's[2] calculations based on the figures yielded by analysis have led to the same conclusion as have those of Hüfner,[3] who estimated the power hæmoglobin possesses of binding gases: the molecular weight of hæmoglobin is 16669.

[1] E. Riegler, *Zeitschr. f. analyt. Chem.* **43**. 539 (1904).

[2] A. Jaquet, *Zeitschr. f. physiol. Chem.* **14**. 289 (1889).

[3] G. Hüfner, *Arch. f.* (*Anat. und*) *Physiol.* 1894, p. 130.

	C	H	N	S	Fe	O	P	
Horse. .	54·87	6·97	17·31	0·65	0·47	19·77	...	Hoppe-Seyler [1]
,, . .	54·40	7·20	17·61	0·65	0·47	19·67	...	Hüfner [2]
,, . .	54·76	7·03	17·28	0·67	0·45	19·81	...	Otto [3]
[,, . .	51·15	6·76	17·94	0·3899	0·335	23·421	...	Zinnofsky [4]]
,, . .	54·81	7·01	17·06	0·6	0·468	19·86	...	Nencki [5]
,, . .	54·56	7·15	17·33	0·43	...	...	...	Schulz [6]
Dog . .	53·85	7·32	16·17	0·39	0·43	21·84	...	Hoppe-Seyler [7]
,, . .	54·57	7·22	16·38	0·568	0·336	20·43	...	Jaquet [8]
,, . .	...	...	16·43	...	...	...	...	v. Noorden [9]
Pig . .	54·17	7·38	16·23	0·66	0·426	21·364	...	Otto [10]
,, (Met-H	53·99	7·18	16·19	0·66	0·45	21·58	...	Otto [10]
Cow . .	...	...	...	...	0·336	...	...	Hüfner [11] and Jaquet
Guinea-pig.	54·12	7·36	16·78	0·58	0·48	20·68	...	Hoppe-Seyler [7]
Squirrel .	54·09	7·39	16·09	0·59	0·4	21·44	...	,, ,,
Goose. .	54·26	7·10	16·21	0·54	0·43	20·69	0·34	,, ,,
,, . .	...	...	...	...	...	...	0·77	Gscheidlen [12]
Cock . .	52·47	7·19	16·45	0·8586	0·335	22·5	0·197	Jaquet [8]

The analyses in this table all refer to oxyhæmoglobin, with the exception of the one by Otto, which represents methæmoglobin, but the latter possesses the same composition as does oxyhæmoglobin. The analyses show that hæmoglobin is distinguished among albumins by its high C- and N-percentage, and this explains its great heat value, for the latter, according to Stohmann and Langbein,[13] amounts to 5885·1 cal. The dissociation-products of globin have already been given on p. 70; the hæmatin amounts, according to Schulz [14] and Lawrow,[15] to between 4-4·5 per cent. See below, p. 508.

The sulphur is partly in the form of cystin in the globin, while the iron, is contained in the hæmatin.

Whether there are different kinds of hæmoglobin Cohnheim believes to be still undecided, but Gamgee [16] has pointed out that although

[1] F. Hoppe-Seyler, *Zeitschr. f. physiol. Chem.* **1**. 121 (1877).

[2] G. Hüfner and Bucheler, *ibid.* **8**. 358 (1884).

[3] J. C. Otto, *Pflüger's Archiv f. d. ges. Physiol.* **31**. 240 (1883).

[4] O. Zinnofsky, *Zeitschr. f. physiol. Chem.* **10**. 16 (1885).

[5] M. Nencki, *Schmiedeberg's Archiv f. experiment. Pathol. und Pharmak.* **20**. 332 (1885).

[6] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **24**. 449 (1898).

[7] F. Hoppe-Seyler, *Med.-chem. Unters.* p. 366 (1868).

[8] A. Jaquet, *Zeitschr. f. physiol. Chem.* **12**. 285 (1888).

[9] C. v. Noorden, *ibid.* **4**. 9 (1879).

[10] J. C. Otto, *ibid.* **7**. 57 (1882).

[11] G. Hüfner, *Arch. f. (Anat. u.) Physiol.* 1894, p. 130.

[12] R. Gscheidlen, *Pflüger's Archiv f. d. ges. Physiol.* **16**. 421 (1878).

[13] F. Stohmann and H. Langbein, *Journ. f. prakt. Chem.* [2] **44**. 336 (1891).

[14] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **24**. 449 (1898).

[15] D. Lawrow, *ibid.* **26**. 343 (1898).

[16] A. Gamgee, *Schäfer's Textbook of Physiol.* **1**. 204 (1898).

"there is in the hæmoglobin of all animals absolute identity of the essential iron-containing nucleus, *i.e.* of that moiety of the molecule on which its colour and its physiological function depend, that at the same time there is such a difference in the ratio of S : Fe in the hæmoglobin of certain animals as renders it highly probable, or rather certain, that in the hæmoglobin of different animal groups the albuminous moiety of the complex molecule differs. Such being the case, it is not surprising that certain of the physical characters of hæmoglobin, such as crystalline form and solubility, should exhibit variations," and "that hæmoglobins varying in certain physical properties may be formed by the linking of the iron-containing molecule to various polymeric combinations of the same albuminous molecule." See also p. 474.

Gamgee's view seems to the author to be also supported by the fact that the amount of water of crystallisation differs for different animals, amounting to the following percentages:—dog, 3·4 (Hoppe-Seyler); horse, 3·94 (Hüfner); pig, 5·9 (Otto); guinea-pig, 6 (Hoppe-Seyler); and squirrel, 9 (Hoppe-Seyler).[1] In further support may be mentioned the recent work of Ham and Balean (see p. 507), who have shown that the globin radical of hæmoglobin may be replaced by some constituent of egg-white. At present we have no right to suppose that the hæmatin radical in one and the same animal is always linked to exactly the same albuminous moiety, for there is no *a priori* reason against the view held by MacMunn that myohæmatin differs from blood-hæmoglobin.

To put it shortly: the hæmatin of all animals is alike, while the albuminous element is not.

Hoppe-Seyler[2] in 1889 brought forward the view that in living blood special compounds, 'phlebin' and 'arterin,' are present, and that out of them reduced- and oxyhæmoglobin are formed secondarily. Kobert[3] has subsequently defended this view, and Bohr[4] distinguishes also between several modifications of oxyhæmoglobin (see p. 494).

How difficult it is to purify hæmoglobin has already been pointed out, as, according to Abderhalden, hæmoglobin which was crystallised only once still contained up to 15 per cent of extraneous albumins and the hæmoglobin of animals possessing nucleated red blood-corpuscles (birds, reptiles, etc.) contains, even after repeated recrystallisation, phosphorus derived from the nucleic acid of the compounds of the nucleus.

[1] Gamgee's Table on p. 205 of *Schäfer's Textbook of Physiology*, vol. i. (1898).

[2] F. Hoppe-Seyler, *Zeitschr. f. physiol. Chem.* **13**. 477 (1889).

[3] H. U. Kobert, *Zeitschr. f. angewandte Mikroskopie*, V. 6 to 10 (1900); R. Kobert, *Deutsche Arztezeitung*, 1900, Heft 18.

[4] Bohr, *Zentralbl. f. Physiol.* **4**. 242, 254 (1890).

Hæmoglobins differ greatly from one another as regards their solubilities. Preyer[1] states that the hæmoglobin of the ox becomes fluid on being exposed to the air, while the hæmoglobin of the squirrel is only soluble in 597 parts of water, and that of the raven is hardly soluble at all in cold water. The solubilities are greatly increased by warming the solutions: dog's hæmoglobin at 5° is only soluble to the extent of 2 parts in 100 of water; while at 18°, 12 to 15 parts are dissolved. Reduced hæmoglobin is always more soluble than is oxyhæmoglobin. Hæmoglobin resembles albumins, as far as salting out is concerned, *i.e.* it is not salted out by sodium chloride or magnesium sulphate from neutral solutions, but is salted out by a saturated magnesium sulphate + sodium sulphate solution. Schulz[2] gives the precipitation-limits for ammonium sulphate as lying between 6·5 and nearly completely saturated solutions.

Oxyhæmoglobin is an acid according to Kühne[3] and Preyer,[4] and methæmoglobin is also an equally strong if not stronger acid according to Hoppe-Seyler, Menzies,[5] and Jäderholm.[6] Hæmoglobin is not acid. A precipitation by means of acid is not possible, as hæmoglobin is decomposed by extremely small amounts of acid.

The coagulation-temperature of hæmoglobin is 64° according to Preyer;[7] but hæmoglobin becomes gradually decomposed if it be kept for some time at 54°. When quite dry, hæmoglobin may be heated for a long time without becoming denaturalised, but oxyhæmoglobin is very apt to become converted into methæmoglobin. Alcohol denaturalises pure hæmoglobin only slowly, which, again, may be due to all salts having been removed by repeated recrystallisation. Hæmoglobin crystals pass into pseudomorphoses when they are treated with alcohol (Preyer[7] and Nencki[8]); see below. Even before alcohol has completely denaturalised hæmoglobin it alters the dissociation of oxyhæmoglobin, rendering it more like methæmoglobin.[9] It is therefore important not to use alcohol in preparing crystals.[10]

Hæmoglobin, being composed of an albuminous radical and a non-albuminous iron-containing nucleus, it is very interesting to note how

[1] W. Preyer, *Die Blutkristalle*, Jena, 1871, p. 54.
[2] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **24**. 449 (1898).
[3] W. Kühne, *Virchow's Archiv*, **34**. 423 (1865).
[4] W. Preyer, *Zentralb. f. d. med. Wissensch.* 1867, No. 18.
[5] J. A. Menzies, *Journ. of Physiol.* **17**. 402 (1895).
[6] Axel Jäderholm, *Zeitschr. f. Biol.* **20**. 419 (1884).
[7] W. Preyer, *Pflüger's Archiv*, **1**. 395 (1868).
[8] M. Nencki, *Schmiedeberg's Archiv f. experim. Pathol. und Pharm.* **20**. 332 (1885).
[9] A. Löwy, *Zentralbl. f. Physiol.* **13**. 449 (1899); G. Hüfner, *Arch. f.* (*Anat. und*) *Physiol.* 1901, Suppl. p. 187.
[10] G. Hüfner, *Arch. f.* (*Anat. und*) *Physiol.* 1901, Suppl. p. 187.

these two fractions so act upon one another as to prevent either giving the reactions which it otherwise would. Kühne showed in 1866 that the albumin reactions are only obtained when the hæmoglobin has been decomposed and its iron-containing fraction, the hæmatin, has been set free; while, for example, cupric and ferrous sulphates, mercuric chloride, silver nitrate, neutral and basic lead acetates precipitate ordinary albumin, they produce no effect on oxyhæmoglobin, but do precipitate the globin as soon as by decomposition of the hæmoglobin it has become separated from the hæmatin. On the other hand, the iron cannot be demonstrated in hæmoglobin till the latter is decomposed, and thereby the 'masked' iron is converted into ionic iron.

How readily acids change hæmoglobin is fully discussed later on (p. 496). Why the salts of the heavy metals such as mercuric chloride do not precipitate (Preyer [1]) is difficult to say, but if we bear in mind that pure salt-free albumins are also not precipitated, the explanation already offered by the author seems to hold good: Salt-free albumins are in the true sense of the word dead, because, by the removal of all electrolytes, the electrical dissociation of amino-acids is prevented, and from the active amino-acids we pass to the inactive ring-compounds (see p. 211), and it is quite conceivable that the Hg-kation of corrosive sublimate is not sufficiently strong to convert the amino-acids in the globin radical from their pseudo-acid pseudo-basic state into chemically active open-chain compounds. Salkowski [2] and Formanek [3] have found that hæmoglobin differs from other albumins in being precipitated when it is shaken with a little chloroform, and that it does not become altered hereby.

Hoppe-Seyler [4] and others have drawn attention to the great resistance which hæmoglobin offers to putrefying organisms; oxyhæmoglobin becomes changed into reduced hæmoglobin, but does not undergo any further change. Hæmoglobin also resists trypsin very strongly, especially as long as it is in living red corpuscles.[5] "To what extent this immunity of hæmoglobin depends on the admixture of antiferments, or is simply a widely distributed general property of pure colloidal albumins, is as little understood as in the case of serum-albumins" (Cohnheim). The author is not convinced of the existence of antiferments: given a ferment and an albumin capable of being acted upon, then a third

[1] W. Preyer, *Plüger's Archiv*, **1**. 395 (1868).

[2] E. Salkowski, *Deutsche medizin. Wochensch.* 1888, No. 16; *Zeitschr. f. physiol. Chem.* **31**. 329 (1900).

[3] E. Formanek, *ibid.* **29**. 416 (1900).

[4] F. Hoppe-Seyler, *Zeitschr. f. physiol. Chem.* **1**. 121 (1877).

[5] H. Sachs, *Münchener medizin. Wochenschr.* 1902, p. 189.

substance may either render the albumin unattackable, as do formic or acet-aldehyde when they alter albumin in such a way that it is no longer acted upon by trypsin, while it is still digested by pepsin, or the third substance may alter the ferment, *e.g.* OH-ions acting on pepsin, in which case the addition of fresh unaltered ferment will result in normal digestion. The author also believes that the resistance offered by hæmoglobin to putrefaction and to tryptic digestion depends on the same cause.

Hæmoglobin differs from globin and all simple albumins in being dextro-rotatory according to Gamgee and Croft Hill,[1] who found

Hæmoglobin	$a_C = +10{\cdot}4$
Globin	$a_C = -54{\cdot}2$

Gamgee[2] has further observed that hæmoglobin and its various compounds with gases are diamagnetic in an electrical field, while hæmatin is strongly magnetic.

Histohæmatins have been carefully investigated by MacMunn.[3] They are allied to the hæmochromogens and subserve a respiratory function, as "their bands are intensified by alkalies and enfeebled by acids, intensified by reducing agents and enfeebled by oxidising agents." MacMunn has described the following bands :—[4]

Stomach wall of cat (blood-free)—

α λ613–λ593
β λ569–λ563
γ λ556–λ551

Kidney of cat—

α λ613–λ596·5
β λ569–λ563
γ λ556–λ550

That special histohæmatin found in muscle MacMunn has called 'myohæmatin.' Its absorption spectrum "is practically the same throughout the whole animal kingdom." It is best studied in the papillary muscles of the heart.

Heart of hare—

α λ613–λ600
β λ569–λ563
γ λ556–λ550

[1] A. Gamgee and Croft Hill, *Ber. d. deutsch. chem. Ges.* **36**. I. 913 (1903).
[2] A. Gamgee, *Proc. Roy. Soc.* **68**. 503 (1901).
[3] MacMunn, *Phil. Trans.*, London, **177**. 235 (1886).
[4] Quoted from M'Kendrick's textbook of *Physiology*, **1**. 138 (1888); see also *Journ. of Physiol.* **8**. 51 (1887).

Heart of rat—

α λ613–λ596·5
β λ596–λ563
γ λ556–λ550

The hæmatin-derivative occurring in the muscles of vertebrates, is, according to MacMunn, a special 'myohæmatin,' with definite derivatives, all of which differ spectroscopically from ordinary hæmoglobin. Hoppe-Seyler,[1] Levy,[2] and Mörner[3] are of the opinion that the hæmoglobin found in muscles is the same as that met with in red blood-corpuscles, and that the displacement of the absorption bands of muscle hæmoglobin towards the red end of the spectrum [the centre of the two bands of blood oxyhæmoglobin being at λ577 and 540, and those of myohæmoglobin at λ581 and λ543] does not signify a difference in the hæmoglobins.

Mörner[4] has suggested that the proteid-constituent of the pigment may be different in hæmoglobin and myohæmatin. Halliburton[5] says: "I think myself there can be no doubt that myohæmatin is a derivative of hæmoglobin, but whether the muscular tissue is capable of producing the change in spite of the reagents added or whether the reagents added are mainly responsible for the change, one cannot at present say."

Halliburton also quotes Copeman[6] who mixed defibrinated and slightly diluted blood with minced muscle and kept it for three weeks in the absence of air at 36°, and so obtained the spectrum of myohæmatin. On mixing blood with minced liver or other tissues Copeman obtained a hæmochromogen-spectrum; the myohæmatin-bands disappear when the myohæmatin-solution is heated to near the boiling point, according to Copeman and Halliburton.

Since the globin radical may be replaced by egg-white as shown by Ham and Balean see (p. 507), there is nothing against the conception of hæmatin uniting with myosinogen or with other albumins to form true respiratory pigments (Mann).

The Crystals of Hæmoglobin and Oxyhæmoglobin

Oxyhæmoglobin crystallises more readily than does any other albumin, and has been known in its crystalline form for a very long

[1] F. Hoppe-Seyler, *Zeitschr. f. physiol. Chem.* **14**. 106 (1889).
[2] L. Levy, *ibid.* **13**. 309 (1888).
[3] K. A. H. Mörner, *Maly's Jahresber. f. Tierchemie*, **27**. 456 (1897).
[4] Mörner, *Nord. med. Ark.* (Stockholm, Festband, 1897).
[5] Halliburton, *Biochemistry of Muscle and Nerve*, 1904, p. 29.
[6] S. Monckton Copeman, *Proc. Physiol. Soc.* Nov. 8 (1890); and *Journ. of Physiol.* **9**. p. xxii.

time. To prepare oxyhæmoglobin crystals proceed thus: Free the red blood-corpuscles as thoroughly as possible from all the albumins of the blood-plasma or blood-serum by decantation, or, still better, by means of the centrifuge, suspending the corpuscles in isotonic salt-solution and centrifugalising them repeatedly. Now lake the corpuscles by adding distilled water, or by repeated freezing and thawing, or by the addition of a little ether, which is then allowed to evaporate. Schuurmans-Stekhoven[1] disintegrates the corpuscles without the addition of any chemicals by shaking them vigorously with particles of asbestos. On cooling laked blood, crystals separate out with the more readily crystallisable species of blood. Crystals in small amounts may be easily obtained by laking defibrinated rat's blood and then allowing the water to partially evaporate; Ewald[2] allows evaporation to take place after having covered laked blood with a microscopic cover-glass; with the blood of the squirrel good results are obtained by simply mixing the blood with Canada balsam and then adjusting the cover-glass.[3] To prepare oxy-hæmoglobin crystals in larger quantities it is necessary to add alcohol to the laked blood according to the directions given by Hoppe-Seyler:[4] cool the laked blood to zero centigrade, add one quarter the volume of alcohol also cooled to zero, and keep the mixture for several days at -5° to -10°. The crystals are then dissolved in a little water warmed up to 35°; the insoluble residue, consisting of the stromata of the red corpuscles, is filtered off, and the filtrate is again exposed to the cold after the addition of alcohol. If this procedure is then repeated several times more, a very pure, ash-free preparation is obtained. Zinnofsky[5] lakes the blood directly by the addition of ether, without having previously removed the albumins of the plasma; he then dissolves the stromata by the addition of very dilute ammonia, and removes them by very careful neutralisation with hydrochloric acid. Jaquet[6] adds to the blood of hens one-third its volume of ether at 35°, as otherwise the nucleated red corpuscles set into a jelly. Schuurmans-Stekhoven places the laked blood into a dialysing tube and then suspends the latter in 45 per cent alcohol; as soon as crystallisation commences,

[1] Schuurmans-Stekhoven, *Maly's Jahresb. f. Tierchemie*, **31**. 212 (1901).

[2] A. Ewald, *Zeitschr. f. Biol.* **22**. 459 (1886).

[3] Professor Francis Gotch tells me that he first heard of this method from a student who worked in Krukenberg's laboratory, and that the method was discovered by a pupil of Krukenberg's.—The author.

[4] F. Hoppe-Seyler, *Med.-chem. Untersuchungen*, p. 169 (1867); *Handbuch der physiol.-chem. Analyse.*

[5] O. Zinnofsky, *Zeitschr. f. physiol. Chem.* **10**. 16 (1885).

[6] A. Jaquet, *ibid.* **14**. 289 (1889).

the blood is taken out of the tube and is put on ice. By this means the addition of alcohol is reduced to a minimum.

Schulz[1] prepares hæmoglobin crystals by an entirely different method, based on Hofmeister's principle of preparing crystals of egg-albumin: the laked blood is mixed with an equal bulk of saturated ammonium-sulphate solution to precipitate the globulins; in the filtrate the hæmoglobin crystallises out and may then be repeatedly recrystallised from half-saturated ammonium-sulphate solutions. To prevent too rapid crystallisation, it is best to cool the laked blood by placing it on ice, then to add ammonium-sulphate solution, and finally to allow crystallisation to take place at the ordinary room-temperature. Hæmoglobin crystals prepared by this method are large and well formed, but they contain ammonium sulphate, which has to be removed by dialysis. According to Schulz[2] one is apt to get hæmoglobin crystals along with serum-albumin crystals if serum be employed which contains hæmoglobin.

According to Hoppe-Seyler the slightly soluble hæmoglobins from the blood of the dog, horse, guinea-pig, squirrel, and rat crystallise readily, as does also the blood of the goose, duck, and pigeon. The more soluble hæmoglobins, *e.g.* those of man, ox, sheep, pig, and rabbit, do not crystallise easily; while the hæmoglobins of the mouse, mole, and bat crystallise somewhat more readily. The last blood to be obtained in a crystalline form was that of the cat (Krüger[3] and Abderhalden[4]). Further information regarding the hæmoglobin crystals of a large number of animals is given by Preyer;[5] the literature on blood crystals has been put together by Kobert[6] and Schulz.[7]

For preparing hæmoglobin crystals the blood of the dog or that of the horse is most suitable; pig's hæmoglobin also crystallises readily according to Otto,[8] although it is very soluble.

It is much more difficult to make reduced hæmoglobin crystallise, as it is much more soluble. Kühne[9] was the first to succeed, and subsequently followed Gscheidlen,[10] Ewald,[11] Nencki,[12] Gürber,[13] and

[1] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **24**. 449 (1898).

[2] F. N. Schulz, *Die Kristallisation von Eiweissstoffen*, Jena, Fischer, 1901.

[3] Fr. Krüger, *Zeitschr. f. Biologie*, **26**. 452 (1890); *Zeitschr. f. physiol. Chem.* **25**. 256 (1898).

[4] E. Abderhalden, *ibid.* **24**. 545 (1898).

[5] W. Preyer, *Blutkristalle*, Jena, 1871.

[6] H. U. Kobert, *Zeitschr. f. angewandte Mikroskopie*, V. 6 to 10 (1900).

[7] F. N. Schulz, *Die Kristallisation von Eiweissstoffen*, Jena, Fischer, 1901.

[8] J. Otto, *Zeitschr. f. physiol. Chem.* **7**. 57 (1882).

[9] W. Kühne, *Virchow's Archiv*, **34**. 423 (1865).

[10] R. Gscheidlen, *Pflüger's Archiv f. d. ges. Physiol.* **16**. 421 (1878).

[11] A. Ewald, *Zeitschr. f. Biologie*, **22**. 459 (1886).

[12] M. Nencki, *Ber. d. deutsch. chem. Ges.* **19**. I. 28 and 410 (1886).

[13] Gürber, *Sitzungsber. d. physik.-mediz. Ges. zu Würzburg*, 1893 (Reprint).

others. Gscheidlen recommends to let blood undergo putrefaction, as hæmoglobin is not altered thereby, while Gürber removes the substances preventing crystallisation, by means of dialysis. For the crystallisation of reduced hæmoglobin the same methods are used as were given above for oxy-hæmoglobin.

Oxyhæmoglobin and hæmoglobin crystallise generally in plates, prisms, or needles, belonging to the rhombic system, but those of the squirrel belong to the hexagonal system, and that from other rodents may be prepared as hexagonal plates (Halliburton[1]). Halliburton and Reichert,[2] by mixing blood of guinea-pigs and of rats in definite proportions, have also prepared derivatives of tetrahedra and spindle-shaped crystals, while the normally occurring hæmoglobin crystals of the guinea-pig form true tetrahedra. Uhlik[3] obtained from horse's blood, in addition to the ordinary prismatic, rhombic crystals, even after putrefaction had set in, hexagonal holo-hedric crystals in six-sided plates on employing low temperatures. The different forms of crystals pass into one another on recrystallisation, and therefore no significance is attached to the form of the crystals (Cohnheim). That the author does not share this view has been pointed out on p. 470. The older literature dealing with hæmoglobin crystals will be found in the important paper of Rollet.[4] Accurate measurements of the angles have been made by Rollet and v. Lang.[5] Those bloods which do not crystallise readily form as a rule only microscopic crystals, while Hüfner and Bücheler[6] obtained from horse's blood large needles measuring 2 to 3 mm. in length and 0·5 mm. in thickness. Gscheidlen once prepared a hæmoglobin crystal measuring 3·5 cm. in length.

The optical properties of the crystals have been studied by Preyer and more thoroughly by Ewald.[7] They are silky, doubly refractile, and not transparent; they also possess a very marked pleochroism. This last property is especially well seen in the crystals of reduced hæmoglobin, for when viewed with only one Nicol prism they show three distinct axial colours, namely, a bluish purple, a reddish purple, and no colour. The crystals of oxyhæmoglobin show the pleochroism less distinctly, but still quite definitely; according to the position of the Nicol's prism they are either of a dark scarlet tint, or of a bright yellowish red. Pleochroism is also shown by the other hæmo-

[1] W. D. Halliburton, *Quart. Journal of Micr. Sc.* **28**. 181 (1888).
[2] Edw. T. Reichert, *Amer. Journ. of Physiol.* **9**. 97 (1903).
[3] M. Uhlik, *Pflüger's Arch.* **104**. 64 (1904).
[4] Rollet, *Sitzb. d. kaiserl. Akad. d. Wiss. Wien, math.-naturw. Klasse*, **46**. 65 (1862).
[5] V. v. Lang, *Wiener Akademie*, **46**. 1862 (according to Preyer).
[6] G. Hüfner and Bücheler, *Zeitschr. f. physiol. Chem.* **8**. 358 (1884).
[7] A. Ewald, *Zeitschr. f. Biol.* **22**. 459 (1886).

globin derivatives to be mentioned later on: methæmoglobin and hæmin are dark blackish brown and pale yellowish brown; methæmoglobin, in crystals, is colourless; CO-hæmoglobin is purple and white. According to the axial plane they affect the spectrum in a different manner, as the absorption-bands become shifted either towards the red or towards the violet end of the spectrum. Ewald draws attention to the fact that similar phenomena are also observed if a substance be dissolved in media possessing different dispersions, and points out what care is required in interpreting small spectroscopic differences such as are shown by the hæmoglobin derivatives under different conditions.

Blood crystals are denaturalised and are converted into pseudomorphoses on being kept, or on being allowed to dry, or when acted upon by alcohol, but their power of refracting light doubly they may retain for a considerable time.[1] Crystals which have become insoluble owing to the action of alcohol, but which still give the typical spectrum and which are still doubly refractile, Nencki[2] has called 'parahæmoglobin.'

The Gaseous Compounds of Hæmoglobin and its Optical Properties

During its passage through the lungs the blood of vertebrates becomes saturated with oxygen, and during its passage through the rest of the body it parts with this oxygen, which is taken up by the tissues; arterial blood, containing oxygen, is of a bright-red colour, while venous blood, poor in oxygen, is of a darker-red, and even purple colour; in cases of asphyxia the blood is almost black. A solution of hæmoglobin on being brought together with atmospheric oxygen absorbs one molecule of oxygen for each molecule of hæmoglobin, and becomes converted thereby into oxyhæmoglobin. The chief characteristic of the two hæmoglobins, namely, of oxy- and reduced hæmoglobin, is their absorption-spectrum; Hoppe-Seyler[3] was the first to describe the spectrum of oxyhæmoglobin, while Stokes[4] first prepared and studied the reduced hæmoglobin.[5] The best method for reducing

[1] W. Preyer, *Blutkristalle*, Jena, 1871.

[2] M. Nencki, *Schmiedeberg's Arch. f. exper. Pathol. u. Pharmak.* **20**. 332 (1885).

[3] F. Hoppe-Seyler, *Virchow's Archiv.* **33**. 446 (1862); *Zentralbl. f. d. med. Wissenschaften*, 1864, pp. 261, 817, 834; *Med.-chem. Untersuch.* p. 169 (1867).

[4] G. G. Stokes, *Philosoph. Magazine and Journ. of Science*, **27**. 4th Ser. p. 388 (1864); *Proc. Roy. Soc.* 1864, June 16 (according to Neumeister's textbook).

[5] Stoke's solution for reducing hæmoglobin is prepared as follows: Two grams of ferrous sulphate are dissolved in 100 cc. of a 3 per cent watery solution of tartaric acid,

oxyhæmoglobin is that of Curtius-Hüfner.[1] Curtius recommended salts of hydrazin, and Hüfner[2] then introduced hydrazin-hydrate, because the only products of its decomposition are nitrogen and water:

$$H_2N - NH_2 . H_2O + O_2 = N_2 + 3H_2O.$$

For a full account of the different reducing methods consult Gamgee's account in Schäfer's *Textbook of Physiology*, vol. i., p. 230 (1898).

Subsequently Hüfner and v. Noorden[3] have devoted great care to the visible spectrum, while Soret[4] and especially Gamgee[5] have studied the absorption-bands in the violet and ultra-violet regions.

A more complete account of the optical properties of the hæmoglobin derivatives than that given here will be found in the papers of Formánek,[6] Ziemke and Müller,[7] and Schulz.[8] The latter has studied hæmatoporphyrin very carefully.

The theory and methods of spectro-photometry are fully explained by Gamgee in Schäfer's *Textbook of Physiology*, vol. i., p. 213 (1898).

Fraunhofer's lines:—

A	$=\lambda 7606{\cdot}1$	expressed in tenth-metres $=1+10^{-10}$ metres.
B	$=\lambda 6869{\cdot}1$	
C	$=\lambda 6563{\cdot}9$	
D_1	$=\lambda 5896{\cdot}8$	
D_2	$=\lambda 5890{\cdot}7$	
E	$=\lambda 5270{\cdot}6$	
b	$=\lambda 5183{\cdot}0$	
F	$=\lambda 4862{\cdot}1$	
G	$=\lambda 4308{\cdot}5$	
H	$=\lambda 3969{\cdot}2$	
K	$=\lambda 3934{\cdot}1$	

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and then ammonia is added till the solution becomes alkaline. This mixture should be always freshly prepared.

[1] Curtius, *Journ. f. prakt. Chem.* **39**. 27 (1889).

[2] Hüfner, *Bestimmung d. Sauerstoffcapacität d. Blutfarbstoffs*, p. 156.

[3] G. Hüfner, *Journ. f. prakt. Chem.* [2] **22**. 362 (1880)'; C. v. Noorden, *Zeitschr. f. physiol. Chem.* **4**. 9 (1879); in both papers will be found the theoretical exposition of his methods; G. Hüfner, *ibid.* **1**. 317 (1877); **1**. 386 (1878); **3**. 1 (1878); **8**. 358 (1884); **10**. 218 (1886); **12**. 568 (1888); **13**. 285 (1888). The final conclusions will be found in the following papers: G. Hüfner, *Archiv für (Anat. und) Physiol.* 1890, p. 1; 1894, pp. 130, 209; 1895, p. 213; 1901, Suppl. p. 187.

[4] Soret, 'Recherches sur l'absorption des rayons ultra-violets par diverses substances,' *Arch. d. sc. phys. et nat. Genève*, 1878, pp. 322, 359.

[5] Arthur Gamgee, *Zeitschr. f. Biol.* **34**. 505 (1896). See also Schäfer's *Textbook of Physiol.* vol. i. (1898).

[6] J. Formánek, *Zeitschr. f. analytische Chem.* **40**. 505 (1901); according to *Maly's Jahresber.* **31**. 223.

[7] E. Ziemke and Franz Müller, *Arch. f. (Anat. u.) Physiol.* 1901, Suppl. p. 177.

[8] Arthur Schulz, *ibid.* 1904, Suppl. p. 271.

Nature of Light-waves

Colour.	Vibration frequency in million million.	Wave-length in centimeters.
Ultra-red	370	·00008100
Red	428	·00007000
Orange-red	483	·00006208
Orange	502	·00005972
Orange-yellow	510	·00005879
Yellow	516	·00005808
Green	569	·00005271
Blue-green	590	·00005082
Cyan-blue	604	·00004960
Blue	634	·00004732
Violet-blue	684	·00004383
Violet	739	·00004059
Ultra-violet	833	·00003600

The mean visible wave-frequency is about 508 to 510 million million vibrations per second.

Oxyhæmoglobin

Oxyhæmoglobin possesses three well-defined absorption-bands α, β, and γ, and probably a fourth band in the red, see below. The bands α and β lie between the Fraunhofer's lines D and E, of which the narrower but more sharply defined band α commences close to D:

α $\lambda 582$–$\lambda 571$
β $\lambda 550$–$\lambda 526$
γ $\lambda 424$–$\lambda 404$

The second band β reaches, according to Hüfner's spectro-photometric measurements, its maximum intensity between

$\lambda 542{\cdot}5$ and $\lambda 531{\cdot}5$

It is to the eye less intense than is the band α, but in spectro-photometric measurements this is not the case according to Hüfner. The quotient of the absorption coefficients at the place of the maximal absorption in band β, and at the place of greatest light between the bands α and β, is according to Hüfner in the case of oxyhæmoglobin 1·578. Müller[1] finds, however, that the quotient of blood freshly drawn from the ears of healthy dogs is frequently as low as 1·47 to 1·48, and warns against implicit faith in Hüfner's figures, which were obtained from purified oxyhæmoglobin crystals. [See later, p. 482.] The third band γ, of about the same intensity as band β, reaches its maximum intensity at $\lambda 414$, close to the line *h*. The

[1] Franz Müller, *Pflüger's Arch.* **103**. 541 (1904).

bands are still well defined, according to Hoppe-Seyler and Gamgee, in layers 1 cm. thick and containing 0·1 gramme of oxyhæmoglobin in 1 litre of water. Gamgee draws attention to the fact that hæmoglobin absorbs both the most visible as also the most actinic light.

Piettre and Vila[1] on examining fresh, newly laked blood of guinea-pigs, or solutions of oxyhæmoglobin in tubes 20 cm. long, have observed in the red a new band ($\lambda = 634$). The band is at once formed at body temperature, and after some time at room temperature. It is not seen after diluting blood with normal salt solution.

Reduced Hæmoglobin

Reduced hæmoglobin possesses two bands, α and β, of which only one occurs in the yellow-green, lying fairly in the middle between D and E, and therefore in the clear area between the α and β bands of oxyhæmoglobin. The band α of reduced hæmoglobin is broader than the band β of oxyhæmoglobin, but it is less intense.

α $\lambda 597$–$\lambda 535$
$\lambda 573$–$\lambda 542$ (darkest part), MacMunn.[2]
β $\lambda 436$–$\lambda 415$

For the same place as he used in the case of oxyhæmoglobin, Hüfner gives the quotient of the extinction-coefficient as 0·7617. The band β in the violet is most intense at $\lambda 425$. The band is therefore narrower than in the case of oxyhæmoglobin, and displaced somewhat towards the red end. A hæmoglobin solution on being shaken with air absorbs oxygen, and the spectrum of hæmoglobin is changed into that of oxyhæmoglobin, and the same holds good for blood or laked blood. The oxyhæmoglobin is reconverted into hæmoglobin by reducing agents, such as Stoke's solution (see p. 478), or ammonium sulphide, or hydrazin hydrate (Hüfner). Siegfried[3] and Novi[4] use also hydrosulphite, which apparently does not reduce completely. The union of hæmoglobin with oxygen is a very loose one, as oxygen is given off on reducing the atmospheric pressure, and in a vacuum even the whole of the oxygen may be removed. If an indifferent gas such as hydrogen or nitrogen be passed for some time through a solution of oxyhæmoglobin, the oxygen is also completely driven off. That the carbonic acid, which is so abundant in venous blood, also displaces oxygen is discussed more fully on p. 489. Whether

[1] Piettre and Vila, *Compt. Rend.* **140**. 390 (1905).
[2] M'Kendrick's *Textbook of Physiology*, **1**. 123 (1888).
[3] M. Siegfried, *Arch. f.* (*Anatomie u.*) *Physiol.* 1890, p. 385.
[4] Ivo Novi, *Pflüger's Arch. f. die gesamte Physiol.* **56**. 289 (1894).

between oxyhæmoglobin and reduced hæmoglobin there exist a transition form, called by Siegfried 'pseudo-hæmoglobin,' is doubtful. Siegfried[1] believed, and Novi[2] has confirmed Siegfried's statement, that in pseudo-hæmoglobin there is still present a certain amount of oxygen, although spectroscopically the pseudo-hæmoglobin resembles reduced hæmoglobin; but Hüfner[3] does not believe in the existence of pseudo-hæmoglobin, because, he says, the statements of Siegfried and Novi are simply based on visual impressions and not on spectrophotometric measurements.

The amount of oxygen which 1 gramme of hæmoglobin can bind Hüfner has endeavoured to determine by oft-repeated experiments, but the values he obtained did not agree very well owing to the estimation of the percentage strength of hæmoglobin being difficult and the dissociation of the oxyhæmoglobin varying. To overcome the second difficulty he employed CO-hæmoglobin, which dissociates much less. Hüfner states that 1 gramme of hæmoglobin binds 1·338 ccm. of carbon monoxide, and as oxygen and carbon monoxide replace one another in equal volumes, he concluded that 1 gramme of hæmoglobin also united with 1·338 ccm. oxygen. This maximal figure was, however, only obtained approximately when very high oxygen-pressures were employed. Cohnheim remarks: "This number is very constant, and numerous experiments of Hüfner, Dybkowski,[4] Herter,[5] Worm Müller,[6] Setschenow,[7] and others have put it beyond doubt that the maximal oxygen capacity of fresh blood or of hæmoglobin solutions prepared by different methods is identical. Spectroscopically there is also no difference."

Haldane,[8] however, is of the opinion that the evidence in support of Hüfner's hypothesis that 1 gramme of oxyhæmoglobin yields 1·34 cc. of oxygen "is far from satisfactory," as Hüfner's[9] original data gave an average of 1·26 cc. of carbonic oxide per gramme of hæmoglobin, the results of individual experiments varying by as much as 10 per cent from one another. To obtain the corrected figure of 1·34 cc. (which, assuming that Zinnofsky's and Jacquet's determinations of iron are correct, gives a ratio of 1 atom of iron to 2 of

[1] M. Siegfried, *Arch. f. (Anat. und) Physiol.* 1890, p. 385.

[2] Ivo Novi, *Pflüger's Arch. f. die gesamte Physiol.* **56**. 289 (1894).

[3] G. Hüfner, *Arch. f. (Anat. und) Physiol.* 1894, p. 130 (p. 140).

[4] W. Dybkowski, *Hoppe-Seyler's med.-chem. Untersuch.* p. 117 (1866).

[5] F. Herter, *Zeitschr. f. physiol. Chem.* **3**. 98 (1879).

[6] J. Worm Müller, *Untersuch. aus der Leipziger physiologischen Anstalt*, **5**. 119 (1870).

[7] J. Setschenow, *Pflüger's Arch. für die gesamte Physiol.* **22**. 252 (1880).

[8] J. Haldane, *Journ. of Physiol.* **25**. 301 (1900).

[9] Hüfner, *Arch. f. (Anat. u.) Physiol.* 1894, p. 128.

oxygen) Hüfner makes the two assumptions, that carbonic oxide at a tension of about 500 mm. does not saturate hæmoglobin to more than about 93 per cent, while at a tension of about 700 mm. the saturation becomes complete. This assumption is inadmissible in view of the work of Haldane and Lorrain Smith,[1] who showed that the affinity of carbonic oxide for hæmoglobin is a very powerful one. Haldane continues: "Moreover, as the dissociation-curve of CO-hæmoglobin is undoubtedly a rectangular hyperbola, even if we were to admit that the saturation is a good way from being complete at 500 mm. tension of carbonic oxide, we should also have to admit that at 700 mm. the incompleteness of saturation is nearly as great. Hüfner's second assumption is that the coefficient of absorption of carbonic oxide in a 3 or 4 per cent solution of hæmoglobin, as deduced from his actual experiments, is incorrect, and that a value about 10 per cent lower is correct. The hæmoglobin solutions employed by Hüfner were dilute, and consequently the correction needed on account of the coefficient of absorption of carbonic oxide amounted to as much as 50 per cent of the result. The hypothetical correction introduced thus makes a considerable difference in the value obtained." Haldane believes that the uncertainty of the determinations of the oxygen capacity of hæmoglobin from electro-photometric observations is the cause of the want of agreement between the results Hüfner obtained with the ferricyanide method (see next paragraph) and with the spectro-photometer, though possibly there may also have been an error due to bacterial decomposition of the hæmoglobin.

Determination of the Oxygen Capacity of Blood by Ferricyanide

Haldane[2] was the first to show that the percentage of oxygen capable of being taken up in combination with the hæmoglobin of blood may readily be determined by chemical means, as the combined oxygen is liberated rapidly and completely on the addition of a solution of potassium ferricyanide to laked blood, and that the liberated oxygen may easily be measured with an apparatus similar to that used by Dupré[3] for determining urea in urine. In a second paper Haldane[4] gives a full description of the modified Dupré apparatus used by him in determining the oxygen capacity of blood, and also precise information as to the treatment of the blood. The result obtained by the ferricyanide method represents only the

[1] J. Haldane and Lorrain Smith, *Journ. of Physiol.* **22**. 253 (1898).
[2] John Haldane, *ibid.* **22**. 298 (1898).
[3] Dupré, *Journ. Chem. Soc.* **1**. 534 (1877).
[4] John Haldane, *Journ. of Physiol.* **25**. 295 (1899-1900).

oxygen in combination with hæmoglobin, while that given by the pump includes also the oxygen in simple solution, and Haldane has therefore "deducted from the result by the pump the percentage of oxygen (about 0·63 with blood saturated at 13°) which would be in solution. This amount was calculated on the assumption that the coefficient of absorption of oxygen in blood is $\frac{1}{6}$th less than it is in water; Paul Bert's experiments[1] on the nitrogen and oxygen of the blood of animals in compressed air having shown that this value is probably correct."

The absorption coefficients of blood and of its constituents for gases at 15° and 38°, according to Bohr's[2] quite recent account, are as follows (see the figure on p. 488):—

	Oxygen.		Nitrogen.		Carbon dioxide.	
	15°	38°	15°	38°	15°	38°
Water . .	0·0342	0·0237	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	0·0098	0·825	0·459

Haldane, in addition to the correction for the absorption coefficient, made also a very slight additional correction in his ferricyanide method, on the assumption that any excess of nitrogen over the percentage present in blood saturated with air at the same temperature, was due to the accidental presence of air in the pump. Comparing the results given by the blood-pump and the ferricyanide method for defibrinated and for oxalated ox-blood, they were found to be identical. Thus 100 volumes of blood yielded 22·38 volumes of oxygen by the blood-pump and 22·39 volumes by the ferricyanide method. The best apparatus for estimating the oxygen and the carbonic acid in such small quantities of blood as 1 cc. is that of Haldane and Barcroft,[3] which is also based on the potassium ferricyanide method. On employing Haldane's ferricyanide method,

[1] Paul Bert, *La pression barométrique*, 1878, p. 661. Haldane remarks: "Bert's experiments on blood saturated with compressed air outside the body apparently indicate a much higher coefficient of absorption, but in all probability these experiments were vitiated by the presence in the saturated blood of air-bubbles caused by the shaking."

[2] Christian Bohr, 'Absorptionscoefficienten des Blutes und des Blutplasmas für Gase,' *Skand. Arch. f. Physiol.* **17**. 104 (1905). See also Nagel's *Handbuch der Physiologie des Menschen*, vol. i. p. 54 (1905).

[3] Haldane and Barcroft, *Journ. of Physiol.* **28**. 232 (1902).

v. Zeynek [1] and Hüfner [2] obtained in the best experiments about 13 per cent less oxygen than was present in the oxyhæmoglobin solutions when judged by the spectro-photometer. Apart from the doubts as to the reliability of the data on which Hüfner has based his spectro-photometric calculation (see above p. 482), Haldane points out that the only factors liable to give too low figures by the ferricyanide method are, firstly, incomplete laking of blood, as ferricyanide cannot act on the hæmoglobin in the corpuscles, and, secondly, the presence of bacteria. Müller [3] fully concurs with Haldane that the ferricyanide method gives the same results as does the blood-pump, but draws attention to the fact that bacteria are not the only agents by means of which blood, which has left the arteries, may after a time show a diminution in the amount of removable oxygen, for if the analyses of Bohr,[4] and even those of Hüfner and v. Zeynek, are examined in an unbiassed manner, there is no doubt that many samples of blood, after having been kept for some time aseptically, do not give the maximal oxygen capacity of 1·34 ccm. per gramme of hæmoglobin, and he accounts for the loss of oxygen by assuming that the blood of some individuals contains "easily oxidisable substances, which combine with the loosely bound oxygen of kept blood or with the oxygen which is set free by ferricyanide, while the oxygen is *in statu nascendi.*" He thus explains the differences obtained occasionally on examining kept blood by the ferricyanide method as due to the auto-consumption of the oxygen by the blood. Certain individuals do not seem to possess the radical which absorbs the oxygen, and in this case the ferricyanide gives the same results with freshly drawn and with kept blood, if bacterial action be excluded. The chemical interaction between blood and potassium ferricyanide is discussed on p. 492.

Normal oxyhæmoglobin is very readily dissociated, while hæmoglobin crystals, in the preparation of which alcohol was used, do not dissociate so readily. This was first shown by Loewy,[5] and then confirmed by Hüfner.[6] That the dissociation of oxyhæmoglobin is greater at higher temperatures has been shown by Hüfner,[6] who has also given tables to show the inter-relationship of the oxygen tension and the percentage saturation of hæmoglobin. As his tables are based on calculation, and not on actual observation, they have

[1] v. Zeynek, *Arch. f.* (*Anat. u.*) *Physiol.* 1899, p. 460.

[2] Hüfner, *ibid.* 1899, p. 491.

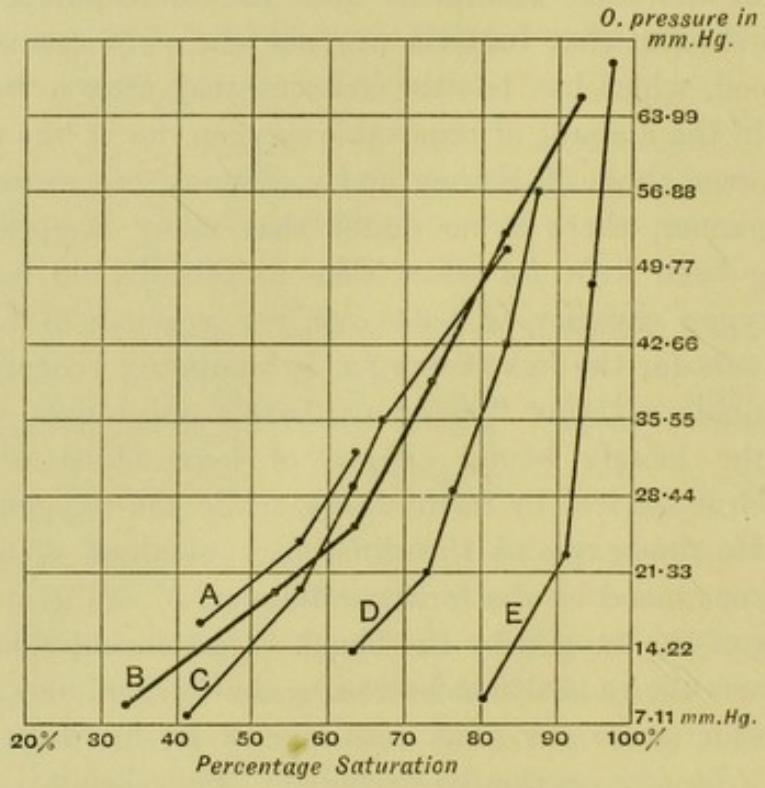
[3] Franz Müller, *Pflüger's Arch.* **103.** 541 (1904).

[4] Bohr, *Skand. Arch.* **3.** 101 (1891).

[5] A. Loewy, *Zentralbl. f. Physiol.* **13.** 449 (1889).

[6] G. Hüfner, *Arch. f.* (*Anat. u.*) *Physiol.* 1890, p. 28; 1901, Suppl. p. 187; *Zeitschr. f. physiol. Chem.* **10.** 218 (1886).

been omitted. Hüfner's tables cannot be used for the direct estimation of the oxygen capacity of blood under varying barometric pressures, according to Schumburg and Zuntz and O. Cohnheim.[1] Loewy and Zuntz[2] showed that the dissociation of oxyhæmoglobin varies according to the amount of dilution of the hæmoglobin, as had previously also been observed by Hüfner and Bohr, and that laked blood behaves differently from normal opaque blood. The more concentrated a hæmoglobin solution the less oxygen does it absorb under equal pressures, and therefore laked blood shows a different behaviour from



A, Loewy and Zuntz (dog's blood); B, Loewy (human blood); C, Paul Bert; D, Hüfner's new curve; E, Hüfner's old curve.

normal blood because the same quantity of hæmoglobin is distributed over, say, twice the volume. Loewy[3] states that this dilution is also the reason why Hüfner's observations, which are based on laked blood, differ from the older observations of Wolffberg and Strassberg made in Pflüger's laboratory on normal blood, for according to Strassberg[4] hæmoglobin is saturated to 60 per cent under an oxygen tension of 25 mm. Hg, while Hüfner found the saturation in his older

[1] Schumburg and N. Zuntz, *Pflüger's Archiv*, **63**. 461 (1896); O. Cohnheim, *Ergebnisse der Physiol.* II. **1**. 625 ff. (1903).

[2] A. Loewy and N. Zuntz, *Arch. f.* (*Anat. u.*) *Physiol.* 1904, p. 166.

[3] A. Loewy, *ibid.* p. 565.

[4] G. Strassberg, *ibid.* **4**. 454, and **6**. 65; Wolffberg, *Pflüger's Arch.* **4**. 465 (1870), and **6**. 23 (1872).

observations to amount to 90 per cent, and in his more recent investigations to 75 per cent. If Hüfner's results were correct, then blood ought to be saturated to the extent of 90 per cent if the O_2-tension in the alveolar air amounts to 30 mm. Hg, but under these conditions animals exhibit distinct indications of want of oxygen. Loewy [1] explains the want of oxygen which manifests itself in heights of 4000-5000 m. as due to a too great dissociation of the oxyhæmoglobin, and also points out that working with normal blood there exist considerable 'individual' differences between the bloods of different persons and dogs as regards oxygen capacities. The mean dissociation-tension of oxyhæmoglobin in man is given in the above Figure, in which Loewy has also included the results obtained by other observers. From the curve of human blood Loewy calculates the following values :—

	Saturation.
Partial pressure of oxygen equals 10	35·77
,, ,, ,, 15	44·52
,, ,, ,, 20	53·36
,, ,, ,, 25	62·40
,, ,, ,, 30	67·29
,, ,, ,, 35	71·09
,, ,, ,, 40	74·51
,, ,, ,, 45	77·81
,, ,, ,, 50	81·11

Or if the partial pressures of oxygen be expressed in percentages of atmospheric pressure :—

	Saturation of oxyhæmoglobin.
Pressure of oxygen corresponding to 2 per cent of an atmosphere	43·19
,, ,, ,, 3 ,, ,,	55·73
,, ,, ,, 4 ,, ,,	65·75
,, ,, ,, 5 ,, ,,	71·20
,, ,, ,, 5·5 ,, ,,	73·72
,, ,, ,, 6 ,, ,,	75·90
,, ,, ,, 7 ,, ,,	80·73

The specific oxygen capacity of blood is defined by Bohr [2] as that amount of oxygen expressed in cubic centimetres at 0° and 760 mm. pressure which is bound by one gramme of iron of the blood at body temperature (37°-38°) under an oxygen tension of 150 mm. or one atmosphere. The most recent determinations on the absorption of gases by the blood made by Krogh,[3] and by Bohr, Hasselbalch, and Krogh,[4] are based on the specific oxygen capacity of blood. For the determination of the amount of iron present in the blood, the amount of iron per 100 grammes of blood was estimated in Bohr's laboratory by

[1] A. Loewy, *Arch. f. (Anat. u.) Physiol.* 1904, p. 233.
[2] Chr. Bohr, *Skand. Arch. f. Physiol.* **3**. 101 (1891).
[3] August Krogh, *ibid.* **16**. 390 (1904).
[4] Chr. Bohr, K. Hasselbalch, and A. Krogh, *ibid.* p. 402.

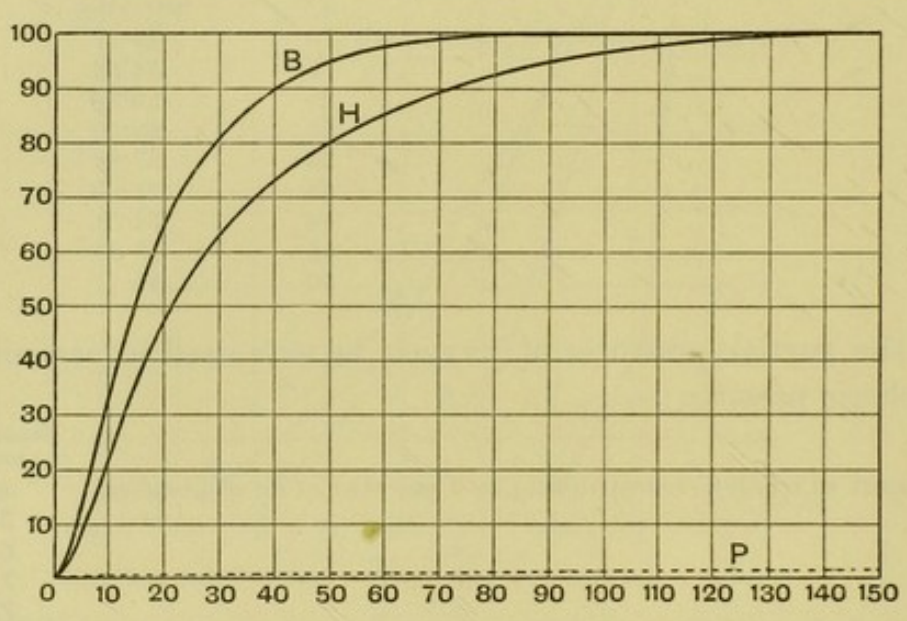
Vilh. Maar, who used A. Neumann's method,[1] and the specific oxygen capacity was then calculated from the amount found. The coagulation of blood and bacterial action were prevented by the addition of 1 : 1000 potassium oxalate or 2 : 1000 sodium fluoride.

Arterial blood taken from the arteria maxillaris externa of the horse while it was feeding showed—

Milligrammes of iron in 100 grammes of blood .	31·75
Specific weight of blood	1·054
Specific oxygen capacity	394·000

Venous blood from the vena maxillaris showed a specific oxygen capacity of 387.

The following Figure represents the differences in the amounts of oxygen which are bound by normal blood, B; a corresponding hæmoglobin solution and blood plasma, P.



Curves representing the oxygen capacity of B, normal blood or hæmochrome; of H, hæmoglobin, and of P, plasma. (Combined from Bohr's Article in *Nagel's Physiology*.)

The CO_2 Capacity of Blood

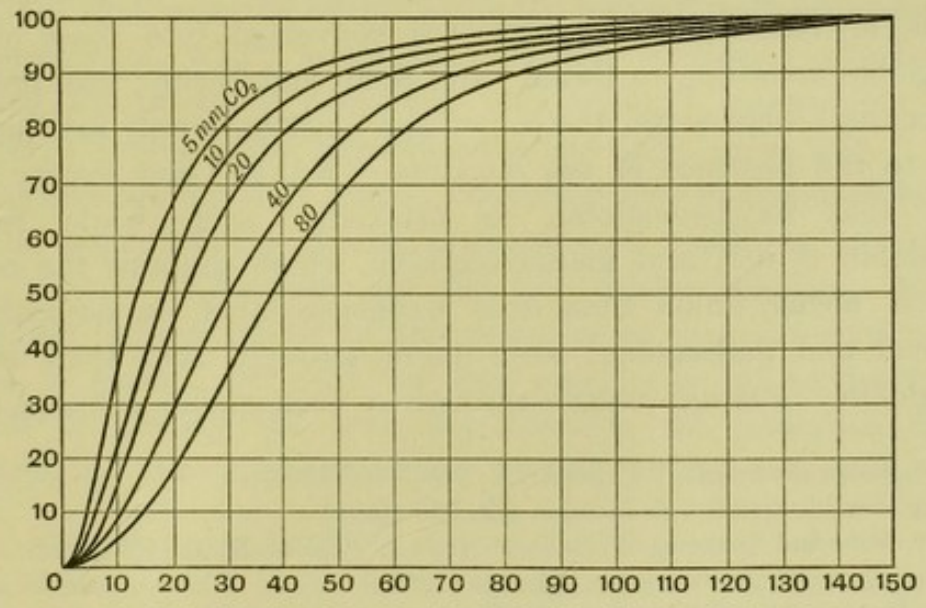
That hæmoglobin combines with carbon dioxide has been known for a long time; but Bohr[2] was the first to point out that CO_2 if under a certain pressure will diminish the oxygen capacity, while the reverse does not hold good. His preliminary observations were, however, inconstant as regards quantitative data, and therefore the whole question was reinvestigated with fresh blood, containing what Bohr calls the genuine blood-colouring matter or hæmochrome.[3] He arrived

[1] A. Neumann, *Zeitschr. f. physiol. Chem.* **37**. 115 (1902).
[2] Chr. Bohr, *Skand. Arch. f. Physiol.* **3**. 47 (1892).
[3] Chr. Bohr, *Zentralbl. f. Physiol.* **17**. 682 (1903-1904).

at the conclusion that CO_2 exerts a strong depressant action on the amount of oxygen absorbed by the blood whenever the oxygen tension is low, while its effect disappears almost completely if oxygen be under the partial pressure of one atmosphere or 150 mm. Hg. In the most recent paper by Bohr, Hasselbalch, and Krogh,[1] the inter-relationship of CO_2 and O_2 for normal dog's blood at 38° is expressed in the following table :—

Oxygen tension.	Percentage absorption of oxygen in the presence of				
	5	10	20	40	80 mm. CO_2.
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
25	76	67	54	40	22
30	82	74·5	63·5	50	31
35	86	79·5	71	58	40
40	89	84	77	66·5	49
45	91	87·5	82	73	56
50	92·5	90	86	78·5	62·5
60	95	93·5	90·5	86	73
70	97	95·5	94	91	80·5
80	98	97	96	94·5	87
90	98·5	98	97	96	91·5
100	99	98·5	98	97	95
150	100	100	100	99·8	99·5

These figures are represented in the following curves :—



There seems to be no interference with the absorption of CO_2 what-

[1] Chr. Bohr, K. Hasselbalch, and A. Krogh, *Skand. Arch.* **16**. 402 (1904).

ever the pressure of the oxygen is, as already pointed out, and this older view of Bohr has been confirmed by his recent investigations.[1]

From a biological point of view the results obtained by Bohr are of great interest, because "even a high CO_2-tension of the blood in the lungs will exert no measurable influence on the absorption of oxygen by the blood on its passage through the lungs, as here the oxygen tension is high; but when the blood reaches the tissues, the oxygen tension becomes reduced, while simultaneously the CO_2-tension is raised, and thereby the giving off of oxygen by the blood will be greatly facilitated, and therefore the amount of oxygen present in the blood will be made use of to a much greater extent than would otherwise be the case. If we assume the oxygen tension of venous blood to be 25 mm. Hg, then on the assumption that CO_2 exerted no influence on the absorption of O_2, there would pass into the plasma only 24 per cent of the amount of O_2 present in the red corpuscles, and only this amount would be available to the tissues. If, however, the CO_2-tension rises simultaneously to 40 or to 80 mm. Hg, then 60 per cent, and in the latter case even 78 per cent of the oxygen, may be given off by the corpuscles, without the tension falling beneath 25 mm. Hg."

At present it is still impossible to make any definite statement as to how oxygen is united to the hæmoglobin molecule, but it is generally assumed that the power of absorbing oxygen is dependent on the iron, or the iron-containing radical hæmatin (p. 508). Oxygen is absorbed normally, only, if the hæmoglobin be in solution, but crystals of reduced hæmoglobin are converted into those of oxyhæmoglobin according to Ewald[2] and Bohr and Torup;[3] as, however, these crystals were moist, the conversion must have only been possible owing to the presence of the moisture. Kühne[4] and Preyer[5] have shown that oxyhæmoglobin is markedly acid, while reduced hæmoglobin is not, and methæmoglobin, which contains the oxygen in much firmer union than does oxyhæmoglobin, is, according to Menzies[6] and Jäderholm,[7] even more strongly acid than is oxyhæmoglobin. Passing oxygen through a hæmoglobin solution makes

[1] Chr. Bohr, *Zentralbl. f. Physiol.* **17**. 713 (1903-1904).

[2] Aug. Ewald, *Zeitschr. f. Biologie*, **22**. 459 (1886).

[3] Chr. Bohr and S. Torup, *Skandinav. Arch. f. Physiol.* **3**. 69 (1891).

[4] W. Kühne, *Virchow's Archiv*, **34**. 423 (1865).

[5] W. Preyer, *Zentralbl. f. d. med. Wissenschaften*, 1867, No. 18; and in *Pflüger's Archiv*, **1**. 395 (1868).

[6] J. A. Menzies, *Journ. of Physiol.* **17**. 402 (1895).

[7] Axel Jäderholm, *Zeitschr. f. Biologie*, **20**. 419 (1884).

the latter more acid according to Kühne; in methæmoglobin, which shows different spectra according as to whether it is in an acid or in an alkaline solution, a current of hydrogen acts like an alkali (? by carrying off CO_2), while oxygen acts like an acid, according to Jäderholm. Mention must also be made of Hoppe-Seyler's[1] observation that oxyhæmoglobin is converted into reduced hæmoglobin on being gently warmed with ammonia. These investigations deserve to be repeated in the light of our present knowledge regarding the migration of radicals in a molecule (see below). In coagulating hæmoglobin by heat and so denaturalising it, the larger amount of oxygen and other gases present remain in the coagulum according to Hermann and Steger;[2] similarly when oxyhæmoglobin is dissociated into globin and hæmatin, oxygen is bound up.

Methæmoglobin

That oxyhæmoglobin readily changes into a new compound of a brownish colour which possesses in addition to the bands of oxyhæmoglobin an additional band in the red end of the spectrum was first observed by Hoppe-Seyler,[3] who called this new substance methæmoglobin. He was of the opinion that it resulted from a partial reduction of oxyhæmoglobin, a view we now know to be wrong. Gamgee[4] then showed that nitrites, including amyl-nitrite, nitroglycerine, etc., were especially apt to give rise to methæmoglobin; and that methæmoglobin holds the oxygen so firmly that the latter cannot be removed by boiling *in vacuo* or by the action of carbonic oxide, but that reducing agents instantly convert methæmoglobin into oxyhæmoglobin, which latter then by the continued action of the reducing substances is converted into reduced hæmoglobin. See, however, Haldane's view regarding the last point, on p. 493.

Oxyhæmoglobin is so readily converted into methæmoglobin, that if it be kept without special precautionary measures having been taken, part of it becomes changed into methæmoglobin. It seems likely that the diminution in the power of oxygen-dissociation which ensues if alcohol be used for the preparation of oxyhæmoglobin crystals is already the first step towards the formation of methæmoglobin; the latter is further formed by the action of a great

[1] F. Hoppe-Seyler, *Zentralbl. f. d. med. Wissenschaften*, 1864, p. 817.

[2] L. Hermann and Th. Steger, *Pflüger's Arch. f. d. ges. Physiol.* **10**. 86 (1875).

[3] F. Hoppe-Seyler, *Zentralbl. f. d. med. Wissenschaften*, 1864, No. 53, and 1865, p. 65. See also *Med. chem. Untersuch. Berlin*, p. 378, and *Zeitschr. f. physiol. Chem.* **2**. 150, 155 (1878).

[4] A. Gamgee, *Philosoph. Trans.* **158**. I. 159 and 589 (1868).

many other reagents. Dittrich[1] has shown that both oxidising substances—ozone, iodine, chlorates, permanganates, nitrates, potassium ferricyanide; and reducing bodies—nascent hydrogen, palladium hydrate, nitrites, pyrogallol, allantoin, hydroquinone, etc.; as also other compounds—anilin, toluidin, acetanilid, acetphenetidin, glycerine, etc., lead to the formation of methæmoglobin. Haldane has shown that the methæmoglobin formed by the action of nitrites on hæmoglobin is mixed with NO-hæmoglobin.[2] The best method for preparing methæmoglobin is to act on oxyhæmoglobin with potassium ferricyanide, according to Hüfner,[3] Külz,[4] Otto,[5] v. Mering,[6] Jäderholm,[7] v. Zeynek,[8] and Hüfner.[9]

Acid-hæmoglobin, which Harnack considers to be a definite substance formed along with methæmoglobin, is discussed on p. 495.

The conversion of oxyhæmoglobin into methæmoglobin may also occur in the living blood,[10] provided that the substances have the power of entering the blood-corpuscles, as can, for example, amyl-nitrite, dinitrobenzene, and antifebrin; the chlorates can enter the corpuscles of the cat, dog, and man, but not those of rabbits and other herbivora (Haldane), and potassium ferricyanide cannot enter at all. Haldane,[11] Makgill, and Mavrogordato have shown that in poisoning with nitrites, dinitrobenzene, etc., which also form methæmoglobin in the blood, death occurs from want of oxygen, exactly as in CO-poisoning. To Haldane's researches into the action of ferricyanides on oxyhæmoglobin attention has already been drawn in connection with the determination of the oxygen capacity of blood (see p. 483). The chemical changes induced by potassium ferricyanide Haldane explains thus: "If ferricyanide be added to diluted blood, it will be found that the solution now gives with ferric chloride a blue colour indicating the presence of ferrocyanide. Evidently, therefore, the ferricyanide is reduced to ferrocyanide. The oxygen rendered avail-

[1] P. Dittrich, *Schmiedeberg's Arch. f. experiment. Path. u. Pharm.* **29**. 247 (1891).

[2] J. Haldane, *Journ. of Physiol.* **21**. 160 (1897).

[3] G. Hüfner, *Zeitschr. f. physiol. Chem.* **8**. 366 (1884).

[4] G. Hüfner and R. Külz, *ibid.* **7**. 366 (1883).

[5] G. Hüfner and J. G. Otto, *ibid.* **7**. 65 (1882); J. G. Otto, *Pflüger's Archiv f. d. ges. Physiol.* **31**. 245 (1883).

[6] v. Mering, *Zeitschr. f. physiol. Chem.* **8**. 186 (1883).

[7] Axel Jäderholm, *Zeitschr. f. Biologie*, **16**. 1 (1880); **20**. 419 (1884).

[8] R. v. Zeynek, *Archiv f. (Anat. und) Physiol.* 1899, p. 460.

[9] G. Hüfner, *ibid.* 1899, p. 491.

[10] v. Mering, *Zeitschr. f. physiol. Chem.* **8**. 186 (1883); P. Dittrich, *loc. cit.*; A. Dennig, *Deutsch. Arch. f. klin. Medizin*, **65**. 524 (1900); according to *Maly's Jahresberichten*, **30**. 169.

[11] J. Haldane, R. H. Makgill, and A. E. Mavrogordato, *Journ. of Physiol.* **21**. 160 (1897).

able in this reaction doubtless passes into the hæmoglobin molecule. The whole process may be provisionally represented by the following equation when ferricyanide is acting on reduced hæmoglobin :—

$$Hb + 4Na_3(Cy_6Fe) + 4NaHCO_3 = HbO_2 + 4Na_4(Cy_6Fe) + 4CO_2 + 2H_2O.$$

In this equation the symbol HbO_2 represents methæmoglobin. When the ferricyanide is acting on oxyhæmoglobin, the following equation represents the supposed process :—

$$Hb\begin{matrix}O\\|\\O\end{matrix} + 4Na_3(Cy_6Fe) + 4NaHCO_3 =$$

$$O_2 + Hb\begin{matrix}O\\\diagdown\diagup\\O\end{matrix} + 4Na_4(Cy_6Fe) + 4CO_2 + 2H_2O.$$

In this case $Hb\begin{matrix}O\\|\\O\end{matrix}$ represents oxyhæmoglobin, and $Hb\begin{matrix}O\\\diagdown\diagup\\O\end{matrix}$ methæmoglobin. The reason for employing these different symbols is that there are some grounds for believing that in oxyhæmoglobin the oxygen atoms are united together, whereas in methæmoglobin this is probably not the case (see also p. 483).

The conversion of oxyhæmoglobin into methæmoglobin by indifferent media or simply by the 'time-factor' might be explained through the migration of hydrogen atoms, analogous to the transition of a ketone into an enole modification, as described by Brühl.[1] The increasedly acid character of methæmoglobin agrees well with this conception, as pointed out by v. Zeynek.

By reducing agents, and especially by ammonium sulphide and Stokes' reagent, methæmoglobin is converted far more readily than is oxyhæmoglobin [2] into reduced hæmoglobin, and the latter is changed instantly into oxyhæmoglobin, provided free oxygen is present, but not otherwise, as Haldane has shown.[3]

The peculiar action of another reducing agent, namely, nitric oxide, on methæmoglobin has been studied by Hüfner and Külz,[4] who found that, although methæmoglobin does not yield oxygen to the blood-pump (Gamgee), it parts with exactly the same amount of oxygen as does oxyhæmoglobin when subjected to NO, and that therefore both methæmoglobin and oxyhæmoglobin must contain the same amount of readily dissociable oxygen.

[1] J. W. Brühl, *Zeitschr. f. physiol. Chem.* **30**. 1 (1899).
[2] J. Haldane, *Journal of Physiol.* **22**. 298 (1898).
[3] J. Haldane, *ibid.* **22**. 302 (1898).
[4] Hüfner and Külz, *Zeit. f. physiol. Chem.* **7**. 366 (1883).

Bohr[1] distinguishes several modifications of hæmoglobin, which differ from one another in their oxygen-capacity, and has called these modifications α-, β-, γ-, δ-hæmoglobin. He has noticed similar differences also in living blood, but Hüfner[2] has suggested that Bohr's observations depend on a partial conversion of hæmoglobin into methæmoglobin or similar compounds. Marchand[3] has likewise described phenomena which speak for a gradual conversion into methæmoglobin.

Methæmoglobin, either as solid or in acid or neutral solutions, is not red as is oxyhæmoglobin, but brown, like English porter; in alkaline solutions it is, however, red. Hüfner[4] was the first to prepare it in a pure crystalline form from dog's, pig's, and horse's blood, after Gamgee had already succeeded in 1868 in obtaining crystals in combination with nitrites. Pure crystals resemble grey-brown, doe-coloured needles with a peculiar silky lustre. The method used for preparing methæmoglobin crystals is the same as that used for oxyhæmoglobin, after the latter has been converted into methæmoglobin by a little potassium ferricyanide. It has the same composition as oxyhæmoglobin, see p. 493. 100 ccm. water dissolve 5,851 grm. at 0°; and much larger quantities at higher temperatures.

Methæmoglobin possesses in acid and in alkaline solutions different spectra, which have been investigated most carefully by Jäderholm,[5] Araki,[6] Gamgee,[7] and Haldane. In acid solutions it possesses two bands; the first very marked band in the red-orange, between C and D and close to C, with its greatest intensity, according to

Gamgee, ...	$\lambda 633$—$\lambda 623$,
Araki, ...	$\lambda 648$—$\lambda 629$,
Dittrich,[8]	$\lambda 632$.

A feebler band—which, examined by the spectro-photometric method of Hüfner, is, however, as intense as the other—lies in the bright blue region of the spectrum between G and F, close to F:

$$\lambda 500—\lambda 495.$$

If methæmoglobin is not in acid solution, or not quite free from

[1] Chr. Bohr and Sophus Torup, *Skand. Arch. f. Phys.* **3**. 69 (1891); Chr. Bohr, *ibid.* **3**. 76, 101 (1891); Fr. Tobiesen, *ibid.* **6**. 273 (1895); Chr. Bohr, *Zentralbl. f. Physiol.* **4**. 249 (1890). [2] G. Hüfner, *Arch. f. (Anat. u.) Physiol.* 1894, p. 130.

[3] F. Marchand, *Virchow's Arch.* **77**. 488 (1879).

[4] G. Hüfner and J. G. Otto, *Zeitschr. f. physiol. Chem.* **7**. 65 (1882); G. Hüfner, *ibid.* **8**. 366 (1884); G. Hüfner, *Arch. f. (Anat. u.) Physiol.* 1899, p. 491; R. v. Zeynek, *ibid.* 1899, p. 460.

[5] Axel Jäderholm, *Zeitschr. f. Biolog.* **16**. 1 (1880); **20**. 419 (1884).

[6] Tr. Araki, *Zeitschr. f. physiol. Chem.* **14**. 405 (1890).

[7] A. Gamgee, *Zeitschr. f. Biolog.* **34**. 505 (1896).

[8] Paul Dittrich, *Schmiedeberg's Arch. f. experim. Pathol. u. Pharmak.* **29**. 247 (1891).

oxyhæmoglobin, it will also show the two absorption bands of the latter lying between D and E (see p. 480). (Ray-Lankester,[1] Araki, and Menzies.[2]) In the extreme violet Gamgee has described a band identical with that found in oxyhæmoglobin, and therefore probably also due to oxyhæmoglobin. According to Gamgee, the band in the violet lies between h and L. In greatly diluted solutions it is restricted to between K and H, in stronger solution it extends to M, and in very strong ones into the ultraviolet.

Alkaline methæmoglobin possesses, according to Jäderholm, three bands, two on either side of the D-line, which frequently become confluent, and a third at E. Their respective centres lie at

$$\lambda 602\,; \qquad \lambda 578\,; \qquad \lambda 539.$$

In solutions so strong that the two first bands become confluent, an absorption-band is seen on the violet side of the D-line, which crosses the D-line and extends up to the red.[3] This band and that at E correspond again with those found in oxyhæmoglobin. v. Zeynek has examined alkaline methæmoglobin solutions spectro-photometrically; the absorption-quotient measured at the same place as in oxyhæmoglobin is 1·185; the absorptive power of methæmoglobin is therefore considerably less than that of oxyhæmoglobin, a factor which may have to be taken into account under certain conditions.

Haldane has pointed out to the author that neutral methæmoglobin solutions really consist of a mixture of alkaline and of acid methæmoglobins, for on faintly acidifying a neutral solution by shaking with expired air, containing about 4 per cent of CO_2, the band of alkaline hæmatin is wiped out, unless oxy- or nitric-oxide-hæmoglobin be present.[4] Haldane has also observed that a methæmoglobin solution made by diluting blood with water, if subjected to the vacuum, shows, as soon as the CO_2 has been boiled out, the spectrum of alkaline methæmoglobin. If air is allowed to come into contact with this methæmoglobin solution it is reconverted into neutral methæmoglobin.

Photo-methæmoglobin is discussed under cyan-methæmoglobin.

[*Acid-hæmoglobin*]

Hæmoglobin is dissociated by stronger acids in a short time into hæmatin and globin, but if feeble organic acids or exceedingly dilute

[1] Ray-Lankester, *Quarterly Journ. of Mic. Sc.* **10**., N.S., 402 (1870).

[2] E. A. Menzies, *Journ. of Physiol.* **17**. 402 (1895).

[3] E. Ziemke and Franz Müller, *Arch. f.* (*Anat. u.*) *Physiol.* 1901, Suppl. p. 177.

[4] In this way oxyhæmoglobin may readily be demonstrated.

mineral acids are allowed to act for a limited time on hæmoglobin there is formed acid-hæmoglobin, according to Harnack (see, however, below). Stokes and Hoppe-Seyler,[1] Preyer[2] and Strassburg,[3] have considered Harnack's acid-hæmoglobin[4] to be methæmoglobin, and have supposed dilute acids to act in the same way as do potassium ferricyanide, amylnitrite, etc.

Acid-hæmoglobin, according to Harnack, is brown, like methæmoglobin, and resembles the latter also greatly in its absorption-spectrum, but the band in the red lies more towards the red end, namely, on either side of the C-line, while the methæmoglobin band only reaches up to C; the band of acid-hæmatin, which also lies in the red, is still more to the red than is the acid-hæmoglobin band. Strassburg has pointed out that the oxygen capacity of hæmoglobin is inversely proportional to the amount of acid which is added to the hæmoglobin; the greater the amount of acid, the less oxygen is taken up, and Ham and Balean,[5] who have made a very careful study of the effect produced on hæmoglobin by the addition of different strengths of acid, as already stated on p. 474, have observed that a strength of acid can be found which only displaces the carbon-dioxide, leaving oxyhæmoglobin in solution. If, however, the acid is increased in strength, a small quantity of oxygen is liberated, and on spectroscopic examination there is seen a faint band in the red, placed between the position of the methæmoglobin and acid-hæmatin bands, but more nearly approaching that of acid-hæmatin; there are also present the bands of oxyhæmoglobin. If now ammonium sulphide be added, the band in the red disappears and those of oxyhæmoglobin give place to the broad band of reduced hæmoglobin, and at the moment of adding the acid, the dark band of hæmochromogen (*i.e.* reduced hæmatin) appears along with the broader band of reduced hæmoglobin, but the hæmochromogen band rapidly fades, leaving only the bands of reduced hæmoglobin. Ham and Balean believe this to be conclusive proof that on the addition of weak acids to oxyhæmoglobin there results a mixture of oxyhæmoglobin and acid-hæmatin, while if sufficient acid be added to replace one-half of the replaceable oxygen, only one strong band, characteristic of acid-hæmatin, is found.

The author is therefore inclined to regard Harnack's acid-hæmoglobin as simply a mixture of oxyhæmoglobin and acid-hæmatin.

[1] F. Hoppe-Seyler, *Virchow's Arch.* **29**. 233 (1864); *Zentralbl. f. d. mediz. Wissensch.* 1865, p. 65.

[2] W. Preyer, *Pflüger's Arch. f. d. ges. Physiol.* **1**. 395 (1868); and in *Blutkristalle*, Jena, 1871. [3] G. Strassburg, *Pflüger's Arch. f. d. ges. Phys.* **4**. 454 (1871).

[4] E. Harnack, *Zeitschr. f. physiol. Chem.* **26**. 558 (1899).

[5] C. E. Ham and H. Balean, *Journ. of Physiol.* **32**. 312 (1905).

The fact that 'acid-hæmatin,' or as Cohnheim puts it, 'acid-hæmoglobin,' is also formed by the prolonged action of CO_2 is of especial biological interest in connection with Bohr's recent work on the dissociation of oxyhæmoglobin by CO_2, as explained on p. 490.

Kathæmoglobin

Kathæmoglobin is a compound which v. Klaveren[1] believes to have prepared by the action of alkalies on hæmoglobin. It is intermediate between hæmoglobin and hæmatin, judged by its spectrum, and is probably a mixture of alkaline hæmatin and oxyhæmoglobin. (The author.)

Carbonic Oxide Hæmoglobin

Lothar Meyer in 1858 was the first to observe that oxygen is replaced in hæmoglobin by an equal volume of CO. The first full account is given by Hoppe-Seyler[2] in 1864. CO-hæmoglobin differs from oxyhæmoglobin in possessing a pinkish colour; the foam is violet. The crystals are isomorphic with those of oxyhæmoglobin, but are darker and of a more bluish tint. According to Ewald,[3] their pleochroism is not strong but very pretty, as with altered nicols the colour changes from a purple red to nearly white. Its absorption-bands are very similar to those of oxyhæmoglobin, but displaced somewhat nearer to D; the second band is also less intense; measured at the same place as oxyhæmoglobin, the quotient of the absorption-coefficient is 1·13, according to Hüfner and Külz.[4] No differences were observed between solutions of CO-hæmoglobin and fresh blood containing CO; neither was there any difference between the blood of different animals. According to Gamgee, a band lies in the violet between h and G; it is narrower than is the corresponding oxyhæmoglobin band and placed more towards the red end. Its centre lies at λ420·5.

Bock[5] showed that the dissociation-curve of CO-hæmoglobin rises very sharply up to a tension of about 0·5 mm., and afterwards very slowly. Hüfner finds, with a tension of 0·5 mm. carbonic oxide, that a hæmoglobin-solution becomes saturated to 87 per cent at 31°. Haldane and Lorrain Smith[6] found that at 15° and a tension of

[1] K. H. L. v. Klaveren, *Zeitschr. f. physiol. Chem.* **33.** 293 (1901).

[2] F. Hoppe-Seyler, *Zentralbl. f. d. med. Wissensch.* 1864, p. 52; *Med.-chem. Untersuchungen*, p. 169 (1867).

[3] A. Ewald, *Zeitschr. f. Biolog.* **22.** 459 (1886).

[4] R. Külz, *Zeitschr. f. physiol. Chem.* **7.** 384 (1883).

[5] Joh. Bock, *Zentralbl. f. Physiol.* **8.** 385 (1894).

[6] J. Haldane and J. Lorrain Smith, *Journ. of Physiol.* **22.** 253 (1897-98).

CO of ·005 per cent of an atmosphere, or 0·035 mm. of Hg, a dilute hæmoglobin solution became 98° per cent saturated with CO, in the absence of oxygen. See further p. 500.

Haldane also showed that the relative saturating powers of carbonic oxide and oxygen are not altered by an increase of the temperature or increased saturation, while the absolute saturating power of both carbonic oxide and of oxygen are diminished by a rise of temperature.

The most characteristic property of the CO-hæmoglobin is its great firmness, as the CO is only given off with great difficulty to the blood-pump. Hüfner and his pupils[1] have repeatedly made use of this property for estimating the volume of the gas united to hæmoglobin, and Hüfner's final estimate of the molecular weight of hæmoglobin has been based on CO-hæmoglobin.

The great firmness of the CO-hæmoglobin is the reason why feeble concentrations of CO are able to displace the oxygen of the hæmoglobin and why carbonic oxide is so poisonous. This gas, by uniting with the hæmoglobin, thereby prevents the taking in of oxygen. Bock,[2] Hüfner,[3] and also Haldane have investigated the avidities of oxygen and carbon monoxide for hæmoglobin, one of the most interesting examples of chemical equilibrium. Haldane[4] has shown that symptoms of CO-poisoning do not manifest themselves, as long as the body is at rest, till the CO, in otherwise normal air, amounts to about ·05 per cent, while urgent symptoms are produced with amounts of 0·2 per cent of CO. In fatal cases of CO-poisoning,[5] the blood is usually about 80 per cent saturated with CO. In recovery from CO-poisoning the CO is driven off, fairly rapidly, through the lungs—none of it is oxidised. The supposed oxidation of carbonic oxide in the living body has been discussed by Haldane.[6]

Identification by the spectroscope is not easy, as the absorption-bands closely resemble those of oxyhæmoglobin, but two methods allow of its ready recognition, namely: firstly, the addition of ammonium sulphide or Stokes' reagent, which produces no change, while in the case of oxyhæmoglobin they convert the latter into reduced hæmoglobin

[1] John Marshall, *Zeitschr. f. physiol. Chem.* **7**. 81 (1882); R. Külz, *ibid.* **7**. 384 (1883); G. Hüfner, *Arch. f. (Anat. u.) Physiol.* 1894, p. 130.

[2] Joh. Bock, *Zentralbl. f. Physiol.* **8**. 385 (1894).

[3] G. Hüfner, *Arch. f. (Anat. u.) Physiol.* 1895, p. 213; *Arch. f. experim. Path. u. Pharm.* **48**. 87 (1902).

[4] J. Haldane, *Journ. of Physiol.* **18**. 430 (1895); see also the older papers by G. Hüfner, *Arch. f. (Anat. u.) Physiol.* 1895, p. 213; H. Dreser, *Schmiedeberg's Arch. f. experim. Path. u. Pharm.* **29**. 110 (1891); F. Hoppe-Seyler, *Zentralbl. f. d. med. Wissensch.* 1865, p. 52; *Zeitschr. f. physiol. Chem.* **1**. 121 (1877).

[5] J. Haldane, *Journ. of Physiol.* **18**. 430 (1895).

[6] J. Haldane, *ibid.* **25**. 225 (1899-1900).

with its characteristic spectrum, as pointed out by Hoppe-Seyler;[1] and secondly, dilution of the suspected blood with water, as recommended by Haldane. Vogel[2] was the first to use very dilute hæmoglobin solutions as a qualitative test for carbonic oxide in air. He shook a small quantity of very dilute blood in a small bottle filled with the air, and subsequently added ammonium sulphide. If the two bands of oxyhæmoglobin were still present, then CO-hæmoglobin had been formed. By this means he detected 0·25 per cent of carbonic oxide. Hempel,[3] by bubbling a large volume of air through a small quantity of blood solution, detected up to 0·06 per cent of CO in air. Haldane's[4] method allows of detecting and estimating as little as ·01 per cent of carbonic oxide, or 0·2 per cent of coal-gas:[5] A clean and dry bottle of 100 to 200 cc. capacity has sucked through it 2 to 3 litres of the suspected air; the bottle is closed by a doubly perforated cork, soaked in paraffin and fitting hermetically, and passing through the cork are two pieces of glass tubing, each with a short piece of india-rubber tubing, with a glass rod at the free end to act as a stopper. For the determination of the CO in the air, dilute about 5 cc. of a solution of defibrinated ox-blood to 200 with water, and introduce the diluted blood into the bottle, taking the following precautions: Remove one of the glass stoppers from the india-rubber tubing, pinching the latter all the time; now insert the pipette with the blood solution into the india-rubber tubing and allow the blood to flow into the bottle by removing the glass rod from the other piece of india-rubber tubing, as hereby sufficient air is allowed to escape to diminish the pressure inside the bottle. After the blood has flowed into the bottle, replace both glass rods in the india-rubber tubing, and shake the bottle fairly vigorously for twenty minutes.[6] A standard solution of carmine is then used for titration. It is made by diluting a stronger ammoniacal solution of carmine until the tint is such that when the solution is mixed with an empirically found proportion of the ·5 per cent blood solution the tint of the mixture exactly resembles, both as regards quality and intensity, a solution of similarly diluted blood when saturated with carbonic oxide or coal-gas. The titration is performed by daylight in two test-tubes of equal diameter, and about 6 cc. of the standard carmine solution are required for 5 cc. of

[1] F. Hoppe-Seyler, *Zentralbl. f. d. med. Wissensch.* 1865, p. 52; *Zeitschr. f. physiol. Chem.* **1**. 121 (1877).

[2] Vogel, *Ber. d. deutsch. chem. Ges.* **10**. 792 (1877); **11**. 235 (1878).

[3] Hempel, *Zeitsch. f. analyt. Chem.* **18**. 402 (1879).

[4] J. Haldane, *Journ. of Physiol.* **18**. 464 (1895).

[5] The latter contains on an average 5 per cent of CO, according to Haldane.

[6] J. Haldane and J. Lorrain Smith, *Journ. of Physiol.* **22**. 233 (1897).

blood solution, the exact proportion depending on the quality of the daylight at the time. The diluted carmine solution should be prepared fresh when required, as its colour distinctly loses its strength when kept for a few days.

From the results of the titration with carmine solution the percentage saturation of the hæmoglobin with carbonic oxide may be easily calculated in the manner illustrated by the following example: To 5 cc. of blood solution 6·2 cc. of standard carmine require to be added to produce the saturation tint. To 5 cc. of blood solution 2·2 cc. of carmine require to be added to produce the tint of blood solution shaken with the air under examination. In the former case the carmine was in the proportion of 6·2 to 11·2; in the latter case in the proportion of 2·2 to 7·2. The percentage saturation of the hæmoglobin in the blood shaken with the air was thus:

$$\frac{2{\cdot}2}{7{\cdot}2} \times \frac{11{\cdot}2}{6{\cdot}2} \times 100 = 55{\cdot}2.$$

With 0·07 per cent CO in air, hæmoglobin becomes half saturated with CO. According to this result, the avidity of CO for hæmoglobin is 300 times greater than that of O_2. Haldane, in some as yet unpublished investigations, has found that with more prolonged shaking blood or blood solution becomes half saturated with only 0·055 per cent of CO, which shows that the affinity of CO for hæmoglobin under perfectly normal conditions is about 380 times greater than that of O_2. The dissociation-curve, according to Haldane's present views, is given on the opposite page.

Carboxyhæmoglobin differs from oxyhæmoglobin also as regards the ease with which it is converted into methæmoglobin. It is not at all acted upon by hydroquinone and pyrocatechin, according to Weyl and Anrep,[1] while oxyhæmoglobin is rapidly converted by these reagents into methæmoglobin; iodine-potassium iodide takes four days and potassium permanganate twenty-four hours to form methæmoglobin. With a number of precipitating reagents, such as caustic soda, caustic soda and calcium chloride, tannic acid or ferrocyanic acid, CO-hæmoglobin preserves its beautiful colour for a long time, while ordinary hæmoglobin is rapidly changed into a dirty brown or green colour. (Hoppe-Seyler, Salkowski,[2] and Wahl.[3]) The same is also the case with sulphuretted hydrogen, which, according to Salkowski,[4] rapidly decomposes oxyhæmoglobin, while carboxyhæmoglobin and

[1] T. Weyl and B. v. Anrep, *Arch. f.* (*Anat. u.*) *Physiol.* 1880, p. 227.

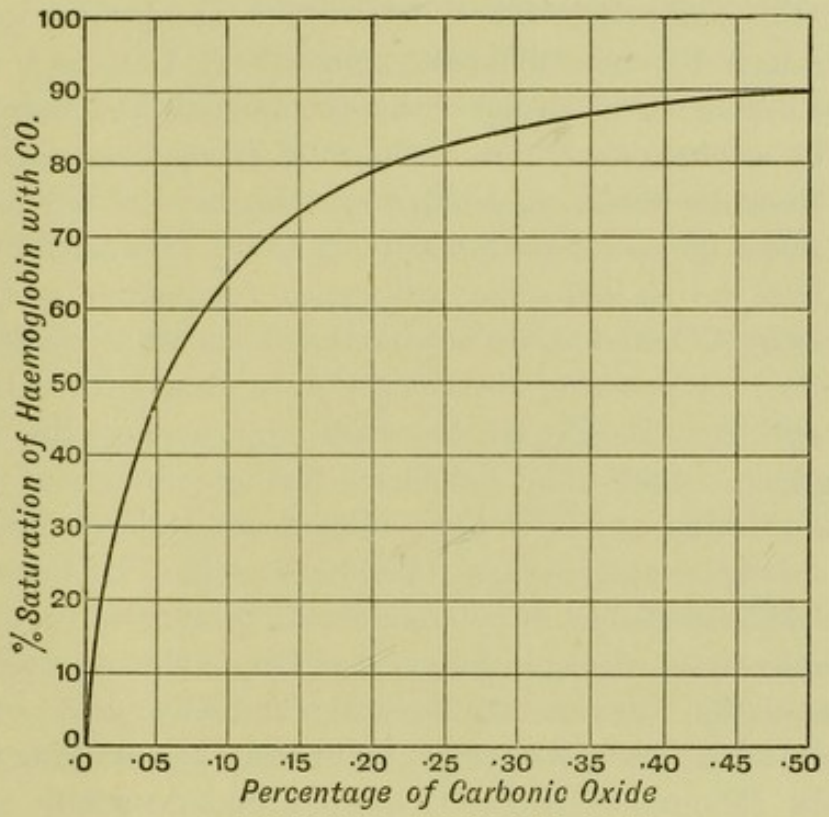
[2] E. Salkowski, *Zeitschr. f. physiol. Chemie*, **12**. 227 (1887).

[3] F. Wahl, *Pflüger's Arch. f. d. ges. Physiol.* **78**. 262 (1900).

[4] E. Salkowski, *Zeitschr. f. physiol. Chem.* **7**. 114 (1882); **27**. 319 (1889).

reduced hæmoglobin may preserve their colour and absorption-bands indefinitely. Haldane and Lorrain Smith[1] state that carboxyhæmoglobin may be recognised in dilutions of 1 : 1,000,000, if oxygen be absent and the effect of CO on hæmoglobin be thereby increased.

A CO-hæmoglobin compound analogous to methæmoglobin does not exist.[2]



CO-hæmochromogen

Pregel[3] has shown that hæmochromogen binds O_2 more firmly than CO, and in this it differs from hæmoglobin. Acethæmatin prepared by Schalfejeff's method[4] and recrystallised with quinine, according to Kuster,[5] is reduced with hydrazinhydrate in a special apparatus which prevents the access of air ; is saturated with CO,[6] and precipitated with a saturated solution of sodium chloride, saturated with CO.

CO-hæmochromogen resembles CO-hæmoglobin by being decomposed with potassium ferricyanide. Each atom of Fe binds 1 atom of CO.

CO-hæmochromogen contains for 1 Fe not 4 but 5 N, because of

[1] J. Haldane and J. Lorrain Smith, *Journal of Physiol.* **22.** 253 (1897-98).

[2] H. Bertin-Sans and J. Moitessier, *ibid.* **113.** 210 (according to *Maly's Jahresber. f. Tierchem.* **22.** 90), (1892).

[3] Fritz Pregel, *Zeitschr. f. physiol. Chem.* **44.** 173 (1905).

[4] Schalfejeff, *Ber. d. deutsch. chem. Ges.* **18.** abstract 232 (1885), also *Zeits. f. phys. Chem.* **30.** 390 (1900).

[5] Kuster, *Zeits. f. phys. Chem.* **30.** 391 (1900).

[6] Prepared from oxalic acid and concentrated H_2SO_4 and preserved over caustic potash solution.

a salt-like combination formed between the blood pigment and the dilute ammonia, in which hæmatin was dissolved before being reduced with hydrazin. The same observation was first made by Zeynek.[1]

Carbohæmoglobin or CO_2 Hb

Under this name Bohr [2] has described a number of loose compounds formed between different amounts of CO_2 and a given quantity of hæmoglobin. None of these compounds has, however, been prepared in a pure state. According to Torup,[3] carbohæmoglobin shows an absorption-band at λ553, while the band of reduced hæmoglobin possesses its maximum intensity at λ559. Cohnheim says that CO_2Hb cannot be classed along with such compounds as COHb or NOHb, because CO_2 and O_2 do not mutually replace one another (see below), and as the power of absorbing CO_2 is even greater than that of uniting with O_2. The CO_2Hb dissociates even more readily than does oxyhæmoglobin. Bock [4] and Bohr have further pointed out that both COHb and MetHb can bind CO_2. Cohnheim believes that hæmoglobin is partly converted into 'acid-hæmoglobin' by the action of CO_2, but acid-hæmoglobin, as pointed out on p. 496, being in all probability acid-hæmatin, it would follow that CO_2 is able to cause a temporary dissociation between the hæmatin and the globin radicals, a point of great interest in connection with the CO_2 carrying-power of hæmoglobin (Mann). That CO_2 and O_2 under certain conditions do react upon one another has been shown by Bohr and his pupils, see p. 489.

Nitric Oxide-hæmoglobin

Hæmoglobin forms also a compound with one molecule of nitric oxide, NO, as first described by Hermann.[5] The affinity of NO for hæmoglobin is even greater than that of CO, and therefore CO is driven out of its combination with hæmoglobin by NO, a fact which Hüfner, Külz, and Marshall have made use of in determining the amount of CO in combination with hæmoglobin.

Kisskalt [6] first noticed that meat becomes red when it is boiled in water containing a nitrite, and suggested that the red colour of salted

[1] Zeynek, *Zeitschr. f. physiol. Chem.* **25.** 492.

[2] Chr. Bohr, *Festschrift für Ludwig*, p. 164 (according to *Maly's Jahresber. f. Tierchem.* **17.** 115) (1887); *Skandinav. Arch. f. Physiol.* **3.** 47 (1891); **8.** 161 (1898).

[3] Sophus Torup, *Maly's Jahresber. f. Tierchem.* **17.** 115 (1887).

[4] Joh. Bock, *Scandinav. Arch. f. Physiol.* **8.** 363 (1898).

[5] L. Hermann, *Arch. f. (Anat. u.) Physiol.* 1865, p. 469.

[6] Kisskalt, *Arch. f. Hygiene*, **35.** 11 (1899).

meat might be due to the presence of nitrite, and Kobert[1] attributed the red colour to a union of nitrites with methæmoglobin. Haldane[2] then showed that the colour of uncooked red meat is simply due to pure NO-hæmoglobin, while in boiled meat it is due to NO-hæmochromogen. Nitric oxide first reduces hæmatin to hæmochromogen, and then combines with the latter. Nitric oxide-hæmochromogen was first prepared by Linossier[3] by bubbling nitric oxide through hæmatin solutions. According to Haldane, NO acts on oxyhæmoglobin, reduced hæmoglobin, and methæmoglobin in this way:

(1) Hæmoglobin readily combines either with nitric oxide to form NO-hæmoglobin or with nascent oxygen to form methæmoglobin.

(2) Nitrous acid easily yields up simultaneously nitric oxide and oxygen.

(3) Nitric oxide readily combines with either oxygen or hæmoglobin.

If no extraneous oxygen is present, as when reduced hæmoglobin is acted on by the nitrite, both the nitric oxide and the oxygen yielded up by the nitrite will combine with the hæmoglobin; and as far more nitric oxide than oxygen is yielded up, more NO-hæmoglobin than methæmoglobin will be formed. On the other hand, when oxyhæmoglobin or methæmoglobin is acted on, the nitric oxide will be free either to combine with hæmoglobin or with the extra oxygen available, and a balance will be struck depending on the relative strengths of the various affinities. The conversion of nitrates into nitrites is brought about by the tissues themselves, as they contain a reducing substance which acts even in the presence of antiseptics.

NO-hæmoglobin forms crystals which are isomorphic with those of oxyhæmoglobin; its solutions are bright red and do not exhibit dichroism, according to Hermann. NO-hæmoglobin shows two absorption-bands which are less defined than are those of oxyhæmoglobin, and the band near D extends beyond D towards the red;[4] the absorption-band in the violet is the same as in the case of CO-hæmoglobin, according to Gamgee. NO-hæmoglobin is as difficult to reduce as is CO-hæmoglobin.

Haldane, Makgill, and Mavrogordato[5] have shown the poisonous action of nitrites to be due to their action on the hæmoglobin, and the

[1] R. Kobert, *Pflüger's Arch.* **82**. 603 (1900).

[2] Haldane, *Journ. of Hygiene*, **1**. 115 (1901).

[3] Linossier, *Compt. Rend.* **104**. 1296 (1887).

[4] Haldane, *Journ. of Hygiene*, **1**. 115 (1901). See also R. Kobert, *Pflüger's Arch.* **82**. 603 (1900).

[5] J. Haldane, R. H. Makgill, and A. E. Mavrogordato, *Journ. of Physiol.* **21** 160 (1897).

consequent paralysis of the oxygen-carrying power of the blood. In compressed oxygen this action is abolished, and with very large doses nitrites then act as direct poisons to the tissues. Nitroglycerine, nitrobenzene, and hydroxylamin act in mice and rabbits as direct tissue poisons before producing symptoms due to the decomposition of the hæmoglobin or to nitrite formation. Dinitrobenzene decomposes the hæmoglobin into a product which is incapable of carrying oxygen.

Sulph-hæmoglobin

Hoppe-Seyler[1] first observed that by the action of H_2S on oxyhæmoglobin the hæmoglobin molecule is destroyed, there being formed a greenish compound which Araki[2] has called sulpho-methæmoglobin.

He also described a true sulph-hæmoglobin with higher sulphur-contents and with an absorption-band in the red,[3] but the real explanation we owe to Harnack.[4] By the action of H_2S on reduced hæmoglobin, sulph-hæmoglobin is said to be formed, but it has not as yet been prepared in a pure state. It exhibits the absorption-band of reduced hæmoglobin in the green, but in addition a distinct band in the orange-red, between C and D, but nearer to C, without, however, reaching this line; it lies therefore considerably more towards the violet end than do the bands of methæmoglobin or of hæmatin. On converting hæmoglobin into sulph-hæmoglobin the solution becomes of a darker red. Harnack proved that we are really dealing with a compound of hæmoglobin with H_2S, and that neither 'acid-hæmoglobin' (see p. 495) nor methæmoglobin give rise to sulph-hæmoglobin. Whether hæmoglobin can be regenerated from its H_2S compound could not be determined, but is probable. If H_2S acts on oxyhæmoglobin or on hæmoglobin in the presence of air, there is also formed at first sulph-hæmoglobin, but this is succeeded by a complete decomposition of the hæmoglobin, leading to complete disappearance of all typical absorption-bands of hæmoglobin. Araki failed also in obtaining normal hæmatin. The decomposed solution is of a dirty greenish colour, but the latter does not depend on any definite coloured substance. It has been suggested that the decomposition is due to rapidly alternating processes of oxidation and of reduction, owing to the combined action of O_2 and H_2S. (Cohnheim.)

[1] F. Hoppe-Seyler, *Zentralbl. f. d. med. Wissensch.* 1863, Nr. 28.
[2] Trasaburo Araki, *Zeitschr. f. physiol. Chem.* **14**. 405 (1890).
[3] F. Hoppe-Seyler, *Med.-chem. Untersuchungen*, p. 151 (1866).
[4] E. Harnack, *Zeitschr. f. physiol. Chem.* **26**. 558 (1899).

Cyan-methæmoglobin or CNHb

Kobert[1] showed that a substance which he calls cyan-methæmoglobin is formed by acting with hydrocyanic acid or one of its salts on a solution of methæmoglobin. In doing so the brown solution of methæmoglobin is changed into a bright-red colour with a distinct yellow tinge specially well seen in thin layers.

Haldane[2] has shown that cyan-methæmoglobin is identical with Bock's[3] photo-methæmoglobin. The formation of the latter is not directly due to the action of light on methæmoglobin but to the action on methæmoglobin of hydrocyanic acid liberated in consequence of the decomposition, by the action of light, of ferricyanide present in the methæmoglobin, for photo-methæmoglobin is only obtained from methæmoglobin in the preparation of which ferricyanide has been used, as, notwithstanding repeated crystallisation, ferricyanide cannot be got rid off. The HCN liberated by the action of light acts on methæmoglobin. It does not displace the oxygen of the latter but becomes linked on to some other radical of the hæmoglobin or hæmochromogen molecule. When the oxygen is taken out of the cyan-methæmoglobin molecule, the CN is eliminated also, but not so if the oxygen is taken out of the cyanhæmatin molecule. If blood solution, to which ferricyanide has been added, be allowed to stand for two or three days, cyan-methæmoglobin is formed in consequence, perhaps, of putrefactive changes leading to decomposition of the ferricyanide. Amylnitrite added in excess may also give rise to the CN-hæmoglobin spectrum on account, doubtless, of the presence of traces of HCN in the reagent.

Hoppe-Seyler's cyanhæmatin is not identical with cyan-methæmoglobin, as asserted by Szigeti.[4]

Cyanhæmatin, according to Haldane, is formed by adding an excess of cyanide to an alkaline solution of hæmatin. Its spectrum is narrower and less diffuse than that of cyan-methæmoglobin. On the addition of ammonium sulphide to cyanhæmatin the spectrum is changed immediately as the single band is replaced by two bands, resembling those of oxyhæmoglobin as regards position and relative breadth, but the two bands are not so well separated as in oxyhæmoglobin, and they are also slightly nearer the violet end of the spectrum.

[1] R. Kobert, *Cyanmethämoglobin und der Nachweis der Blausäure*, Stuttgart, 1891 ; *Pflüger's Arch.* **82**. 603 (1900).

[2] J. Haldane, *Journ. of Physiol.* **25**. 230 (1900) ; see also R. v. Zeynek, *Zeitschr. f. physiol. Chem.* **33**. 426 (1901).

[3] J. Bock, *Skandinav. Arch. f. Physiol.* **6**. 229 (1895).

[4] Szigeti, *Maly's Jahresb.* 1893, p. 620.

The spectrum is that of a reduction-product of CN-hæmatin, and is not that of hæmochromogen, as asserted by Szigeti.

Cyan-methæmoglobin, according to Haldane, gives a spectrum resembling that of reduced hæmoglobin. It is not changed at first visibly by the addition of ammonium sulphide, but on warming the solution and allowing it to stand, the cyan-methæmoglobin is gradually reduced to hæmoglobin.

Cyanhæmatin and cyan-methæmoglobin agree in not being affected in any way by a vacuum (Haldane).

v. Zeynek[1] has obtained cyan-methæmoglobin in crystals, and has also examined it spectro-photometrically. In neutral and alkaline solutions it shows a broad band in the green which possesses its greatest intensity of absorption at $\lambda 535$, while reduced hæmoglobin shows the maximum absorption at $\lambda 547$, according to Kobert and v. Zeynek. The absorption-coefficient measured at Hüfner's place equals 1·29, according to Bock and v. Zeynek.

Cyan-methæmoglobin contains one molecule of HCN (=0·158 per cent) for one molecule of hæmoglobin. It is converted into hæmoglobin not only by reducing agents, but also by putrefaction.

Azo-imide Methæmoglobin

L. Smith and C. G. L. Wolf[2] have studied the action of azo-imide or hydrogen nitride $\begin{matrix} N \\ \| \\ N \end{matrix}\!\!>\!N\text{—}H$, which is the only substance known, the an-ion of which is composed of a single N-atom. It resembles hydrocyanic acid in its properties, and forms with methæmoglobin a compound resembling that formed by HCN.[3]

Acetylen-hæmoglobin

This compound has been described by Liebreich and Bistrow[4] in 1868, but probably does not exist, according to Gamgee.[5]

[1] R. v. Zeynek, *Zeitschr. f. physiol. Chem.* **33.** 426 (1901).
[2] Smith and Wolf, *Journ. of Med. Research*, **12.** 451 (1904).
[3] Abstract by Alsberg in *Zentralbl. f. Physiol.* **19.** 41 (1905).
[4] O. Liebreich and A. Bistrow, *Ber. d. deutsch. chem. Ges.* **1.** I. 220 (1868).
[5] Gamgee, *Schäfer's Text-book of Physiology*, **1.** 242 (1898).

The Dissociation-products of Hæmoglobin

On adding to a salt-free solution of pure hæmoglobin a few drops of very dilute acid the hæmoglobin is split up into globin and hæmatin, according to Hoppe-Seyler,[1] Stokes,[2] Preyer,[3] Schulz,[4] and Lawrow.[5] Harnack's intermediate 'acid-hæmoglobin' has already been discussed on p. 495. The 'globin' of Schulz is prepared as follows :—A salt-free hæmoglobin solution is dissociated by a little acid; then alcohol and ether are added. The hæmatin passes into the ether, while the globin remains in the watery alcohol solution. Other observers have proceeded similarly, but if globin is not wanted as such, salt may be added, the solution may be boiled, the amount of acid be varied, etc., when instead of globin there are formed acid-albumin or even more remote transformation-products. v. Zeynek[6] brought about dissociation with pepsin-hydrochloric acid, when the albumin is dissociated into peptones, while the hæmatin separates out as an insoluble mass.

By stronger alkalies, or by boiling, hæmoglobin is also split up into hæmatin and albumin. According to Schulz, 100 parts of hæmoglobin give rise to 86·5 parts of globin and 4·2 parts of hæmatin; of the unknown remainder a certain fraction belongs to the globin. Lawrow found 94·09 per cent globin, 4·47 per cent hæmatin, and only 1·44 per cent unknown bodies, amongst which he could demonstrate fatty acids and ammonia. Hoppe-Seyler, on dissociating hæmoglobin, has also observed the occurrence of formic, butyric, and other acids of the fatty series. The union between globin and hæmatin must be an exceedingly feeble one, as the minutest trace of an acid at once causes dissociation. Notwithstanding this ready dissociation, we are dealing with a true salt formation, as the globin may be replaced by egg-white, according to Ham and Baleau,[7] who believe hæmatin to be an acetyl-compound or similar derivative, but see p. 509. The union between the hæmatin and the globin, according to Hoppe-Seyler, is an esterlike one, and Hüfner[8] also holds that the globin and hæmatin are kept together by one or several atoms of oxygen acting as the link.

[1] F. Hoppe-Seyler, *Virchow's Arch.* **29**. 233 (1864); *Zentralbl. f. d. med. Wissensch.* 1864, p. 261; 1865, p. 65.

[2] G. G. Stokes, *Philosoph. Magaz.* **27**. 4 Ser. 388 (1864).

[3] W. Preyer, *Pflüger's Arch. f. d. gesamte Physiol.* **1**. 395 (1868); *Die Blutkristalle*, Jena, 1871.

[4] F. N. Schulz, *Zeitschr. f. physiol. Chem.* **24**. 449 (1898).

[5] D. Lawrow, *ibid.* **26**. 343 (1898).

[6] R. v. Zeynek, *ibid.* **30**. 126 (1900).

[7] C. E. Ham and H. Baleau, *Journ. of Physiol.* **32**. 312 (1905).

[8] G. Hüfner, *Arch. f.* (*Anat. u.*) *Physiol.* 1899, p. 491.

Gamgee [1] has noticed an electrolytic dissociation of hæmoglobin, but was unable to exclude secondary action due to formation of acids.

Hæmatin and its Derivatives

Hæmatin is the non-proteid constituent of hæmoglobin and contains the iron as a ferri-compound. It is a pyrrol derivative, as has been shown by Küster [2] and Nencki [3] and their pupils. All investigations into the chemical structure of the noncrystalline hæmatin start with hæmin, which may be readily obtained in crystals. According to Nencki, hæmin is formed from hæmatin by one OH group of the latter becoming replaced by Cl.

Küster's [4] latest investigations (see p. 517) having shown that hæmatin has the formula

$$C_{34}H_{34}N_4ClFeO_5,$$

the conversion of hæmatin into hæmin would be expressed by the formula

$$C_{34}H_{34}N_4FeO_5 + HCl = C_{34}H_{33}ClN_4FeO_4 + H_2O.$$

The analyses of Nencki and Sieber,[5] Cloetta,[6] Rosenfeld,[7] Bialobrzeski,[8] Mörner,[9] v. Zeynek,[10] and the older ones of Küster [11] give a somewhat different composition. For a long time the formula $C_{32}H_{31}ClN_4FeO_3$ has been in use.[12] The chief reason for these differences is not that

[1] A. Gamgee, *Proc. Roy. Soc.* **68.** 503 (1901); **70.** 79 (1902).

[2] W. Küster, *Über das Hämatin, Habilitationsschr.*, Tübingen, 1896; *Ber. d. deutsch. chem. Ges.* **29.** I. 821 (1896); **30.** I. 105 (1897); *Zeitschr. f. physiol. Chem.* **28.** 1 (1899); G. Hüfner and M. Kölle, *ibid.* **28.** 34 (1899); *ibid.* **29.** 185 (1900); *Ber. d. deutsch. chem. Ges.* **32.** I. 678 (1899); **33.** III. 3021 (1900); **35.** II. 1268 (1902); **35.** III. 2948 (1902); *Liebig's Annalen*, **315.** 174 (1901); *Zeitschr. f. physiol. Chem.* **40.** 391 and 423 (1903); M. Kölle, *Dissertation*, Tübingen, 1898.

[3] M. Nencki and N. Sieber, *Archiv f. experiment. Pathol. u. Pharm.* **24.** 430 (1888); M. Nencki and J. Zaleski, *Zeitschr. f. physiol. Chem.* **30.** 384 (1900); M. Nencki and J. Zaleski, *Ber. d. deutsch. chem. Ges.* **34.** I. 997 (1901); M. Nencki and L. Marchlewski, *ibid.* **34.** II. 1687 (1901); J. Zaleski, *Zeitschr. f. physiol. Chem.* **37.** 54 (1902). Compare also the reports by H. Steudel, *Chem. Zeitschr*, I. Nr. 15 (1902); N. Sieber-Schumoff, *Münch. med. Wochenschr.* 1902, Nr. 45.

[4] William Küster, *Zeitschr. f. physiol. Chem.* **40.** 391 (1903).

[5] M. Nencki and N. Sieber, *Ber. d. deutsch. chem. Ges.* **17.** II. 2270 (1884); *Arch. f. exper. Path. u. Pharm.* **18.** 401 (1884); **20.** 325 (1885); **24.** 430 (1888).

[6] M. Cloetta, *ibid.* **36.** 349 (1895). [7] M. Rosenfeld, *ibid.* **40.** 137 (1898).

[8] M. Bialobrzeski, *Ber. d. deutsch. chem. Ges.* **29.** III. 2842 (1896).

[9] K. A. H. Mörner, *Maly's Jahresber. f. Tierchemie*, **27.** 145 (1897).

[10] E. v. Zeynek, *Zeitschr. f. physiol. Chem.* **25.** 492 (1898).

[11] W. Küster, *Ber. d. deutsch. chem. Ges.* **29.** I. 821 (1896).

[12] M. Nencki and N. Sieber, *Ber. d. deutsch. chem. Ges.* **17.** II. 2270 (1884); *Arch. f. exper. Path. u. Pharm.* **18.** 401 (1884); **20.** 325 (1885); **24.** 430 (1888); E. v. Zeynek, *Zeitschr. f. physiol. Chem.* **25.** 492 (1898); W. Küster, *Ber. d. deutsch. chem. Ges.* **29.** I. 821 (1896).

hæmin has the tendency of crystallising with portions of such solvents as amylalkohol, acetic acid, etc., as believed by Nencki and Sieber, and Schalfejew,[1] but is due to impurities, according to Küster (see p. 517). Schalfejew had also observed the presence of an impurity which could only be removed by repeated recrystallisation.

Hæmatoporphyrin.—By the action of acids under certain conditions described below, hæmatin is decomposed into a ferrous salt and the iron-free compound called hæmatoporphyrin. On dissolving, for example, hæmatin or hæmin in strong sulphuric acid, and then filtering the mixture through asbestos, a clear purple-red solution is obtained. Another method of preparing hæmatoporphyrin, namely, that of Hoppe-Seyler,[2] is to enclose hæmatin or hæmin dissolved in strong HCl in a sealed tube and to heat the latter up to 130°. Nencki and Sieber[3] act on hæmin, first at room-temperature and then on the water bath, with glacial acetic acid saturated with hydrobromic acid. According to Schulz[4] and Hoppe-Seyler-Thierfelder,[5] hæmochromogen, *i.e.* reduced hæmatin, passes readily into hæmatoporphyrin on being treated with dilute acids. By these means are obtained purple or deep red solutions, which deposit hæmatoporphyrin on being diluted with water and on being neutralised with caustic soda till the reaction is only faintly acid. (See also p. 468.)

The simplest and best method for preparing hæmatoporphyrin is, however, that of Laidlaw.[6] It is based on the observation that "whereas oxyhæmoglobin yields hæmatin to all strengths of mineral acids (except concentrated sulphuric acid), reduced hæmoglobin treated in the same manner gives hæmatoporphyrin." "The presence of oxygen confers stability to the iron of blood pigments, and the simplest explanation seems to be that the oxygen of oxyhæmoglobin is in direct relation to the iron," for while 2 per cent hydrochloric acid can split off the iron from the pigment in the reduced condition, it requires the strongest hydrochloric acid, aided by heat and pressure, to effect the cleavage in the oxidised pigment. By acting on reduced hæmoglobin with acids there is formed in the first instance hæmochromogen, and the latter is then converted into the iron-free hæmatoporphyrin. For reducing oxyhæmoglobin Laidlaw uses hydrazin-hydrate, as its

[1] M. Schalfejew, *Chem. Zentralbl.* **18**. 232. (1885); *Maly's Jahresb.* **15**. 138 (1885).

[2] F. Hoppe-Seyler, *Zentralbl. f. d. med. Wiss.* 1864, p. 261; and in *Med.-chem. Untersuch.* p. 523 (1870).

[3] M. Nencki and N. Sieber, *Monatsh. f. Chem.* **9**. 115 (1889). See also *Schmiedeberg's Arch. f. experiment. Path. u. Pharm.* **24**. 430 (1888).

[4] A. Schulz, *Arch. f.* (*Anat. u.*) *Physiol.* 1904, Suppl. p. 271.

[5] Hoppe-Seyler-Thierfelder, *Handbuch d. physiol. u. pathol. Analyse*, 1903, p. 282 (seventh edition).

[6] P. P. Laidlaw, *Journ. of Physiol.* **31**. 465 (1904).

bye-products are negligible, and with such reduced hæmoglobin 15 per cent HCl in the cold yields a pure hæmatoporphyrin. Hæmatoporphyrin by uniting with iron becomes hæmochromogen, which by oxidation is changed into hæmatin, as will be shown later (see p. 524). The formation of hæmatoporphyrin, using the old formula for hæmatin (see p. 508) occurs readily and almost quantitatively, according to the equation :[1]

$$C_{32}H_{32}N_4FeO_4 + 2H_2O + 2HBr = 2C_{16}H_{18}N_2O_3 + FeBr_2 + H_2.$$

Hæmatin (one molecule) = Hæmatoporphyrin (two molecules).

Or using the new formula according to Zaleski :[2]

$$C_{34}H_{33}O_4N_4ClFe + 2H_2O + 2HBr = C_{34}H_{38}O_6N_4 + FeBr_2 + HCl$$

Hæmin (one molecule) = Hæmatoporphyrin (one molecule).

No hydrogen is given off during the formation of hæmatoporphyrin according to Zaleski.

Mesoporphyrin.—On carefully reducing acet-hæmin or hæmatoporphyrin with hydriodic acid and phosphonium iodide, Nencki and Zaleski obtained mesoporphyrin, to which they attributed the formula $C_{16}H_{18}N_2O_2$. After Nencki's death his pupil Zaleski continued the research,[3] and using a very detailed method has arrived at the result that free mesoporphyrin has the formula

$$C_{34}H_{38}O_4N_4.$$

Mesoporphyrin differs from hæmatoporphyrin in containing two oxygen atoms less. Zaleski has prepared the following compounds of meso porphyrin :

$C_{34}H_{38}O_4N_4 . 2HCl$	Mesoporphyrin hydrochloride
$C_{34}H_{38}O_6N_4 . 2HCl$	Hæmatoporphyrin hydrochloride
$C_{34}H_{36}O_4N_4 . (C_2H_5)_2$	Mesoporphyrin ethylester
$C_{34}H_{36}O_4N_4 . Zn$	Zinc (or Cu) salt of mesoporphyrin
$C_{34}H_{34}O_4N_4 . (C_2H_5)_2Cu$	Copper salt of mesoporphyrin ethylester.

Hæmopyrrol : Nencki and Zaleski, by further reduction of mesoporphyrin with stronger hydriodic acid and more phosphonium iodide, obtained hæmopyrrol :

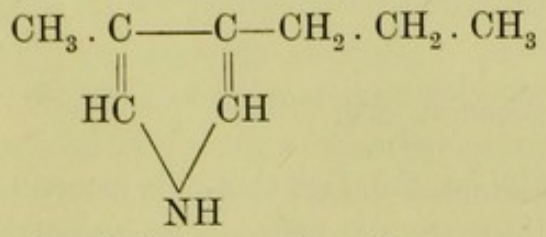
$$C_8H_{13}N,$$

[1] M. Nencki and N. Sieber, *Monatshefte f. Chemie*, **9**. 115 (1889) ; and in *Schmiedeberg's Archiv. für experiment. Pathol. und Pharmakol.* **24**. 430 (1888) ; W. Küster, *Berichte d. deutsch. chem. Gesellschaft*, **30**. I. 105 (1897).

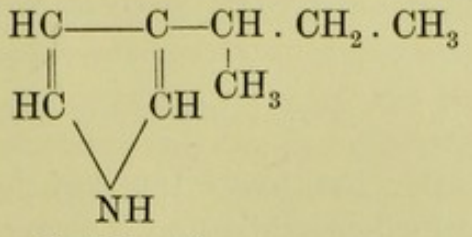
[2] J. Zaleski, *Zeitschr. f. physiol. Chem.* **37**. 74 (1902).

[3] J. Zaleski, *ibid.* **37**. 54 (1902).

which is probably identical with methylpropylpyrrol, although it may perhaps be butylpyrrol:

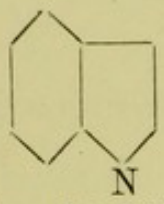


3-methyl 4-*n*-propylpyrrol.

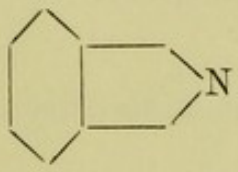


Butylpyrrol.

According to an abstract by Czapek[1] in the *Zentralbl. f. Physiologie*, Küster[2] believes that in hæmatoporphyrin and in phylloporphyrin there is not found a normal indolring, but an iso-indolring:



Normal indolring.



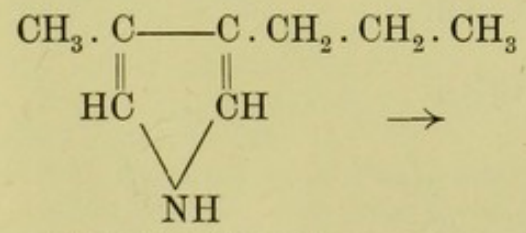
Iso-indolring.

Hæmatinic Acids

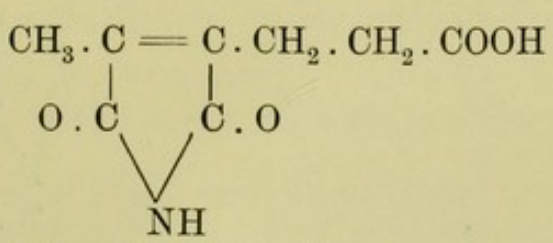
By oxidising hæmatin dissolved in glacial acetic acid with a watery solution of sodium bichromate at the temperature of the water bath, Küster[3] obtained two ether-soluble acids, which crystallised readily and which he called hæmatinic acids:

Anhydride of tribasic hæmatinic acid	$C_8H_8O_5$.
Dibasic hæmatinic acid	$C_8H_9NO_4$.

These acids are formed, according to Küster,[4] Nencki and Zaleski,[5] by the oxidation of methylpropylpyrrol or hæmopyrrol, thus:



Methylpropylpyrrol.



Dibasic hæmatinic acid.

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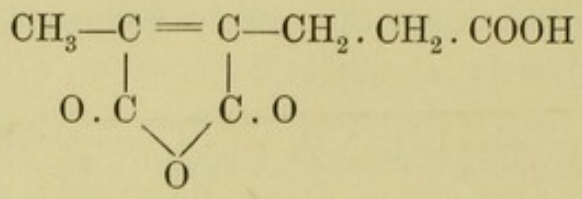
[1] Czapek, *Zentralbl. f. Physiol.* **18**. 591 (1904).

[2] W. Küster, *Ber. d. deutsch. bot. Ges.* **22**. 339 (1904).

[3] W. Küster, *Zeitschr. f. physiol. Chem.* **28**. 1 (1899); **29**. 185 (1900).

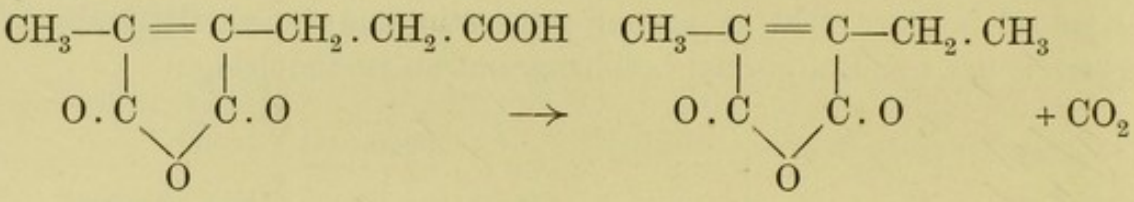
[4] W. Küster, *Berichte d. deutsch. chem. Gesellschaft*, **35**. III. 2948 (1902).

[5] M. Nencki and J. Zaleski, *ibid.* **34**. I. 997 (1901); J. Zaleski, *Zeitschr. f. physiol. Chem.* **37**. 54 (1902).



Partial anhydride of tribasic hæmatinic acid.

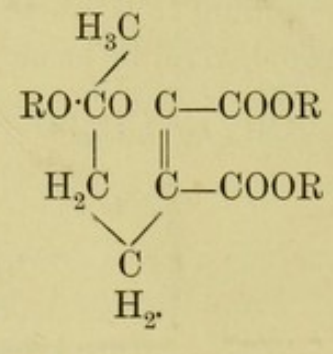
Küster has based this last formula on the fact that this compound by further oxidisation is changed into succinic acid. The anhydride of tribasic hæmatinic acid becomes dibasic by splitting off carbonic acid (Kölle)[1] according to the equation :



Anhydride of tribasic hæmatinic acid. Anhydride of dibasic hæmatinic acid.

This dibasic anhydride, according to Galler,[2] is identical with ethyl-methyl-maleic acid anhydride : $C_7H_8O_3$.

On dissolving one gramme of hæmatin in 60 volumes of hot glacial acetic acid, and oxidising as quickly as possible with 12·5 grammes of CrO_3, corresponding to 21 atoms of oxygen for each hæmatin molecule, and at once distilling off the glacial acetic acid, Küster[3] showed in his third paper that the hæmatinic acids were obtained in such amounts that at least three molecules of $C_8H_9O_4N_1$, if not, indeed, four molecules, could be obtained from one molecule of hæmatin. For this reason he considers the hæmatinic acids to be the most important dissociation-products of hæmatin. Küster succeeded in synthetising the ester of tribasic hæmatinic acid :



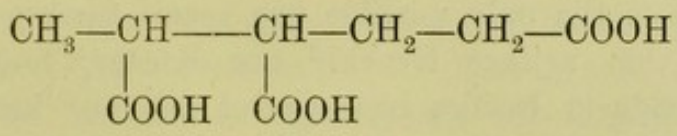
This compound must be able to undergo intramolecular condensation between the methyl and the neighbouring carboxethyll group. With sodium ethylate a substance is obtained which gives a colour reaction with ferric chloride. This same ester, when heated with alcoholic

[1] M. Kölle, *Dis.* Tüb. 1898 ; W. Küster, *Ber. d. deuts. chem. Ges.* **35**. 2948 (1902).
[2] Galler, *ibid.* **35**. 2948 (1902). [3] W. Küster, *Zeit. f. phys. Chem.* **44**. 391 (1905).

ammonia in a tube to 130°, gives rise to a brown fluid, which on exposure to the air becomes deep blue, while hæmatinic acid itself splits off CO_2, and is converted into the imide of methylethyl-maleic acid. After removal of the alcohol and the ammonia a watery solution is obtained which shows two feeble bands in the region of the oxy-hæmoglobin bands. Another ester, resembling the one just described, Küster obtained from the hæmatinic acid $C_8H_9O_4N$.

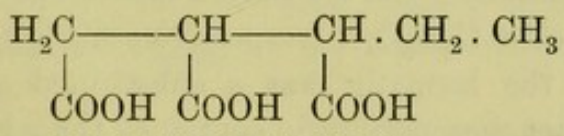
All these reactions favour the view that hæmopyrrol is 3-methyl-4-*n*-propylpyrrol, but by comparing the oxidation-product of hæmopyrrol with the synthetically prepared imide, Küster and Haas [1] have shown that the two compounds are not identical, as the difference in the melting-points amounts to 7°.

By reducing methylethyl-maleic anhydride, hæmotricarboxylic acids are obtained:[2]—



Hæmotricarboxylic acid.

These carboxylic acids are isomeric, although not identical, with the synthetically prepared ethyltricarballylic acid:



Ethyltricarballylic acid.

And this fact supports the formula of butylpyrrol given above for hæmopyrrol.

That hæmopyrrol is a pyrrol-derivative is proved [3] by the fact that an ethereal solution of hæmopyrrol [4] combines with a freshly prepared watery solution of benzene-diazonium [5] chloride to form an azo-dye-compound, which is soluble in alcohol with a cherry-red and in

[1] Küster and Haas, *Ber. d. deutsch. chem. Ges.* **37**. 2470 (1904).

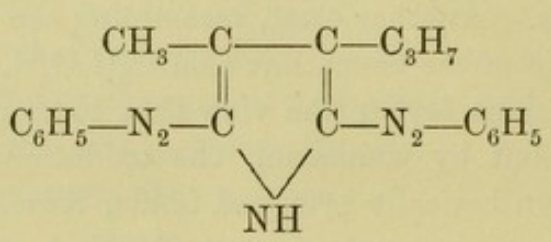
[2] M. Kölle, *Dissertation*, Tübingen, 1898; W. Küster, *Ber. d. deutsch. chem. Ges.* **35**. III. 2948 (1902).

[3] H. Goldmann and L. Marchlewski, *Zeitschr. f. physiol. Chem.* **43**. 415 (1905).

[4] The hæmopyrrol was made according to Nencki-Zaleski's method: 5 grammes of hæmin, 100 grammes of hydriodic acid, 100 grammes of glacial acetic acid, and about 8 grammes of phosphonium iodide.

[5] The discovery of the fact that pyrrol and some of its homologues react with diazonium compounds was first made by Fischer and Hepp (*Ber. der deutsch. chem. Ges.* **19**. 2251 (1886). Pyrrol in acid solutions yields mono-azo-dyes, while in alkaline solutions in the presence of two molecules of the diazonium-compound there are formed di-azo-dyes. Both α and β hydrogen-atoms of pyrrol may be substituted. (See p. 43 for formula of pyrrol.)

chloroform with a bluish-violet colour. Goldmann, Hetper, and Marchlewski[1] showed subsequently that the substance in question is the hydrochloride of hæmopyrrol-diazodibenzene. The free azo-dye, on the supposition that hæmopyrrol is really methyl-propyl-pyrrol and not another homologue of pyrrol, has this constitution:



Hæmopyrrol-di-azoditoluene was prepared analogously.

Hæmatoporphyrin can therefore be built up directly from four molecules of hæmopyrrol or of one of its oxidation-products. That this synthesis is the only possible one, seems further to be proved by the fact that neither Nencki[2] nor Küster[3] found any other dissociation-products besides hæmopyrrol and the hæmatinic acids. The maximum yield of hæmopyrrol[2] and of the hæmatinic acids[3] obtained from hæmatoporphyrin at first only amounted to 32 and 50 per cent respectively, and Küster,[4] being able to account for only 16 out of the 34 C-atoms and for only 2 out of the 4 N-atoms by the two hæmatinic acids, $C_8H_9O_4N$ (respectively $C_8H_8O_5$), concluded that only one-half of the hæmatin was a substituted pyrrol, but when Zaleski showed that mesoporphyrin contained the whole of the carbon, namely 34C, Kutscher[5] realised that his old view was wrong, and that greater importance will have to be attached to the hæmatinic acids than has been done in the past. On reinvestigating the formation of hæmatinic acids (see p. 512) he now holds that at least three molecules of $C_8H_9O_4N$, if not indeed four molecules, can be obtained from hæmatin.

Description of the Individual Dissociation-products:

Hæmin

Hæmin was first prepared by Teichmann in 1853 by the action of glacial acetic acid and a little sodium chloride on warmed blood.[6]

[1] H. Goldmann, J. Hetper, and L. Marchlewski, *Zeits. f. phys. Chem.* **45**. 176 (1905).
[2] M. Nencki and J. Zaleski, *Ber. d. deutsch. chem. Ges.* **34**. I. 997 (1901).
[3] M. Kölle, *Dissertation*, Tübingen, 1898.
[4] W. Küster, *Zeitschr. f. physiol. Chem.* **28**. 1 (1899), and **29**. 185 (1900).
[5] W. Kutscher, *ibid.* **44**. 391 (1905).
[6] L. Teichmann, *Zeitschr. f. rat. Med.* N.F. **3**. 375 (1853); **8**. 141 (1857).

To prepare hæmin Nencki and Sieber[1] take 400 grammes of red blood-corpuscles, freed from serum and coagulated with alcohol; add 1600 grammes of amyl-alcohol; heat up; add 20 to 25 ccm. of hydrochloric acid and simmer for 10 minutes. On cooling, crystals of hæmin separate out; the yield from 1 litre of blood is 1·5 to 3 grammes of hæmin crystals. Cloetta[2] and Rosenfeld[3] wash the blood-corpuscles with sodium sulphate instead of with sodium chloride, and extract the alcohol coagulum with hot ethyl-alcohol containing sulphuric or oxalic acid. The crystals formed on cooling are recrystallised from hot alcohol containing HCl. Mörner[4] coagulates diluted blood with sulphuric acid, extracts with alcohol containing H_2SO_4, and after the addition of HCl heats the mixture up to the boiling-point. Schalfejew's[5] method gives the purest hæmin and also the largest yield. He proceeds as follows: To 1 volume of defibrinated blood, filtered through a linen cloth, are added 4 volumes of glacial acetic acid heated up to 80°; after the mixture has cooled to between 55°-60°, heat up again at once to 80°. On allowing the mixture to cool crystals are formed; after 10-12 hours pour off supernatant fluid and wash the crystalline precipitate in a high cylinder with 5-6 volumes of water; after repeated washings filter off the crystals and wash the crystals on the filter with water, alcohol, and ether. One litre of blood yields 5 grammes of crystals of the same size, and belonging to the triclinic system, forming thin rhomboidal plates.

The chemical nature of the crystals formed by Schalfejew's method is discussed below.

Hæmin forms microscopic, small brown plates, which are known as Teichmann's crystals.[6] For medico-legal purposes,[7] it is customary to mix some blood with a little sodium chloride and glacial acetic acid on a microscopic slide, to cover the preparation with a cover-glass, to boil the acetic acid, and to add 2 to 3 additional drops of acid. It is much better to use potassium iodide, as the crystals are then much larger (E. Wace Carlier). Hæmin crystals show, according to Ewald,[8] a distinct pleochroism, being either deep black or pale yellowish

[1] M. Nencki and N. Sieber, *Ber. d. deutsch. chem. Ges.* **17**. II. 2270 (1884); and in *Schmiedeberg's Arch. f. exper. Path. u. Pharm.* **18**. 401 (1884); **20**. 325 (1885).

[2] M. Cloetta, *ibid.* **36**. 349 (1895).

[3] M. Rosenfeld, *ibid.* **40**. 137 (1898).

[4] K. A. H. Mörner, *Maly's Jahresber. f. Tierchemie*, **27**. 145 (1897).

[5] M. Schalfejew, *Chem. Zentralbl.* **18**. 232 (from the Russian) (1885); *Maly's Jahresber.* **15**. 138 (1885).

[6] M. Schalfejew, *Maly's Jahresberichte*, **15**. 138 (1885). Compare also H. U. Kobert, *Wirbeltierblut in mikrokristallinischer Hinsicht*, Stuttgart, Enke, 1901.

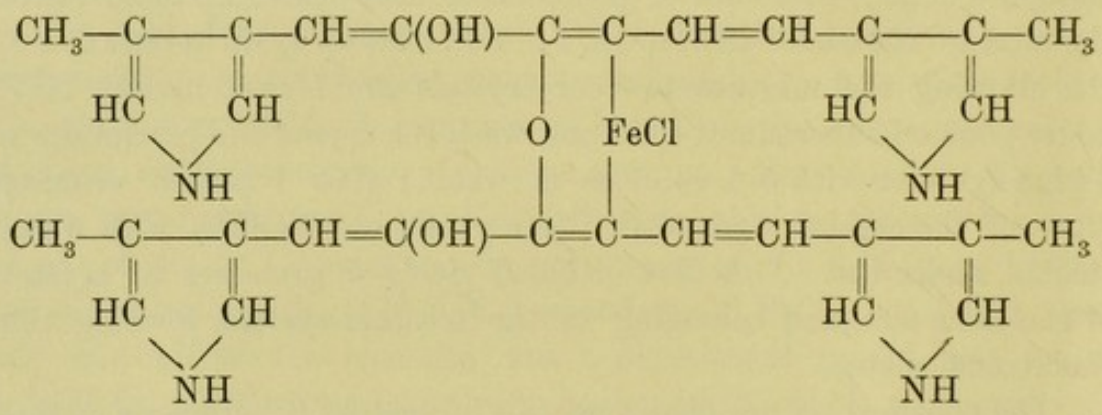
[7] F. Hoppe-Seyler, *Med.-chem. Untersuch.* p. 366 (1868); p. 523 (1870).

[8] August Ewald, *Zeitschr. f. Biol.* **22**. 459 (1886).

brown; they are insoluble in water, hardly soluble in ether, and slightly soluble in alcohol and chloroform, according to Bialobrzeski.[1]

Hæmin bears to hæmatin the same relation as does an ester to a tertiary alcohol, as the halogen is very readily split off by alkalies. The acid character of hæmatin is, further, conditioned by a hydroxyl group, which is not the same as the one which is replaced by the halogen in converting hæmatin into hæmin. The chlorine is bound therefore, according to Küster,[2] in the hæmin, to a carbon and not to a nitrogen atom. Nencki and Sieber[3] were, however, the first to point out that the chlorine was probably united to carbon or to iron.

On the assumption that the hæmatoporphyrin molecule is built up of two hæmopyrrol molecules, and that the two hæmatoporphyrin molecules in hæmin are kept together by the iron-atom present in hæmatin, Nencki and Zaleski give the following formula for hæmin :—



Six distinct empirical formulæ have been given to hæmin :[4]—

Nencki and Sieber's amyl-alcohol extract :	$C_{32}H_{31}O_3N_4ClFe$.[5]
Acet-hæmin of Schalfejew :	$C_{34}H_{33}O_4N_4ClFe$.[5]
β-hæmin of Mörner :	$C_{34}H_{35}O_4N_4ClFe$.[6]
Hæmin (Cloetta, Rosenfeld) :	$C_{30}H_{35}O_3N_3ClFe$.[7]
v. Zeynek, after peptic digestion :	$C_{34}H_{34}O_4N_5ClFe$.[8]
Küster's hæmin :	$C_{34}H_{33}O_4N_4ClFe$.[4]

[1] M. Bialobrzeski, *Ber. d. deutsch. chem. Ges.* **29**. III. 2842 (1896).

[2] W. Küster, *ibid.* **29**. I. 821 (1896).

[3] M. Nencki and N. Sieber, *Schmiedeberg's Arch. f. exper. Path. u. Pharm.* **20**. 326 (1886).

[4] W. Küster, *Zeit. f. physiol. Chem.* **40**. 391 (1904).

[5] Nencki and Sieber, *Arch. f. experim. Path. u. Pharm.* **18**. 401 ; **20**. 325 ; **24**. 430 (1884-1888).

[6] Mörner, *Nordisk med. Ark. Festband*, 1897, Nos. 1 and 27.

[7] Cloetta, *Arch. f. exp. Path. u. Pharm.* **36**. 352 (1895). See also Rosenfeld, *ibid.* **40**. 137 (1898).

[8] v. Zeynek, *Zeit. f. physiol. Chem.* **30**. 126 (1900).

The formation of hæmatin out of Schalfejew's acet-hæmin Zaleski [1] expresses by the equation :

$$\underset{\text{Acet-hæmin.}}{C_{34}H_{33}ON_4ClFe} + 2HBr + 2H_2O = \underset{\text{Hæmatin.}}{C_{34}H_{38}O_6N_4} + FeBr_2 + HCl.$$

According to this view Schalfejew's acet-hæmin is no longer regarded as an acetyl-hæmin, nor does the β-hæmin of Mörner represent a propionyl-hæmin, or an ethyl-ester of 'acet-hæmin.' As little as it is possible to split off acetic acid from 'acet-hæmin,' [2] as little can an alphyl-oxy group or a propionic or acetic acid remainder be obtained from β-hæmin.[3]

Küster is of the opinion that all the hæmins enumerated above (acet-hæmin, Nencki and Sieber's hæmin, β-hæmin, Cloetta-Rosenfeld's hæmin) are one and the same ; that only one hæmin exists, with the formula :

$$C_{34}H_{33}O_4N_4ClFe.$$

The discrepancies in the formulæ given above are attributed by Küster to insufficient recrystallisation. He also adduces direct proof that these 'hæmins' are really identical. On treating the different hæmins with cooled anilin, the chlorine atom is removed, and amorphous products are obtained, all of which, after careful washing in very dilute acetic acid, and after long extraction with ether, agree in giving the formula :

$$C_{34}H_{32}O_4N_4Fe.$$

This compound is called a 'de-hydrochlorid-hæmin.' By the same methods as are employed in making acet-hæmin, the de-hydrochlorid-hæmin may be reconverted into acet-hæmin. By treating the latter with watery solutions of alkalies, the chlorine becomes replaced by a hydroxyl group, and 'hæmatin' is formed :

$$C_{34}H_{34}O_5N_4ClFe.$$

This formula corresponds to the old one, first given by Hoppe-Seyler, namely :

$$C_{35}H_{35}O_5N_4Fe.$$

Now 'hæmatin' obtained in this way from hæmins cannot be reconverted into hæmin, because the saponification with alkalies does not simply substitute a hydroxyl group for the chlorine, but produces also some other change whereby the configuration of the 'hæmatin' molecule is altered.[4]

[1] Zaleski, *Zeitschr. f. physiol. Chem.* **37**. 54 (1904).
[2] Nencki and Zaleski, *ibid.* **30**. 396-397, 403-404 (1900).
[3] Küster, *Ber. d. deutsch. chem. Ges.* **35**. 2951 (1902).
[4] W. Küster, *Zeitschr. f. physiol. Chem.* **40**. 396 (1904).

It is necessary therefore to distinguish between the 'natural hæmatin' obtained by the action of dilute acids on hæmoglobin, and the 'artificial hæmatin' prepared from hæmin.

In many cases Küster has observed the analysis of 'artificial hæmatin' to give too low a reading for hydrogen, and is not certain whether the correct formula for 'hæmatin' is not

$$C_{34}H_{32}O_5N_4Fe.$$

This formula he derives from hæmin according to the equation:

$$C_{34}H_{33}O_4N_4ClFe + NaOH + O = C_{34}H_{32}O_5N_4Fe + H_2O + NaCl.$$

Küster has further obtained a de-hydro-hæmatin:

$$C_{34}H_{32}O_4N_4Fe,$$

by precipitating with dilute acids de-hydrochlorid-hæmin dissolved in alkalies or in alkaline carbonates. De-hydro-hæmatin cannot be converted into hæmin.

Küster[1] has also prepared the acetic acid and hydrobromic acid esters; Nencki and Zaleski[2] several esters of hæmin, such as the dimethyl-, ethyl-, and mono-amyl-ester. Nencki and Zaleski believed the hæmin prepared by Schalfejew's[3] method to be the acetyl-ester of hæmin, but this, according to Zaleski[4] and Küster,[5] is not the case. The readiness with which hæmin forms addition-compounds depends on the presence of two hydroxyl groups.[6] The oxidation-products of the different hæmins are identical, according to Küster.[7]

Küster[8] has also succeeded in introducing up to eight molecules of anilin, $C_6H_5 . NH_2$, into hæmin by boiling hæmin with anilin. At first four molecules of anilin are introduced by removing eight hydrogen atoms by oxidation, yielding the compound $C_{58}H_{52}O_4N_8Fe$, and later another two or four molecules of anilin are joined with a simultaneous addition of water and the splitting off of a molecule of ammonia yielding a compound 'anilinohæmin' with the formulæ:

$$C_{70}H_{65}O_5N_9Fe \quad \text{and} \quad C_{82}H_{79}O_5N_{11}Fe.$$

Although there may be some doubt as to the exact number of

[1] W. Küster, *Ber. d. deutsch. chem. Ges.* **29**. I. 821 (1896).

[2] M. Nencki and J. Zaleski, *Zeitschr. f. physiol. Chem.* **30**. 384 (1900).

[3] M. Schalfejew, *Maly's Jahresberichte*, **15**. 138 (1885); compare also H. U. Kobert, *Wirbeltierblut in mikrokristallinischer Hinsicht*, Stuttgart, Enke, 1901.

[4] J. Zaleski, *Zeitschr. f. physiol. Chem.* **37**. 54 (1902).

[5] W. Küster, *ibid.* **40**. 391 (1903).

[6] M. Nencki and Zaleski, *ibid.* **30**. 384 (1900).

[7] W. Küster, *ibid.* **29**. 185 (1900).

[8] William Küster, *ibid.* **40**. 423 (1904).

anilin-molecules which may be introduced into hæmin, there is no doubt that anilin is introduced, because the molecular weight of the new compound is raised, and the percentage amount of iron is lowered without any iron being split off.

The compound resulting from the first interaction of hæmin and anilin is acetone-insoluble, while the final products are acetone-soluble. As all compounds are insoluble in caustic soda, it is assumed that the hydroxyl groups of hæmin play a part in the reaction.

Hetper and Marchlewski [1] prepared hæmin by means of propionic acid, and obtained hæmin crystals which were somewhat larger than 'acet-hæmin' crystals, but they differed in no other respect.[2]

The chloroform solutions of hæmin are made thus :—Hæmin is dissolved in a mixture of chloroform and quinine, and then a few drops of acetic acid are added :

Band α λ655—630
β λ655—534
γ λ524—497

In concentrated solutions the bands β and γ fuse, and a feeble line is seen at the Na-line. Very similar bands are obtained with the dimethyl-ether of hæmin. Hæmin in chloroform + quinine, cinchonin, or ammonia, but without acetic acid, shows two bands :

α λ615—582
β λ506—475 (ill-defined)

Dimethyl-hæmin has the same spectrum. Addition of acetic acid produces the three bands given above. Acetic acid and chloroform solutions of hæmin and its dimethyl ethers in very dilute solutions show a band on the Tl-line. By addition of quinine this band is changed to the Kβ line.[3] Hetper and Marchlewski and Küster agree in that acet-hæmin may be prepared from Mörner's hæmin, but while Küster found no OC_2H_5 group, Hetper and Marchlewski found it in traces.

Hæmatin

Attention has already been drawn on p. 507 to the fact that the dissociation of hæmoglobin into an albuminous fraction and into the ferri-compound : hæmatin, may be brought about by acids, or by alkalies, or by heat, and that we have also to distinguish between 'natural hæmatin' and 'artificial hæmatin' (p. 517).

[1] J. Hetper and L. Marchlewski, *Zeitschr. f. physiol. Chem.* **41**. 38 (1904).
[2] Photographs in *Bull. de l'Acad. des Sc. de Cracovie*, Mai 1904.
[3] Cp. Gamgee, *Zeitschr. f. Biol.* 1896, p. 505.

Hæmatin forms a non-crystalline bluish-black powder, which is insoluble in water, alcohol, ether, chloroform, and in watery solutions of acids; very slightly soluble in glacial acetic acid, or in alcohol or ether containing HCl, but very soluble in all alkaline solutions. When heated beyond 180° C. it undergoes decomposition; when incinerated it gives off hydrocyanic acid, while a residue of 12·6 per cent, consisting of pure oxide of iron, remains behind.

On distilling carefully-dried hæmatin with perfectly dry zinc dust, Milroy[1] obtained three volatile substances, two of which, in their spectroscopic characters resembled hæmatoporphyrin and urobilin, while they differed from the latter markedly in regard to their solubilities. The third substance was probably allied to hæmopyrrol. The products of distillation were condensed in (1) a spiral glass worm surrounded by a water jacket, (2) a coiled tube placed in a mixture of ice and water, and (3) the remaining gas passed through a wash-bottle containing concentrated HCl. This HCl absorbed some pigment which, on spectroscopic examination, showed absorption-bands apparently identical in position and character with those of acid hæmatoporphyrin. The pigment which condensed in the tubes, dissolved in chloroform with a reddish-brown colour, showed a faint green fluorescence, and gave on spectroscopic examination the three bands:

α λ578—λ560
β λ540—λ430
γ λ500—λ402

After distilling off the chloroform the pigment residue was found to be insoluble in caustic alkalies, soluble in concentrated HCl, but precipitated by dilution with water, and readily soluble in alcohol, ether, chloroform, petroleum ether, glacial acetic acid, and benzene. On dissolving the pigment in ether and adding to the latter some concentrated HCl diluted with an equal bulk of water, a brown pigment passed into the ether having an absorption-band, λ506—λ476.

The red watery hydrochloric acid solution showed three bands closely resembling those of hæmatoporphyrin:

α λ598—λ588 dark
β λ578—λ576 faint
λ578—λ562 slightly fainter
γ λ562—λ538 dark

On diluting the acid solutions, extracting the pigment with chloro-

[1] J. A. Milroy, *Proc. of Physiol. Soc.* xxiv.; *Journ. of Physiol.* **31**. (1904).

form, washing the latter thoroughly with water, adding some 5 per cent caustic soda, and shaking gently, gave, when the chloroform had completely separated from the aqueous alkali, the following absorption-bands :

α	$\lambda 617{\cdot}5$—$\lambda 612$	
β	$\lambda 573{\cdot}5$—$\lambda 567{\cdot}5$	pale
	$\lambda 567{\cdot}5$—$\lambda 561$	dark
γ	$\lambda 539$ —$\lambda 527{\cdot}5$	
δ	$\lambda 512{\cdot}5$—$\lambda 492{\cdot}5$	

By a slow distillation of hæmatin with a large excess of zinc dust Milroy obtained a more complete reduction ; the yellow oil obtained gave a vapour which, with a pine-wood strip dipped into hyrochloric acid showed the red pyrrol-reaction, but the colour reactions for pyrrol with isatine and quinine gave negative results.

In alkaline solutions hæmatin is red in thick layers, and greenish in thin layers ; in acid solutions it appears brown. The spectrum of acid hæmatin resembles that of acid methæmoglobin. The band in the red lies, however, nearer the red end, according to Menzies[1] at $\lambda 650$, and does not reach the C-line according to Harnack.[2] The second broad band lies between *b* and F, and the band in the violet, according to Gamgee,[3] resembles that of methæmoglobin, for it is a broad, intense band between *h* and L, which in dilute solutions extends from H to K, and in strong solutions from M into the ultra-violet. In alkaline solutions it only shows one band in the yellow, which commences half-way between C and D and then extends beyond D. There is no distinct band in the violet, as the whole of the violet becomes extinguished (Gamgee).

Pure hæmatin in alkaline solutions cannot be reduced by ammonium sulphide or other weak reducing agents (Hoppe-Seyler and Gamgee),[4] but in the presence of albumin and similar foreign bodies, such as ethylamine, aniline, glycocoll, taurine (Bertin Sans and Montessier),[5] it is changed into a substance called 'reduced hæmatin,' which shows the same absorption-spectrum as does hæmochromogen, a ferro-compound obtained by Hoppe-Seyler on adding alkalies or acids to a solution of reduced hæmoglobin in the absence of all oxygen.

[1] J. A. Menzies, *Journ. of Physiol.* **17**. 415 (1895).
[2] E. Harnack, *Zeitschr. f. physiol. Chem.* **26**. 558 (1899).
[3] A. Gamgee, *Zeitschr. f. Biol.* **34**. 505 (1896).
[4] A. Gamgee, *Schäfer's Textbook of Physiol.* **1**. 251, 252 (1898).
[5] Bertin Sans and Montessier, *Compt. Rend.* 1880.

The synthesis of hæmatin out of hæmatoporphyrin and a ferrous salt by Laidlaw's method is described on p. 524.

Arnold[1] has described a 'neutral hæmatin' which dissolves in neutral dilute alcohol containing salts, and which possesses a spectrum resembling that of oxyhæmoglobin. The existence of this substance has, however, been denied.[2]

Hæmatin is magnetic, and thus differs from the diamagnetic oxyhæmoglobin (Gamgee).[3]

Linossier[4] has described a nitrous oxide-hæmatin, the spectrum of which corresponds to that of NO-hæmoglobin, and which could not be reduced; by oxygen it is split up into hæmatin and nitrous oxide.

Cyanhæmatin has been described on p. 505, along with cyanmethæmoglobin.

Hæmochromogen

It is generally stated that Hoppe-Seyler succeeded in getting hæmochromogen to crystallise, but this statement, according to Gamgee,[5] is erroneous. Donogány,[6] by acting on blood with pyridin and ammonium sulphide, did obtain crystals, and Kobert[7] succeeded by the same method. Hæmochromogen has the appearance of powdered red phosphorus; on being dried more thoroughly it becomes reddish-brown, and in the moist state has to be carefully protected against air, as otherwise it is converted into hæmatin. It is insoluble in water, alcohol, and ether, but readily soluble, with a cherry-red colour, in alkalies; it is precipitated by neutralisation. v. Zeynek supposes that it is formed by one atom of oxygen being removed from two molecules of hæmatin; its ammonia salt has according to him the formula:

$$C_{64}H_{70}Fe_2N_{10}O_7.$$

Hæmochromogen shows three absorption-bands. The α-band lies between D and E, nearer D with its centre at $\lambda 559$ (v. Zeynek); the second or β-band commences at E and extends beyond *b*. The first band is especially intense.

[1] V. Arnold, *Zeitschr. f. physiol. Chem.* **29**. 78 (1900).

[2] K. H. L. van Klaveren, *ibid.* **33**. 293 (1901); *Maly's Jahresb. f. Tierchem.* **30**. 164 and 165 (1900) (V. Arnold and L. Wachholz).

[3] A. Gamgee, *Proc. Roy. Soc.* **68**. 503 (1902).

[4] G. Linossier, *Compt. Rend.* **104**. 1296 [according to *Maly's Jahresberichte*, **17**. 121 (1887)].

[5] A. Gamgee, *Schäfer's Textbook*, **1**. 256 (1898).

[6] J. Donogány, *Maly's Jahresberichte*, 1893, pp. 126-131; *Virchow's Arch.* **148**. 234.

[7] H. U. Kobert, *Zeitschr. f. angew. Microskop.* **5**. Heft 6-10 (1900); and Reprint, P. Wittrin, Leipzig, 1900.

The absorption-bands α and β of natural and synthetised hæmochromogen are as follows :—

	α	β
Natural	λ567–λ547	λ532–λ518 (Gamgee).[1]
	λ565–λ547	λ527–λ514 (Hoppe-Seyler).[2]
Synthetised	λ565–λ545	λ530–λ510 (Laidlaw).[3] (See p. 524.)

The third or γ-band, also intense, lies in the violet between h and g, λ430–λ410, with its centre at λ420, and corresponds, therefore, exactly with the band of carboxyhæmoglobin (Gamgee).

v. Zeynek has prepared hæmochromogen by reducing pure hæmatin with hydrazin-hydrate, which has an advantage, as the decomposition-products of the reducing agent are inert substances :

$$H_2N - NH_2 . H_2O + O_2 = N_2 + 3H_2O.$$

By working in an atmosphere of hydrogen he prevented a subsequent oxidation of the hæmochromogen. Its ammonium salt is formed according to the equation :

$$2(C_{32}H_{32}FeN_4O_4) + 2NH_4 = C_{64}H_{72}Fe_2N_{10}O_7 + O.$$

From the hæmatin liberated by peptic digestion and having the formula $C_{34}H_{35}N_5FeO_5$ v. Zeynek prepared an ammonium salt of hæmochromogen having the probable formula :

$$C_{34}H_{38}N_6FeO_4.$$

On shaking an alkaline solution of hæmochromogen with air it passes into alkaline hæmatin, when only judged by the visible absorption-spectrum, but Gamgee has shown that such an assumption would be altogether wrong, for oxidised hæmochromogen shows no band in the violet, the whole of the violet and ultra-violet becoming indeed much clearer than they are with reduced hæmochromogen. The behaviour of the violet end of the spectrum is therefore exactly contrary to what it is in the case of alkaline hæmatin, for in the latter the whole of the violet and ultra-violet are darkened. On reducing oxidised hæmochromogen, the band of reduced hæmochromogen reappears in the violet.

Hæmochromogen differs from hæmatin, but resembles hæmoglobin in being able to combine with carbonic oxide to form carboxy-hæmochromogen, according to Hoppe-Seyler[4] and Küster.

[1] A. Gamgee, *Physiol. Chem.* **1**. 111 (1880).
[2] Hoppe-Seyler, *Zeitschr. f. physiol. Chem.* **13**. 496 (1889).
[3] P. P. Laidlaw, *Journ. of Physiol.* **31**. 467 (1904).
[4] F. Hoppe-Seyler, *Zeitschr. f. physiol. Chem.* **13**. 477 (1889).

While, according to Hoppe-Seyler, hæmochromogen is the iron-containing radical which, by its union with an albuminous substance, forms hæmoglobin and then becomes enabled to absorb oxygen,[1] Gamgee[2] stated, in 1898, that "probably hæmochromogen does not exist preformed in hæmoglobin and its compounds." He has promised to throw more light on this question.

Hæmatoporphyrin

The methods of preparing hæmatoporphyrin from hæmatin have already been referred to on p. 509. Mulder's[3] iron-free hæmatin, or hæmatoporphyrin, to use Hoppe-Seyler's terminology, may be obtained by either the action of concentrated H_2SO_4 on hæmatin; by treating hæmin with a saturated solution of hydrobromic acid in glacial acetic acid; by the reduction of acid alcoholic hæmatin solutions; by acting on hæmochromogen or hæmoglobin with dilute mineral acids (see p. 508). The hæmatoporphyrin prepared from hæmatin with strong H_2SO_4 is the anhydride of the preparation obtained with hydrobromic acid, according to Nencki and Sieber.[4] The H_2SO_4 preparation is almost insoluble in alcohol, ether, and dilute acids, but readily soluble in alkalies; the Br-preparation is readily soluble in fixed alkalies and their carbonates, and also in dilute mineral acids and alcohol, but only slightly soluble in ether, amyl-alcohol, benzene, and chloroform, somewhat more soluble in acidified amyl-alcohol and acetic ether, and almost insoluble in water and dilute acids.

The reconversion of hæmatoporphyrin into hæmatin by the incorporation of iron has also been accomplished:—Struck by the ease with which iron is removable from reduced hæmatin or hæmochromogen, Laidlaw attempted to replace the iron and was successful. To 1 gramme of hæmatoporphyrin prepared by Nencki's method, dissolved in dilute ammonia and warmed on the water-bath in a flask, to exclude air as much as possible, were added some Stoke's fluid prepared from 2 grammes of ferrous sulphate (see p. 478) and a few drops of a 50 per cent solution of hydrazin-hydrate. After one to two hours, care having been taken to replace the evaporated ammonia and to keep the mixture thoroughly reduced by hydrazin, a solution is obtained which shows the absorption-bands of hæmochromogen. On shaking the solution with air it is converted into alkaline hæmatin.

[1] Hoppe-Seyler, *Zeitschr. f. physiol. Chem.* **13**. 492-493 (1889).

[2] A. Gamgee, *Schäfer's Textbook of Physiol.* **1**. 258 (1898).

[3] Mulder, 'Über eisenfreies Hæmatin,' *Journ. f. prakt. Chem.* **32**. 186 (1844).

[4] Nencki and Sieber, *Sitzb. d. kais. Akad. de Wiss. Wien.* 1889, year 1888, vol. 97, p. 80; and *Arch. f. experim. Pathol. u. Pharm.* **24**. 430 (1888).

To separate the hæmatin proceed as follows: Pour the mixture from the flask into a large evaporating dish; add some strong potash; evaporate ammonia to throw down excess of iron; and a solution of hæmatin in potash remains as the hydrazin is decomposed by the free exposure to the air. Now throw down the hæmatin by the addition of acid, collect the precipitate on a filter-paper, and wash it in dilute HCl till all traces of hæmatoporphyrin have disappeared. Redissolve the precipitated hæmatin, and throw it down once more by the addition of acid.

Hæmatoporphyrin is an acid, forming mono- and di-basic metallic salts; but it also forms a salt with hydrochloric acid which, when recrystallised from alcohol, forms brownish-red needles. Hæmatoporphyrin is precipitated by acetic acid and by barium- and calcium-hydrates. Solutions of hæmatoporphyrin, even if extraordinarily dilute, exhibit a magnificent red fluorescence (Gamgee).[1] The alcoholic solution possesses a beautiful red colour, which on the addition of alkalies becomes more yellow, while by acids it is converted into a violet colour.

In neutral alcoholic solutions hæmatoporphyrin shows five bands, according to Garrod[2] and Schulz:[3]

I. α $\lambda 625$–$\lambda 617$
II. β $\lambda 605$–$\lambda 599$
III. γ $\lambda 584$–$\lambda 555$ { 1. $\lambda 573$–$\lambda 568$; 2. $\lambda 566$–$\lambda 562$; 3. $\lambda 558$–$\lambda 555$ }
IV. δ $\lambda 543$–$\lambda 525$
V. ϵ $\lambda 514$–$\lambda 486$

The Figs. I.-V. on this and the next page show what bands correspond with one another, according to Schulz.

Schulz, for an alcoholic solution containing 1 per cent of H_2SO_4, gives the following bands:

I. α $\lambda 600$–$\lambda 588$
III. β* $\lambda 580$–$\lambda 539$ { 1. $\lambda 580$–$\lambda 571$; 2 + 3. $\lambda 564$–$\lambda 539$ }
IV. γ Absorption from $\lambda 539$–$\lambda 505$ { 1. $\lambda 530$–$\lambda 525$; 2. $\lambda 518$–$\lambda 506$ }

In alcoholic solutions containing 20 per cent of a 25 per cent watery solution of ammonia Schulz saw four bands:

[1] A. Gamgee, *Schäfer's Textbook of Physiol.* **1**. 259 (1898).
[2] Garrod, *Journ. of Physiol.* **13**. 598 (1892), and **15**. 108 (1894).
[3] Schulz, *Arch. f.* (*Anat. u.*) *Physiol.* 1904, Suppl. 270.

I. α λ624–λ614
III. β λ584–λ563
λ571–λ563 darkest
IV. γ λ543–λ525
V. δ λ516–λ485

The so-called 'metallic spectrum' of hæmatoporphyrin, first described by MacMunn in 1889, and then by Hammarsten[1] and Garrod,[2] Schulz obtained by adding zinc chloride to an ammoniacal solution:

III. α λ589–λ570
IV. β λ560–λ526
V. γ λ512–λ501 (See below.)

In acid solutions it possesses a spectrum with two well-marked and several less distinct absorption-bands. The α-band lies between C and D, close to D, while the second or β-band is most intense halfway between D and E; its faint edge almost reaches D.

α λ597–λ587 (Garrod[3] and Nebelthau[4])
β λ557–λ541 „ „

In alkaline solutions it shows four bands (Garrod,[2] Nebelthau,[4] and Gamgee):

α Between C and D . . . λ621–λ610
β Between D and E, close to D . λ590–λ572
γ Between D and E, close to E . λ555–λ528
δ Between *b* and F λ514–λ498
ϵ Between *h* and H, and in strong solutions to K and beyond.

All these bands may be shifted more towards the red or towards the violet end of the spectrum, according to the amount of ammonia or alkali present in the solution. In addition, the method of preparing alkaline hæmatoporphyrin also alters the spectrum. On the addition of an alkaline zinc acetate solution the bands α and δ disappear, while the bands β and γ become sharper and more intense. The ϵ-band is seen in all hæmatoporphyrin-solutions whatever the reaction may be, but it is more evident in alkaline solutions (Gamgee). Hæmatoporphyrin has been found in the urine of people

[1] Hammarsten, *Skandin. Archiv. f. Physiol.* **3**. 319 (1892).
[2] Garrod, *Journ. of Physiol.* **13**. 598 (1892).
[3] A. F. Garrod, *ibid.* **13**. 603 (1892); **17**. 349 (1895).
[4] E. Nebelthau, *Zeitschr. f. physiol. Chem.* **27**. 324 (1899). (Full literature is given.)

suffering from sulphonal poisoning, and also in other diseases, and even in healthy people by MacMunn,[1] Salkowski,[2] Hammarsten,[3] Garrod,[4] Riva and Zoja,[5] and Nebelthau.[6] It is precipitated from urine by the addition of barium- or calcium-hydrate, but most simply by acetic acid, according to Nebelthau. The urine may at once show a burgundy colour, or only develop the same after standing for some time, owing to the hæmatoporphyrin being formed from a colourless chromogen.

Hæmatoporphyrin contains two hydroxyl groups,[7] which may be replaced by methyl groups, there being formed a dimethyl-hæmatoporphyrin.

Mesoporphyrin

This compound was obtained by Nencki and Zaleski[8] by carefully reducing hæmatoporphyrin. According to Zaleski,[9] mesoporphyrin differs from hæmatoporphyrin in containing two oxygen atoms less, owing to two hydroxyl groups of hæmatoporphyrin having been split off (see p. 465). Hæmato- and meso-porphyrin "show in acid and alkaline, in alcoholic and watery solutions the same general distribution of absorption-bands, and only by examining simultaneously the solutions of hæmato- and meso-porphyrin is it possible to see that in the latter all absorption-bands are shifted somewhat towards the violet end of the spectrum" (Marchlewski).[10] Mesoporphyrin also closely resembles hæmatoporphyrin as regards ethereal and ordinary salt formations.

Hæmopyrrol is described by Nencki and Zaleski[11] (see pp. 511, 529).

Hæmatinic Acids

Hæmatinic acids[12] are described in the various papers of Küster (see p. 511).

[1] C. A. MacMunn, *Proc. Roy. Soc. Lond.* **31**. 211 (1880-81), and *Journ. of Physiol.* **6**. 36 (1885), **7**. 243, 249 (1886), **10**. 71 (1889).

[2] E. Salkowski, *Zeitschr. f. physiol. Chem.* **15**. 286 (1891).

[3] O. Hammarsten, *Skandinav. Arch. f. Physiol.* **3**. 319 (1891).

[4] A. F. Garrod, *Journ. of Physiol.* **13**. 603 (1892); **17**. 349 (1895).

[5] A. Riva and L. Zoja, *Maly's Jahresberichte f. Tierchemie*, **24**. 673 (1894).

[6] E. Nebelthau, *Zeits. f. phys. Chem.* **27**. 324 (1899). (Full literature is given.)

[7] M. Nencki and J. Zaleski, *ibid.* **30**. 384 (1900).

[8] M. Nencki and J. Zaleski, *Ber. d. deutsch. chem. Ges.* **34**. I. 997 (1901).

[9] J. Zaleski, *Zeitschr. f. physiol. Chem.* **37**. 54 (1902).

[10] L. Marchlewski, *Anz. Akad. Wiss. Krakau.* 1902, April.

[11] M. Nencki and J. Zaleski, *Ber. d. deutsch. chem. Ges.* **34**. I. 997 (1901).

[12] W. Küster, *Liebig's Annalen*, **315**. 174 (1900); M. Kölle, *Dissertation*, Tübingen, 1898.

Relationships of Hæmatoporphyrin to other naturally occurring Colouring Matters

PHYLLOPORPHYRIN.—From the green colouring matter of plants, the chlorophyll,[1] and from its dissociation product phyllotaonin, Schunk and Marchlewski[2] and Marchlewski[3] have prepared a colouring matter which they call phylloporphyrin. It has the composition:

$$C_{16}H_{18}N_2O,$$

and differs only from hæmatoporphyrin in possessing two oxygen atoms less.[4] Marchlewski[3] obtained from it both hæmopyrrol and also hæmatinic acids. Nencki and Zaleski have made the attempt to convert hæmatoporphyrin into phylloporphyrin, but only succeeded in removing one of the two hydroxyls of hæmatoporphyrin, and so reached a product intermediate between hæmatoporphyrin and phylloporphyrin, which they therefore called mesoporphyrin (see p. 527). Spectroscopically mesoporphyrin already resembles phylloporphyrin, according to Marchlewski.

The red colouring matter of the blood and the green chlorophyll of plants being closely allied substances, are also probably related to the lipochromes (Marchlewski).[5]

HÆMATOIDIN.—Derivatives of hæmatoporphyrin occur also in the animal body, for Virchow[6] discovered in 1847 in blood extravasations hæmatoidin in well-formed rhombic crystals of a brick-red or deep ruby colour. According to Nencki and Zaleski[7] hæmatoidin is identical with mesoporphyrin, as both exhibit the same colour changes.

UROBILIN is a reduction compound of hæmatin or hæmatoporphyrin, as has been shown by Hoppe-Seyler,[8] Nencki and Sieber,[9] and le Noble.[10] Urobilin occurs normally in the urine and in the fæces, and is also

[1] L. Marchlewski, *Die Chem. d. Chloroph.* Leipzig and Hamburg, L. Voss (1905).

[2] E. Schunck and Marchlewski, *Liebig's Annalen*, **278**. 329 (1894); **284**. 81 (1895); **288**. 209 (1895); **290**. 306 (1896).

[3] L. Marchlewski, *Bull. de l'Acad. des Sciences de Cracovie, Cl. Math. et Nat.* 1902, January and April, pp. 1 and 223.

[4] M. Nencki, *Ber. d. deutsch. chem. Ges.* **29**. III. 2877 (1896); M. Nencki and J. Zaleski, *ibid.* **34**. I. 997 (1901); compare also with the papers by Nencki, Sieber, and Steudel quoted on p. 508.

[5] L. Marchlewski, *Zeitschr. f. physiol. Chem.* **38**. 196 (1903).

[6] R. Virchow, *Virchow's Archiv.* **1**. 379 and 411 (1847).

[7] M. Nencki and J. Zaleski, *Ber. d. deutsch. chem. Ges.* **34**. I. 997 (1901).

[8] Hoppe-Seyler, *ibid.* **7**. II. 1065 (1874).

[9] M. Nencki and N. Sieber, *Arch. f. experim. Path. u. Pharm.* **18**. 401 (1884); *Ber. d. deutsch. chem. Ges.* **17**. II. 2270 (1884); *Monatsh. f. Chem.* **9**. 115 (1889).

[10] C. le Nobel, *Pflüger's Archiv.* **40**. 501 (1887).

formed when hæmopyrrol is oxidised by the air.[1] If rabbits be fed on hæmopyrrol they excrete urobilin. It contains, as does hæmatin, four molecules of hæmopyrrol (Nencki and Zaleski),[1] and possesses, according to Maly,[2] the formula :

$$C_{32}H_{40}O_7N_4.$$

BILIRUBIN.—This colouring matter of the bile is also a derivative of hæmatoporphyrin, for Virchow,[3] Jaffe,[4] and Salkowski[5] have shown that bilirubin greatly resembles hæmatoidin or mesoporphyrin, if it is not identical with it, and Maly[2] has further prepared urobilin—or hydrobilirubin—by a simple reduction of bilirubin. Küster,[6] finally, has succeeded in getting the same hæmatinic acids from bilirubin as he obtained from hæmatin. (For a fuller account of these pigments see Roscoe and Schorlemmer's *Handbook of Organic Chemistry*, **9**. 309 (1901).)

HÆMOCYANIN. — Instead of the iron-containing hæmoglobin, cephalopods possess in their blood a proteid containing copper. This copper-albuminate the discoverer, Frédéricq,[7] has called hæmocyanin. Subsequently it has been very thoroughly investigated by Henze,[8] who was the first to prepare it in a pure state. Hæmocyanin may be obtained in well-formed crystals by using the Hofmeister-Hopkins method of salting out (see p. 325). Henze obtained the following percentage composition :

$$C_{53.66}H_{7.33}N_{16.09}S_{0.86}Cu_{0.38}O_{21.67}.$$

It gave all the colour- and precipitation tests of albumins and the biuret reaction, without copper having to be added. It is soluble in water, in salt solutions, and in alkalies. Magnesium sulphate does not salt out, and the limits for ammonium sulphate lie between 3·5, and 10. Its coagulation-temperature lies between 68° and 72°. Towards acids it is as sensitive as is hæmoglobin, becoming decomposed into albumin and copper, but hæmocyanin is not a copper salt, as it does not give the reactions of copper-ions without having been decomposed.

Hæmocyanin binds oxygen, and gives off the latter, when a stream of hydrogen, carbonic oxide, and especially carbon dioxide[7] is passed

[1] M. Nencki and J. Zaleski, *Ber. d. deutsch. chem. Ges.* **34**. I. 997 (1901).

[2] R. Maly, *Zentralbl. f. d. med. Wiss.* 1871, No. 54 ; *Liebig's Annalen*, **161**. 368 (1872) ; **163**. 77 (1872) ; *Pflüger's Archiv*, **20**. 331 (1879).

[3] R. Virchow, *Virchow's Archiv*, **1**. 379 and 411 (1847).

[4] M. Jaffe, *Virchow's Archiv*, **47**. 405 (1869) ; *Zentralbl. f. d. med. Wiss.* 1869, p. 177.

[5] E. Salkowski, *Hoppe-Seyler's Med.-chem. Unters.* p. 436 (1871).

[6] W. Küster, *Ber. d. deutsch. chem. Ges.* **35**. II. 1268 (1902).

[7] L. Frédéricq, *Arch. de Zool. expér.* **7**. 535 (1878).

[8] M. Henze, *Zeitschr. f. physiol. Chem.* **33**. 370 (1901). Here the older literature.

through the solution. When reduced it forms a colourless compound, but when oxidised it exhibits a pure blue. Henze[1] has analysed hæmocyanin and found its nitrogen to be distributed in the following manner:

ammonia-	N	. .	0·73— 1·08 per cent
di-amino-	N	. .	4·34— 4·70 per cent
mono-amino-	N	. .	9·50—10·66 per cent
hæmin-	N	. .	0·26— 0·66 per cent

In the mono-amino acid fraction were found leucin, tyrosin, and glutaminic acid; while the di-amino acids are represented by lysin and histidin; arginin could not be demonstrated with certainty, nor could a carbohydrate radical, although hæmocyanin gives a positive reaction with Molisch's test.

According to Krukenberg[2] hæmocyanin shows no absorption bands. The oxygen capacity has not yet been determined, but is, according to Henze, less than that of hæmoglobin. Hæmocyanin is the only albuminous substance found in the blood of cephalopods, and it subserves respiratory purposes. Halliburton[3] has given a list of animals in which hæmocyanin has so far been found.

Phyco-erythrin, the red colouring matter met with in certain seaweeds, the Florideæ, frequently crystallises out at the death of the cells. Molisch[4] first recognised its albuminous character; he isolated it, and succeeded in getting it to crystallise from its solutions.

Phycocyan is a similar, blue colouring matter found in the Cyanophyceæ. It was obtained in crystals belonging to the monoclinic system by Molisch,[5] who employed Hofmeister's method of fractional salting out. The crystals give the Millon and xanthoproteic reactions; they are converted by alcohol into pseudomorphoses, and unite with acid and basic dye-stuffs.

Colouring Matter from the Fins of Crenilabrus pavo.—v. Zeynek[6] has observed in the fish Crenilabrus pavo a blue colouring matter during the spring time. He has isolated it and shown it to be an albuminous compound.

II. The Glyco-Proteids

The glyco-proteids are albuminous substances, amongst the dissociation-products of which is found a carbohydrate or the derivative of a carbohydrate.

[1] M. Henze, *Zeitschr. f. physiol. Chem.* **43**. 290 (1904).
[2] F. C. W. Krukenberg, *Zentralb. f. d. med. Wiss.* 1880, No. 23.
[3] W. D. Halliburton, *Journ. of Physiol.* **6**. 300 (1885).
[4] H. Molisch, *Bot. Zeitschr.* 1894, p. 177. [5] H. Molisch, *ibid.* 1895, p. 131.
[6] R. v. Zeynek, *Zeitschr. f. physiol. Chem.* **34**. 148 (1901); **36**. 668 (1902).

The properties of this carbohydrate have already been discussed from p. 154 to p. 164. It is an unknown aminated polysaccharid which does not reduce, and the amino-group of which is not free; on being boiled with alkalies or with acids it gives rise to glucosamin. In the case of the mucin forming the covering of the eggs in frog's spawn glucosamin is replaced by galactosamin, according to Schulz and Ditthorn;[1] while in chondro-mucoid its place is taken by a hexo-amino acid, according to Orgler and Neuberg.[2] Neuberg[3] has found in the albumin prepared from the yolk of the egg, in addition to glucosamin, another carbohydrate acid.

Glyco-proteids differ from the nucleo-proteids and from hæmoglobin in not readily dissociating into the albumin moiety and the prosthetic group. The carbohydrate is only set free by boiling with mineral acids or by the intense action of alkalies, and by both of these procedures the albumin fraction is broken up into crystalline dissociation-products or at least into albumoses. For this reason, and also because other albumins contain a carbohydrate-radical (p. 356), it is doubtful whether we are justified in making a special group of "glyco-proteids." It is quite possible that in the so-called glyco-proteids we are only dealing with a group of albumins in which one of the dissociation-products, namely, the sugar-radical, is present in a larger amount than in the ordinary albumins.

To the glyco-proteids belong the mucins and related compounds, the egg-albumin and the little understood phospho-glyco-proteid. Egg-albumin has already been dealt with amongst the albumins on p. 353, and, therefore, only the sharply defined and readily recognisable class of mucins and mucoids will be discussed now.

Eichwald[4] was the first to observe that a reducing substance may be separated from mucin, and he was the first to regard mucins as composed of an albumin + a sugar-radical. The nature of the mucins was subsequently most thoroughly investigated by Hammarsten, and he defined accurately the features of this group of proteids. The sugar-radical has been most thoroughly investigated by Müller, as already explained on pp. 154-164.

The mucins and mucoids are acid compounds, containing no phosphorus, and yielding a reducing substance on being boiled with acids. Their percentage-composition is remarkable for the low carbon-

[1] F. N. Schulz and F. Ditthorn, *Zeitschr. f. physiol. Chem.* **29**. 373 (1900); **32**. 428 (1901).

[2] A. Orgler and C. Neuberg, *ibid.* **37**. 407 (1903).

[3] C. Neuberg, *Ber. d. deutsch. chem. Ges.* **34**. III. 3963 (1901).

[4] A. Eichwald, *Liebig's Annalen* **134**. 177 (1865).

and nitrogen- and the high oxygen-content, and this is owing to the presence of the carbohydrate group which is rich in oxygen. The high percentage of oxygen results, of course, in a low heat value.[1] Glyco-proteids contain also a relatively large amount of sulphur. Little is known regarding their dissociation-products. Obolensky[2] obtained from an impure mucin, prepared from the submaxillary gland, leucin and tyrosin; Mitjukoff[3] from a pseudo-mucin, lysin and arginin. On decomposing pseudo-mucin by means of strong mineral acids Otori[4] found pseudo-mucin to differ from paramucin in only giving rise to small amounts of humin substances, namely, 2·6265 grammes of humin from 43·369 dry, ash-free (calculated) pseudo-mucin; while Mitjukoff[3] obtained very large amounts from paramucin. Otori gives the following table regarding the composition of pseudo-mucin :—

100 parts of pseudo-mucin yield in	grammes.
Ammonia	0·7517
Guanidin	0·0393
Arginin	0·2875
Lysin	2·6389
Tyrosin	1·089
Leucin	4·677
Oxalic acid	0·1275
Lævulinic acid	1·971
Reducing substance calculated as grape-sugar	0·7333
Insoluble humin substance	6·056

The amount of carbohydrate present varies greatly, being from 3 to 37 per cent. In the submaxillary mucin Müller and Seemann[5] found 42 per cent glucosamin.

All mucins give a violet biuret-reaction like the ordinary albumins, and also the xantho-proteic and lead-sulphide reactions and the tests of Millon and Molisch. The physical properties of the true mucins are discussed below.

The mucins and mucoids are not coagulated by heat, and differ in this respect markedly from the native albumins and from most of the proteids. They become, however, denaturalised when they are acted upon by acids and especially by alkalies, or by alcohol and other precipitating reagents, for they then no longer show their normal mucilaginous character. This transformation or dissociation resembles

[1] P. B. Hawk and W. J. Gies, *Amer. Journ. of Physiol.* V. 387 (1901).
[2] Obolensky, *Hoppe-Seyler's Med.-chem. Unters.* p. 590 (1871).
[3] Kath. Mitjukoff, Dissertation, Bern, *Arch. f. Gynäkol.* **49**. 278 (1895).
[4] J. Otori, *Zeitschr. f. physiol. Chem.* **42**. 453 (1904).
[5] F. Müller and J. Seemann, *Deutsche med. Wochenschr.* 1899, p. 209.

the denaturalisation of the true albumins in being a permanent, irreversible one. Glyco-proteids are pronounced acids, for they redden litmus paper and are precipitable by stronger acids. They resemble the nucleo-albumins in being uncoagulable by heat (which does not exclude the possibility that they become denaturalised) and in being acid in character, but they differ from the nucleo-albumins in possessing no phosphorus and in containing a carbohydrate. Being acids, most glyco-proteids are precipitated by acetic acid, but they are only slightly soluble in an excess of this acid, while globulins, nucleo-albumins, and nucleo-proteids are readily soluble in an excess of acetic acid. Mineral acids also precipitate glyco-proteids, but the precipitate dissolves more readily in an excess of such acids. In solutions of alkalies, alkali-carbonates, and in ammonia, all glyco-proteids are readily soluble, neutral and in some cases even acid salts being formed. Excess of an alkali, however small, quickly denaturalises and decomposes glyco-proteids.

(a) The Mucins

Mucins are found in most of the slimy fluids occurring in the body, and they cause the sliminess. Even when greatly diluted they form more or less adhesive solutions, which may be pulled out into viscous threads. They are excreted normally, partly by the goblet cells found on the surface of all mucous membranes, such as the respiratory and alimentary systems, the bile-ducts, urinary passages, etc., and partly by deeply situated mucous glands, and in particular by the submaxillary gland. Mucins are also found amongst invertebrates —for example, in snails, the skin of which is covered with mucin. Other bodies closely related to the mucins and forming the transition to the mucoids are found in connective tissues—for example, in tendons, the vitreous humour, the umbilical cord, etc., and will be discussed under the heading of the mucoids. In some animals the mucins are replaced by nucleo-proteids, which latter also possess the same slimy character.

The mucin of the submaxillary gland of the ox, apart from older investigations, has been studied specially by Obolensky [1] and Landwehr,[2] by Hammarsten [3] and his pupil Folin ; [4] the mucin of the respiratory tract by Friedrich Müller ; [5] that of the bile by Landwehr,[2]

[1] Obolensky, *Hoppe-Seyler's Med.-chem. Untersuch.* p. 590 (1871).

[2] H. A. Landwehr, *Zeits. f. phys. Chem.* **5**. 371 (1881) ; **6**. 74 (1881) ; **9**. 361 (1885).

[3] O. Hammarsten, *ibid.* **12**. 163 (1887). [4] O. Folin, *ibid.* **23**. 347 (1897).

[5] Friedrich Müller, *Zeitschr. f. Biol.* **42**. 468 (1901), (here will be found a review of the older papers by himself and his pupils).

Hammarsten,[1] Neumeister,[2] Winternitz,[3] and Brauer;[4] the mucins of the snail[5] and the eggs of the perch[6] by Hammarsten; that of frog's spawn by Giacosa[7] and Schulz and Ditthorn;[8] that of hag-fish (Myxina) by Waymouth Reid.[9] The mucins of the gastric and the intestinal mucous membranes have not been specially investigated, although judging by histological staining reactions the gastric mucous cells are much less acid than are the intestinal ones (Mann). The glucosamin prepared from these two mucins is, however, the same, and also resembles that prepared from the mucin of the respiratory tract. The mucilaginous substances of the ovarial cysts are discussed below. The following percentage analyses have been made:—

C	H	N	S	O		
48·84	6·80	12·32	0·84	31·2	Submaxillary gland (ox)	Hammarsten.[10]
48·17	6·91	10·8	1·42	31·7	Sputum	Müller.[11]
50·3	6·84	13·62	1·71	27·53	Snail, mantle	Hammarsten.[5]
50·45	6·79	13·66	1·6	27·50	Snail, foot	Hammarsten.[5]
49·09	6·69	13·04	1·54	...	Eggs of perch	Hammarsten.[6]
52·90	7·2	9·24	1·32	29·34	Frog's spawn	Giacosa.[7]
43·92	6·03	8·8	...	...	Frog's spawn	Schulz and Ditthorn.[8]
49·8	6·9	10·27	1·25	31·78	Pseudo-mucin (ovaries)	Hammarsten.[12]
49·65	7·64	11·16	...	...		Otori.[13]
51·76	7·76	10·7	1·09	28·69	Paramucin (ovaries)	Mitjukoff.[14]

Whether the great differences shown depend on the existence of different mucins or on the want of purity of the preparations is uncertain. In their reactions the different mucins so closely resemble one another that the description of the carefully examined submaxillary mucin, given by Hammarsten, holds good for all the other mucins. For methods of preparation see under bile-mucin, p. 537.

[1] O. Hammarsten, *Königl. Gesellsch. der Wissensch. zu Upsala*, June 15, 1893.

[2] R. Neumeister, *Sitzungsber. der Würzburger physikal.-medizin. Gesellsch.* March 8, 1890 (reprint).

[3] H. Winternitz, *Zeitschr. f. physiol. Chem.* **21**. 387 (1895).

[4] L. Brauer, *ibid.* **40**. 182 (1903).

[5] O. Hammarsten, *Pflüger's Arch. f. d. ges. Physiol.* **36**. 373 (1885).

[6] O. Hammarsten, *Skand. Arch. f. Physiol.* **17**. 13 (1905).

[7] Piero Giacosa, *Zeitschr. f. physiol. Chem.* **7**. 40 (1882).

[8] F. N. Schulz and F. Ditthorn, *ibid.* **29**. 373 (1900); **32**. 428 (1901).

[9] Waymouth Reid, *Journ. of Physiol.* **13**. 340 (1893).

[10] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **12**. 163 (1887).

[11] Fr. Müller, *Zeitschr. f. Biolog.* **42**. 468 (1901), (the older work by himself and by his pupils is here reviewed).

[12] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **6**. 194 (1882).

[13] J. Otori, *ibid.* **42**. 453 (1904), (the percentage calculated from a supposed ash-free substance).

[14] K. Mitjukoff, Dissertation, Bern, and in *Archiv f. Gynäkol.* **49**. 42 (1895).

1. *Submaxillary Mucin*

Mucin forms a white, loose, hardly hygroscopic powder, and may be preserved in this dry state for years without its properties becoming altered. It is only with difficulty soluble in water and neutral salt-solutions; it is insoluble in acids, but on the addition of acetic acid it gives rise to a tough, adhesive curd. It is, however, readily soluble in very dilute alkalies, and then forms a neutral or in some instances a slightly acid solution. Mucins are therefore pronounced acids. A solution containing 0·228 per cent of mucin behaves like a typical mucilaginous solution, being viscous, adhesive, and readily pulled out into threads. From its solutions it is precipitated by acids, specially by acetic acid, but the precipitate, instead of being flocculent, as in the case of ordinary albumins, forms a tough, mucilaginous mass which, if stirred with a glass rod, winds round the latter.

Mucin is either not soluble or only soluble to a slight extent in an excess of acetic acid, while it is readily dissolved by 0·1 to 0·2 per cent HCl, but not so readily as are the nucleo-albumins and the globulins. Mucin is precipitated by acids only if the solutions are poor in salts; it is not precipitated in the presence of sodium chloride or other neutral salts. Mucin, like all other glyco-proteids, is not coagulated on being boiled, and the addition of acetic acid to a boiling solution of mucin does not give rise to a more abundant precipitate than can be obtained by adding acetic acid to a cold solution, and if to the boiling mucin-solution some sodium chloride be added, acetic acid will again cause no precipitate. Brauer has made use of this property in demonstrating coagulable albumin in addition to mucin in pathological bile: he very slightly acidifies the bile, which already contains salts, and then boils it: the coagulable albumin is thrown down, while the unaltered mucin remains in solution. Mucin is not precipitated by alcohol except a sufficient amount of neutral salts be present; in the absence of salts, alcohol only gives rise to a more or less marked opalescence. Mucin is precipitated by nitric acid, and also by copper sulphate, mercuric chloride, ferric chloride, and lead acetate. Potassium bichromate and alum do not give rise to a precipitate, but convert mucin into a slimy, swollen mass. The alkaloidal reagents, tannin, mercury + potassium iodide, etc., in neutral solutions do not precipitate, but they do throw down mucin which has been rendered soluble by the addition of an excess of hydrochloric acid. Potassium ferrocyanide does not precipitate, but, at the most, renders the solution somewhat more viscous; it resembles the neutral salts in preventing the precipitation by means of acids.

Mucin is salted out by saturated sodium-chloride and magnesium-sulphate solutions; the limits for ammonium sulphate in the case of bile mucin are 3·2 and 4·6, according to Brauer.

Mucin is very resistant to acids, but is readily denaturalised by alkalies; if it be kept for some time in feebly alkaline solutions, it is at first still precipitable by means of acetic acid like a typical mucin, but soon it gives rise to a slight flocculent precipitate, and finally the whole of the mucin is thrown down as flocculi, and whenever this happens the solution has lost its typical slimy character, and has become a limpid solution. The mucin is changed by alkalies into an alkali-albuminate, and then possesses different properties and a different composition, for it is now readily precipitated by salts, and may be precipitated by acids if great care be taken in exactly neutralising the solution, as the neutralisation-precipitate is at once dissolved by the smallest excess of acid. The alkali-albuminate differs from mucin further in being precipitated by potassium ferrocyanide + acetic acid. If stronger solutions of alkali are allowed to act on mucin a well-marked giving off of ammonia may be observed. Similar observations were made by Drechsel and Mitjukoff[1] with the closely related para-mucin, and by K. A. H. Mörner[2] and others with the mucoids. In addition to alkali-albuminates, there occur, after some time, in denaturalised mucin solutions, also albumoses possessing the usual characteristics.

Animal gum, discussed on p. 538, is formed by the action of stronger alkalies. (See also the index.)

With pepsin and trypsin, mucin dissolves to a clear, watery solution, containing probably albumoses, according to Friedrich Müller[3] and Mitjukoff;[1] a splitting off of a carbohydrate or other well-marked radical has not been observed.

Towards putrefaction, according to Müller[3] and Giacosa,[4] mucins are very resistant, "as their peculiar physical property makes the entrance of putrefactive bacteria a difficult matter, and as bactericidal bodies may also play a part" (Cohnheim). The real reason, according to experiments made by the author, is the acid nature of the mucins.

With alkalies and the alkaline earths mucin forms soluble soaps; the naturally occurring mucin is sodium mucinate, according to Müller.

To prepare chemically pure mucins is very difficult, as even in strongly mucilaginous fluids they are present in only very minute

[1] Kath. Mitjukoff, Dissert., Bern, *Arch. f. Gynäk.* **49**. fasc. 2 (1895).
[2] K. A. H. Mörner, *Scandinav. Arch. f. Physiol.* **6**. 332 (1895).
[3] Fr. Müller, *Zeitschr. f. Biol.* **42**. 468 (1901).
[4] P. Giacosa, *Zeitschr. f. physiol. Chem.* **7**. 40 (1882).

quantities. Mucin-solutions do not filter at all readily, and the tough mucin-coagula settle only very slowly. Finally, there is always the danger of denaturalisation by means of alkalies or by alcohol.

2. *Bile Mucin*

From the bile of man and dogs mucin may be directly precipitated by the addition of acetic acid or of alcohol, as no other albuminous substances are present, but a disadvantage is that this mucin is contaminated by bile salts and bile pigments, which Paijkull [1] had great difficulty in removing by long-continued dialysis. The mucin of the submaxillary gland Hammarsten prepares as follows: The gland is extracted with water, and then hydrochloric acid is added to the extract to the extent of 0·1 to 0·2 per cent. By this means mucin and the abundantly present nucleo-proteid are both thrown down, but they soon pass into solution again. On diluting this solution containing hydrochloric acid with four times its volume of distilled water, the mucin is again precipitated, while the nucleo-proteid is still kept in solution by the dilute hydrochloric acid. The mucin so obtained is then carefully dissolved in very dilute caustic-potash solution, or even better in ammonia, every precaution being taken to prevent the reaction from becoming alkaline. The dissolved mucin is then precipitated with acetic acid; again dissolved in an alkali, reprecipitated with acetic acid, and this procedure repeated once more. The mucin from the snail was prepared similarly.

3. *Bronchial Mucin*

The mucin of the respiratory passages Friedrich Müller [2] prepared from the glassy, purely mucous sputum of patients suffering from chronic bronchitis. The mucin is freed as much as possible from admixtures of pus, food particles, etc., and is then precipitated with alcohol; the albumin and nuclein are thrown down as flocculent precipitates, while the mucin separates out as fine fibres, which may be separated mechanically from the albumin, etc. The mucin is now repeatedly washed with very dilute hydrochloric acid (0·1-0·2 per cent) and soda-solution, dissolved in very dilute caustic soda, precipitated with acetic acid, and the fibrous precipitate purified by dialysis. The mucin obtained in this way is free from albumin and nucleo-proteids, and with dilute soda-solution still gives an opalescent mucilaginous solution.

[1] S. Paijkull, *Zeitschr. f. physiol. Chem.* **12**. 196 (1887).

[2] Fr. Müller, *Zeitschr. f. Biol.* **42**. 468 (1901).

4. *Snail Mucin*

The mucin of the vineyard snail, *Helix pomatia*, differs in many respects from that of vertebrates; it is relatively readily accessible, and has therefore been investigated repeatedly. The most minute examination has been made by Hammarsten.[1] The mucin is not secreted as such, but as a mucinogen which does not dissolve readily even in alkalies; when dissolved it forms a tough, not very mucous fluid, which gives the reactions of mucin, but which is not precipitated by corrosive sublimate. By the action of alkalies, or much more slowly by mere standing in watery solutions, this mucinogen is converted into typical mucin. The phenomenon that mucous glands excrete mucinogen, which then becomes converted into mucin, has also been observed by v. Uexküll[2] in the case of the sea-urchin, where the change is brought about by the action of sea-water, and analogous instances seem to be common amongst the invertebrates. The mucin of the salivary glands of vertebrates does not pass through the mucinogen stage, according to Holmgren,[3] but is from the very first a true mucin. Considering, however, how readily the granules in mucous cells are altered under the slightest provocation, the existence of a mucinogen stage cannot, according to the author's opinion, be denied for vertebrates.

The mucin of the eggs of the perch, according to Hammarsten,[4] is in ripe eggs normally present as a mucinogen with traces of mucin. In unripe eggs there is comparatively much mucin, and hence during the ripening of eggs mucin is converted into mucinogen. It yields pseudo-mucin and para-mucin.

5. *Pseudo-Mucin*

Scherer[5] described in 1852 two substances from the contents of an ovarial cyst which he called "metalbumin" and "paralbumin." He and subsequently Eichwald[5] could split off a sugar-radical from both these substances, and Landwehr[6] prepared from them, as he did out of mucin, animal gum. These substances have been investigated more thoroughly by Hammarsten,[7] who first called

[1] O. Hammarsten, *Pflüger's Arch.* **36**. 373 (1885).

[2] J. v. Uexküll, *Zeitschr. f. Biol.* **37**. 334 (p. 388) (1899).

[3] E. Holmgren, Hammarsten's account of the Swedish papers in *Maly's Jahresbericht für Tierchemie*, **27**. 36 (1897).

[4] O. Hammarsten, *Skand. Arch. f. Physiol.* **17**. 13 (1905).

[5] According to Hammarsten.

[6] H. A. Landwehr, *Zeitschr. f. physiol. Chem.* **8**. 114 (1883).

[7] O. Hammarsten, *ibid.* **6**. 194 (1882).

them pseudo-mucins. Later on they were also studied by Oerum,[1] Pfannenstiel,[2] Leathes,[3] Zängerle,[4] Steudel,[5] Neuberg and Heymann,[6] and Otori.[7]

In normal Graafian follicles, and also in the so-called hydrops ovarii, Pfannenstiel found only albumins, presumably serum-albumin and serum-globulin, while the proliferating, papillary, or glandular cystomata always contain, according to Oerum and Pfannenstiel, pseudo-mucin, which imparts to them a more or less mucous or viscous character.

Pseudo-mucin, prepared by precipitation with alcohol, according to Hammarsten's method, from cystomic fluids containing no or very little albumin, is a fine, white, very hygroscopic powder. It is readily soluble in water, and if present in low concentrations behaves like mucin; in stronger concentrations—Oerum found in ovarial cystomes 0·88 to 10·83 per cent albuminous bodies—it forms a whitish, tough, and mucilaginous fluid resembling a thick gum-solution. By acidification with acetic acid or hydrochloric acid, pseudo-mucin is not precipitated, and thus differs from the true mucins; neither does nitric acid precipitate, but it renders the solution more opalescent and more viscous. Otherwise pseudo-mucin gives the reactions of true mucins: it is not precipitated by ferrocyanic acid or by being boiled, but it is precipitated by lead acetate, mercuric chloride, and tannic acid. The two reagents last mentioned do not produce, however, a true flocculation, but only lead to the formation of a mucilaginous jelly. Alcohol gives rise to a tough curd, as it does in solutions of mucin; pseudo-mucin is only slowly denaturalised by alcohol.

Pseudo-mucin gives the xantho-proteic reaction and those of Millon and Adamkiewicz. It is not precipitated by magnesium sulphate, even if the reaction be acid, and when boiled with acids liberates glucosamin, which, according to Zängerle, is identical with the glucosamin prepared from true mucin; he obtained 30 grammes of glucosamin from 100 grammes of pseudo-mucin. Neuberg and Heymann found considerable quantities of glucosamin, and believe that the other carbohydrates mentioned by Leathes are absent. The dissociation-products[7] which

[1] H. P. Oerum, *Maly's Jahresbericht f. Tierchemie*, **14**. 459 (1884).

[2] J. Pfannenstiel, *Arch. f. Gynäk.* **38**. 407 (1890).

[3] J. B. Leathes, *Schmiedeberg's Archiv f. experiment. Pathol. und Pharmakol.* **43**. 245 (1899).

[4] Zängerle, *Münchener med. Wochenschr.* 1900, p. 414.

[5] M. Steudel, *Zeitschr. f. physiol. Chem.* **34**. 353 (1901), (good account of older literature bearing on the carbohydrate-radical).

[6] C. Neuberg and F. Heymann, *Hofmeister's Beiträge*, **2**. 201 (1902).

[7] J. Otori, *Zeitschr. f. physiol. Chem.* **42**. 453 (1904).

Otori obtained by acting on pseudo-mucin with boiling mineral acids have already been tabulated in the introduction (p. 534).

6. *Para-Mucin*

A variety of pseudo-mucin, first described by Drechsel and Mitjukoff,[1] and later by Panzer,[2] Leathes,[3] and Steudel,[4] is the so-called para-mucin. Occasionally one finds in all the ovarial cysts, or in some of the cysts of the multilocular tumour, not a fluid, but a trembling jelly, to which Mitjukoff first gave the name of para-mucin. It is insoluble in water, shrinks on the addition of acids, is changed by acid absolute alcohol into a fine, not hygroscopic powder, which is converted again into a jelly on being moistened with a little alkali-solution. On the addition of greater quantities of potassium or sodium hydrate, para-mucin dissolves into a slimy solution, which gives the ordinary mucin-reactions: it is not coagulated by being boiled, and is not precipitated by ferrocyanic acid, although a slight turbidity, depending probably on traces of albumin, may show itself. Tannin, lead-acetate, etc., cause a precipitate, as do also acetic acid and mineral acids. In this last feature para-mucin resembles the true mucins and differs from pseudo-mucin. Excess of mineral acids renders the precipitate soluble.

Amongst the dissociation-products Mitjukoff found lysin and arginin, and at least 12·5 per cent of a reducing substance, which was liberated by boiling with acids; Steudel obtained about 12 per cent of the latter. Glucosamin occurs in para-mucins as it does in other glyco-proteids, as a more complex compound.

In certain cystomata, in addition to pseudo-mucin, are also found considerable quantities of albumin, essentially serum-albumin; this mixture corresponds to Scherer's par-albumin and, as Hammarsten has shown, resembles both in composition and in reactions, a mixture consisting of albumin and pseudo-mucin.

A substance closely resembling pseudo-mucin, but containing only 45·74 per cent carbon and 5·68 per cent of nitrogen, Hammarsten[5] once found in a "ganglion" of unknown origin in the leg of a man.

[1] Kath. Mitjukoff, Dissertation, Bern, and in *Arch. f. Gynäk.* **49**. No. 2 (1895).
[2] Th. Panzer, *Zeitschr. f. physiol. Chem.* **28**. 363 (1899).
[3] J. B. Leathes, *Arch. f. experim. Path. u. Pharmak.* **43**. 245 (1899).
[4] H. Steudel, *Zeitschr. f. physiol. Chem.* **34**. 353 (1901).
[5] O. Hammarsten abstract in *Maly's Jahresber. für Tierchemie*, **22**. 561 (1892).

(b) The Mucoids

Hammarsten has called a number of substances, which closely resemble mucins in their composition and in their reactions, by the name of mucoids. These mucoids differ from mucins either as regards their physical properties or by not being precipitable with acids. They are found partly in solution, as, for example, in blood-serum, in the white of egg, or in ascitic fluid, and partly along with collagen, etc., in the tissues. Their separation from the mucins is quite arbitrary. The mucoids occurring in the vitreous humour, in tendons, and in the umbilical cord are by some called mucoids, and by others mucins, without their properties differing from one another in any marked way. With the view of reserving the name "mucin" for the true mucinous substances secreted by epithelia, Cohnheim calls all substances not derived from epithelium, but from connective tissues "mucoids." The tissue-mucoids which resemble the mucins most will be discussed in the first instance, and subsequently the soluble mucoids.

1. *Mucoid from Tendons and Bones*

Loebisch,[1] Chittenden and Gies,[2] and Cutter and Gies[3] have isolated a substance from tendons which does not differ from the true mucins in its properties. Its composition also resembles that of the mucins,[4] the only exceptional feature being the high sulphur-content:

C	H	N	S	O
48·26	6·49	11·51	2·31	31·43

Levene[5] separated from this mucoid a substance closely resembling chondro-sulphuric acid (see below).

When boiled with acids, or with water under heightened pressure, a high carbohydrate is liberated, which does not reduce, which is slightly dextro-rotatory, and which, by more intense action of acids, is converted into a reducing carbohydrate, which

[1] M. F. Loebisch, *Zeitschr. f. physiol. Chem.* **10**. 40 (1885).

[2] R. H. Chittenden and W. Gies, *Journ. of experiment. Med.* **1**. 186 (according to *Maly's Jahresber. f. Tierchem.* **26**. 32) (1896).

[3] W. D. Cutter and W. J. Gies, *Amer. Journ. of Physiol.* **6**. 155 (1901); compare also A. N. Richards and W. J. Gies, *ibid.* **7**. 93 (1902); G. W. Vandegrift and W. J. Gies, *ibid.* **5**. 287 (1901); S. Bünger and W. J. Gies, *ibid.* **6**. 219 (1901).

[4] M. F. Loebisch, *Zeitschr. f. physiol. Chem.* **10**. 40 (1885); R. H. Chittenden and W. Gies, *Journ. of experiment. Med.* **1**. 186 (according to *Maly's Jahresber. f. Tierchem.* **26**. 32) (1896); W. D. Cutter and W. J. Gies, *Amer. Journ. of Physiol.* **6**. 155 (1901); compare also A. N. Richards and W. J. Gies, *ibid.* **7**. 93 (1902); G. W. Vandegrift and W. J. Gies, *ibid.* **5**. 287 (1901); S. Bunger and W. J. Gies, *ibid.* **6**. 219 (1901).

[5] P. A. Levene, *Zeitschr. f. physiol. Chem.* **31**. 395 (1900).

forms a well-defined osazone;[1] whether this substance is glucosamin has not yet been determined. Tendon-mucoid resists the action of alkalies more strongly than do the mucins. To prepare tendon-mucoid, tendons are extracted with half-saturated lime-water. According to Posner and Gies,[2] tendon-mucoid, in the presence of sulphuric, hydrochloric, or acetic acid, forms relatively stable compounds with alkali-albuminates, acid-albumins, proteoses, gelatine, and the water-soluble albumins of muscle, tendon, blood-serum, and egg-white. Tendon-mucoid reacts, therefore, in exactly the same way as would chondro-sulphuric acid and glyco-thionic acid. These new compounds have an acid reaction; are comparatively insoluble in dilute acids; behave towards precipitating agents as do mucoids; do not become coagulated in neutral solutions; contain more nitrogen than does the mucoid by itself; and when boiled with dilute hydrochloric acid give rise to glyco-thionic acid and a reducing substance. Because of the ease with which tendon-mucoid reacts with the albuminous substances enumerated above, it is probable that mucoid occurs normally in the body in combinations similar to those obtained in the test-tube.

Gies[3] has prepared a substance identical with tendon-mucoid from bones, which he calls "osseo-mucoid." It also possesses a high sulphur-content (2·5 per cent), and contains likewise a paired sulphuric acid, and thus resembles the chondro-mucoid.

2. *Chondro-Mucoid and Chondro-Sulphuric Acid*[4]

Johannes Müller[5] in 1837 called the ground substance of cartilage "chondrin," and believed it to be a special substance; G. J. Mulder[6] in 1838 showed chondrin to contain a definite amount of sulphur. Fischer and Boedeker[7] and de Bary[8] then demonstrated that chondrin, when boiled, gives rise to a reducing substance. Morochowetz[9] first recognised that the ground matrix of cartilage is a mixture of ordinary collagen and a mucin-like substance. The full explanation

[1] R. H. Chittenden and W. Gies, *Journ. of experiment. Med.* **1.** 186 (according to *Maly's Jahresber. f. Tierchem.* **26.** 32) (1896).

[2] E. R. Posner and W. J. Gies, *Amer. Journ. of Physiol.* **11.** 404 (1904).

[3] P. B. Hawk and W. J. Gies, *Amer. Journ. of Physiol.* **5.** 387 (1901).

[4] The author has shortened the term chondroitin-sulphuric acid, used in Mandel's translation of Hammarsten's *Physiological Chemistry.*

[5] Joh. Müller, *Liebig's Annalen*, **21.** 277 (1837).

[6] G. J. Mulder was the first to make quantitative estimations of the amount of sulphur and phosphorus in egg-white, fibrin, and serum.

[7] G. Fischer and C. Boedeker, *ibid.* **117.** 111 (1861).

[8] J. de Bary, *Hoppe-Seyler's Med.-chem. Untersuch.* p. 71 (1866).

[9] L. Morochowetz, *Verhandl. d. naturhist.-med. Ver. Heidelb.* N.F. I. p. 480 (1876).

as to the composition of cartilage, as also an accurate description of the chondro-mucoid, we owe to Mörner,[1] who showed that cartilage, apart from the cells enclosed in it, consists, firstly, of an albumoid, which forms a trabecular network, and, secondly, of collagen and mucoid, which fill the spaces between the trabeculæ (compare with p. 566).

Chondro-mucoid shows the usual reactions of the mucins and mucoids: it dissolves in alkalies to a neutral, thick fluid, and is precipitated by acids. Most of the salts of the heavy metals cause a precipitate, but the alkaloidal reagents do not; tannic acid in particular does not precipitate, even in the presence of salts. Chondro-mucoid even prevents the precipitation of other albumins, such as gelatin, by tannic acid, and this explains the older statements as to the non-precipitability of chondrin, which is a mixture of chrondro-mucoid and of gelatine. The colour-reactions are all positive; ammonium sulphate salts out. The percentage composition[1] of chrondro-mucoid corresponds to that of the mucins; the high sulphur-content, 2·42 per cent, of which 1·8 per cent is due to chondro-sulphuric acid, is specially noteworthy. By the action of acids, and even more readily by that of alkalies, towards which it is very susceptible, it is dissociated, there being formed an albuminate, albumoses, and peptones, a reducing carbohydrate, and chondro-sulphuric acid. Fischer and Boedeker prepared, already in 1861, a nitrogen-containing acid from cartilage; afterwards Krukenberg[2] called this acid "Chondroitsäure," and described it more fully. The acid was first prepared in a pure state by Mörner, who recognised it as a paired sulphuric acid, and who accurately described its properties. Subsequently it was investigated by Schmiedeberg[3] and Orgler and Neuberg.[4] The chondroitin-sulphuric acid or, shortly, chondro-sulphuric acid, is a colloidal substance of unknown constitution. If it be boiled for a short time with acids it becomes decomposed into sulphuric acid and a remainder, which contains no sulphur, and which Schmiedeberg called "chondroitin." It is therefore a paired or ethereal-sulphuric acid.

Chondroitin is a gum-like acid which, by further action of acids, is converted into "chondrosin," an aminated polysaccharid. From this latter Orgler and Neuberg prepared a hexosamin- or tetra-oxy-amino-caproic acid, the exact configuration of which is still unknown. They

[1] C. T. Mörner, *Skandinav. Archiv für Physiol.* **1**. 210 (1889).

[2] F. C. W. Krukenberg, *Sitzungsber. der Würzburger phys.-med. Ges.* 1883 (reprint); F. C. W. Krukenberg, *Zeitschr. f. Biolog.* **20**. 307 (1884).

[3] O. Schmiedeberg, *Archiv f. experiment. Pathol. und Pharmak.* **28**. 355 (1891).

[4] A. Orgler and C. Neuberg, *Zeitschr. f. physiol. Chem.* **37**. 407 (1903).

were able to exclude the presence of both glucosamin and also glycuronic acid, which latter Schmiedeberg has assumed to be present. The hexosamin-acid gives Ehrlich's reaction with p-dimethyl-amino-benzaldehyde (see p. 10), which is believed to be a test for the mono- or di-acetate of glucosamin.

The percentage composition of chondro-sulphuric is

C 35·28 H 4·68 N 3·15 S 6·33 O 50·56 (Mörner).
C 37·1 H 4·83 N 2·71 S 5·5 O 50·14 (Schmiedeberg).

Some other preparations of Schmiedeberg showed slight deviations.

Chondro-sulphuric acid has a strongly acid reaction, and forms with metals neutral salts which, as a rule, are readily soluble. Schmiedeberg prepared amorphous copper-, iron-, and potash salts, as well as a copper-oxide salt. In water the acid is readily soluble, and if sufficiently concentrated is of a gum-like consistence. It is precipitated by stannous chloride, basic lead acetate, mercurous nitrate, ferric chloride, and uranium nitrate, but not by other metals, nor by any acid, or by the alkaloidal reagents. By acetic acid it is, however, precipitated if the acid be largely in excess, and by alcohol if salts are present. It does not reduce, but keeps copper oxide and other metallic oxides in solution by forming soluble salts. Its watery solutions are laevo-rotatory.

With albuminous substances, for example with gelatine, chondro-sulphuric acid forms insoluble salts, which behave like nucleic acids, for they become hydrolytically dissociated in the absence of an excess of acid. The salts of chondro-sulphuric acid, however, do not precipitate albumins, and therefore the mixture of sodium or potassium chondro-sulphate and gelatine, which one obtains by digesting cartilage with pepsin, or by boiling cartilage in a Papin-pot, is only precipitated if the chondro-sulphuric acid is liberated by the addition of other acids; mineral acids in excess re-dissolve the precipitate. This reaction is also of importance in connection with the mucoid found in the urine (see below). The greater part of chondro-sulphuric acid is a constituent of chondro-mucoid, but a small amount, according to Mörner and Schmiedeberg, occurs in cartilage either in the free state or as an alkali-salt. To demonstrate the presence of combined and free chondro-sulphuric acids, Mörner proceeds as follows: The tissues are extracted with caustic potash, the extract is neutralised; the chondro-sulphuric acid is precipitated with alcohol and dissolved in water. This watery solution must show the following reactions:—

1. Give a precipitate with gelatine and acetic acid.
2. Give a precipitate with glacial acetic acid.

3. Give a reducing substance after having been boiled with hydrochloric acid.

4. Give sulphur-reactions.

Mörner,[1] adopting these tests, was able to demonstrate the presence of chondro-sulphuric acid in all varieties of cartilage, in enchondromata, and in the inner layers of the aorta; Lönnberg[2] found it in the skate (Raja batis); traces of it were also found by Mörner[3] and Krawkow[4] in bones, by Krawkow in the ligamentum nuchæ and in the gastric mucous membrane of the pig. It would appear to also occur in the mucoid of tendons and bones (see above). Levene[5] states to have discovered a similar substance in the spleen.

Large amounts were found by Schmiedeberg's pupils Oddi[6] and Krawkow[4] in 'amyloid' (see p. 574), but in this substance it seems to be more firmly united than in chondro-mucoid. According to Krawkow, chondro-sulphuric acid is the cause of amyloid staining with methyl violet. If sodium chondro-sulphate be administered in the food it is excreted in large amounts by the kidneys; traces are also found in the liver. K. Mörner[7] has finally found chondro-sulphuric acid regularly and in not inconsiderable quantities—about 0·05 per cent—in normal urine. This observation is important, because if albumin be present, chondro-sulphuric acid will precipitate it whenever the urine is acidified, and, on the other hand, some albumin-reactions, as, for example, precipitation with tannic acid, will be prevented by it. A certain percentage of the ethereal sulphuric acids belongs, therefore, to chondro-sulphuric acid and not to indoxyl-sulphuric acid, etc.

To prepare chondro-sulphuric acid Mörner proceeds as follows: The cartilage is divided into small pieces, and is then extracted for several days at room temperature with 2 to 5 per cent caustic potash. This extract contains, in addition to chondro-sulphuric acid, an albuminate and the albumoses of the mucoid, a little collagen and albumoid. To remove the albuminate the solution is first neutralised and then rendered slightly acid with acetic or hydrochloric acid; the other albuminous substances are then removed with tannic acid, first in acid and then in slightly alkaline solutions; the tannic acid is precipitated from the slightly acid solution with lead acetate; the lead

[1] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **20**. 357 (1894).

[2] J. Lönnberg, Hammarsten's abstract from the Swedish original in *Maly's Jahresber. f. Tierchem.* **19**. 325 (1889).

[3] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **23**. 311 (1897).

[4] N. P. Krawkow, *Schmiedeberg's Arch. f. exper. Path. u. Pharm.* **40**. 195 (1897).

[5] P. A. Levene, *Zeitschr. f. physiol. Chem.* **37**. 400 (1903).

[6] Ruggero Oddi, *Schmiedeberg's Arch. f. exper. Path. u. Pharm.* **33**. 376 (1893).

[7] K. A. H. Mörner, *Skandinav. Arch. f. Physiol.* **6**. 332 (1895).

is removed with sulphuretted hydrogen and the filtrate freed from salts by dialysis. Finally, the solution is strongly inspissated, and after the addition of some sodium chloride precipitated with alcohol. Schmiedeberg digests the cartilage—he used the nasal septum of the pig—with pepsin-hydrochloric acid, and thus obtains a doughy residue consisting of collagen and chondro-sulphuric acid. The acid is then converted into the cupric oxide potash salt.

3. *The Mucoids of the Vitreous Humour, the Cornea, and the Umbilical Cord*

Virchow[1] was the first to notice that mucin-like substances occur in the vitreous of the eye and in the umbilical cord. The mucoid of the vitreous was then investigated by Mörner[2] and Halliburton and Young.[3] Although the mucoid amounts to only 0·1 per cent of the vitreous humour, it yet conditions the physical properties of the humour, which resembles a very thin jelly. It gives the ordinary mucin-reactions; acetic acid precipitates it from solutions poor in salts; alkalies dissolve the precipitate; the alkaloidal reagents and some of the heavy metals cause precipitation, as do also potassium ferrocyanide + acetic acid and nitric acid. It gives all the ordinary colour-reactions, except Liebermann's reaction, showing that the presence of tryptophane is not well marked. Sodium chloride salts it out from acid solutions, and magnesium sulphate also from neutral solutions. According to Young, heating from 70-72° in slightly acid solutions causes denaturalisation.

The vitreous humour contains, in addition to mucoid, also traces of albumin.

The mucoid of the cornea has been prepared and analysed by Mörner.[4] It resembles the mucins as regards precipitability by acetic acid; it is precipitated by the alkaloidal reagents, except by ferrocyanic acid, and by the salts of the heavy metals except by corrosive sublimate, etc. The ground substance of the cornea contains 20, and that of the sclerotic coat 13 per cent of mucoid; the remainder is collagen (compare with p. 565).

The mucoid of the umbilical cord has been studied by Jernström[5] and Young.[3] It possesses the ordinary character of the mucins; the carbohydrate radical is readily split off by 2 per cent hydrochloric

[1] Rud. Virchow, *Virchow's Archiv.* **4**. 468 (1852).
[2] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **18**. 233 (1893).
[3] R. A. Young, *Journ. of Physiol.* **16**. 325 (1894).
[4] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **18**. 213 (1893).
[5] E. A. Jernström, *Maly's Jahresber. f. Tierchem.* **10**. 34 (1880).

acid in thirty minutes, according to Young; amongst the dissociation-products is found indol.

The analyses of Mörner and Young yield the same figures as obtained with other mucins.

The notochord, which otherwise resembles the umbilical cord, does not contain any mucoid, according to Kossel[1] (compare with p. 578).

4. *Ovi-mucoid.*

Neumeister[2] and Salkowski[3] had observed that white of egg contains, besides the well-known albumin and globulin, another substance which has properties of an albumose. This body Neumeister called pseudo-peptone. Mörner[4] recognised that the new substance was a glyco-proteid, and hence called it ovi-mucoid. It occurs in large quantities in white of egg, forming about one-eighth of the organic constituents, or 1·5 per cent of the solution. The ovi-mucoid resembles the other mucoids in not being coagulated by heat, but it is also not precipitated by acids such as acetic, hydrochloric, or nitric acids, or by metallic salts or most of the alkaloidal reagents. It is, however, precipitated by tannic acid, phosphomolybdic acid, lead acetate + ammonia, and by alcohol. The best method of preparing it consists in first removing the albumin and the globulins of the eggwhite, by heating the latter after slight acidification, and then precipitating the mucoid, in the filtrate, with alcohol. When dried, it forms brittle, transparent lamellæ; a concentrated solution is adhesive like gum; a dilute solution foams strongly, but cannot be pulled out into threads. In cold water it simply swells up, without passing into solution, but on heating it dissolves, and does not separate out on cooling. According to Mörner, it contains

12·65 per cent of N, and 2·2 per cent of S.

The greater part of the sulphur may be split off by boiling with alkalies, but Zanetti[5] finds that boiling with hydrochloric acid liberates sulphuric acid, and this must, therefore, have originally been in the form of ethereal sulphuric acid. In addition to the lead-sulphide reaction, ovi-mucoid also gives the reaction of Millon, and the biuret and xanthoproteic tests, while, according to Mörner, it does not give the reactions of Liebermann or Adamkiewicz, and therefore it does

[1] A. Kossel, *Zeitschr. f. physiol. Chem.* **15**. 331 (1891).

[2] R. Neumeister, *Zeitschr. f. Biolog.* **27**. 309 (1890).

[3] E. Salkowski, *Zentralbl. f. d. med. Wiss.* 1893, No. 31.

[4] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **18**. 525 (1893).

[5] C. U. Zanetti, *Ann. di Chim. e Farmac.* **12**. According to *Maly's Jahresber. f. Tierchem.* **27**. 31 (1897).

not contain tryptophane. Sodium chloride does not salt out ovi-mucoid, while sodium- and magnesium-sulphate do so on boiling; precipitation with ammonium sulphate commences in $\frac{2}{3}$ saturated solutions, and fully saturated solutions are required for a complete separation. When boiled with acids, a reducing substance is split off, which has been carefully investigated by Friedrich Müller and Seemann;[1] this reducing radical is identical with the glucosamin which may be obtained from true mucins, but the glucosamin does not occur in the molecule as such, according to Steudel.[2] Out of 100 grammes ovi-mucoid Seemann obtained 29·4 grammes of glucosamin. Amongst the dissociation-products he obtained acetic acid and a di-ethyl-sulphino-fatty acid. Weydemann[3] prepared from ovi-mucoid Landwehr's 'animal gum,' which resembles that obtainable from mucin and pseudo-mucin.

5. *Serum-mucoid*

Zanetti[4] found in blood-serum a mucoid, which closely resembles ovi-mucoid in its properties and in its composition. Its presence must be taken into account when working at the separation of sugar-radicals from serum-albumin.

6. *Mucoid from Urine*

A mucoid which also resembles ovi-mucoid, but which is more nearly related to the mucins, being precipitable by acetic acid, has been isolated by K. A. H. Mörner[5] from human urine. From 260 litres he obtained 4·3 grammes. It is partly in solution and partly in the form of the so-called 'nubecula.' In some animals it is replaced by a nucleo-albumin, and the nucleic acid from the leucocytes of the urine has also a mucilaginous character (see there). Stähelin[6] describes a body having somewhat different properties, as occurring occasionally in the urine.

7. *Mucoid from Ascitic Fluid*

A number of ascitic fluids formed during different etiological conditions have been investigated by Hammarsten,[7] Paijkull,[8] Umber,[9] and

[1] J. Seemann, Dissertation, Marburg, 1898; F. Müller and J. Seemann, *Deutsche med. Wochenschr.* 1899, p. 209; F. Müller, *Zeitschr. f. Biol.* **42**. 468 (1901).

[2] H. Steudel, *Zeitschr. f. physiol. Chem.* **34**. 353 (1901).

[3] H. Weydemann, Dissertation, Marburg, 1896.

[4] C. U. Zanetti, *Ann. di Chim. e Farmac.* **12**. According to *Maly's Jahresber. f. Tierchem.* **27**. 31 (1897).

[5] K. A. H. Mörner, *Skandinav. Archiv f. Physiol.* **6**. 332 (1895).

[6] Stähelin, *Münchener med. Wochenschr.* 1902, p. 1412.

[7] O. Hammarsten, *Zeitschr. f. physiol. Chem.* **15**. 202 (1891).

[8] L. Paijkull, *Maly's Jahresber. f. Tierchem.* **22**. 558 (1892).

[9] F. Umber, *Zeitschr. f. klin. Med.* **48**. Hefte 5 and 6 (1903); *Münch. med. Wochenschr.* II. p. 1169 (1902).

Stähelin.[1] They found a mucoid, which imparted to the fluid an opalescent appearance and a peculiar viscosity. When pure the mucoid is precipitated by means of acetic acid, but from ascitic fluid only after the other albuminous substances have been removed and the salts have been dialysed off, or after greatly diluting the ascitic fluid. When the coagulable albumins are precipitated this mucoid is also carried down according to Stähelin, but it is possible to bring it again into solution. Umber diluted the ascitic fluid, precipitated with acetic acid, and then purified the precipitate with alcohol and ether. The mucoid is precipitated by the alkaloidal reagents, also by potassium ferrocyanide, nitric acid, copper sulphate, ferric chloride, and lead acetate. It gives all the colour-reactions of albumins. It is completely precipitated by half-saturated ammonium sulphate. As boiling for a short time with acids gives rise to only very minute quantities of a reducing substance, Umber and Stähelin have doubts as to whether this mucoid is a mucin at all, but that it is really a mucin has been shown by von Holst[2] who, working under Hammarsten, has made a very careful study of ascitic and synovial fluids. The ascitic fluid obtained from a patient suffering from cancer ventriculi et peritonei, was of a yellow colour, viscous, and distinctly alkaline to litmus paper. The 'serosa-mucin' was precipitated by 1 per cent acetic acid, then dissolved in just sufficient alkali, and this procedure repeated thrice. The neutral solution obtained by dissolving the precipitated mucin in alkali did not coagulate when it was boiled; it was precipitated by acetic and hydrochloric acid; was insoluble in excess of acetic, but soluble in 0·1 – 0·5 per cent hydrochloric acid. The neutral solution was not precipitated by such alkaloidal reagents as sodium molybdate or potassium + mercury iodide; it did not directly reduce an alkaline copper solution, but did so after having been boiled for half an hour in 2 per cent HCl. By pepsin + hydrochloric acid, a fraction amounting to 8·7 per cent and containing phosphorus could be removed without altering the properties of the serosa-mucin, which now contained neither phosphorus nor iron, and therefore could neither be a nucleo-proteid nor a nucleo-albumin. The analysis of this serosa-mucin is included in the following table of von Holst's :—

[1] Stähelin, *Münchener med. Wochenschr.* 1902, p. 1412.

[2] Gustaf v. Holst, *Zeitsch. f. physiol. Chem.* **43**. 145 (1904).

[TABLE

TABLE SHOWING THE PERCENTAGE COMPOSITION OF THE MUCIN-SUBSTANCES FROM ASCITIC AND SYNOVIAL FLUIDS

	C.	H.	N.	S.	
Mucoid from ascitic fluid .	51·40	6·80	13·01	...	Hammarsten.
Serosa-mucin 1 . . .	51·41	6·68	13·31	1·30	von Holst.
,, ,, 2 . . .	51·43	6·65	13·23	1·25	
,, ,, 1 . . .	51·35	6·72	14·91	1·32	Umber.
,, ,, 2 . . .	50·23	6·87	14·37	1·32	
,, ,, from synovia .	51·05	6·53	13·01	1·34	von Holst.

The presence of nucleo-albumins in synovial fluids is mentioned on p. 407.

The phosphorus-containing albumins found by Stähelin in exudations are probably derived from leucocytes.

8. *Mucoid from the Egg-coverings of Sepia and from Sponges*

The eggs of the octopus family are surrounded by a tough elastic covering, which is the solidified secretion of the nidamental glands. This mucoid has the same composition as have other mucoids, according to v. Fürth.[1]

Boiling with acids liberates 36 to 39 per cent of an aminated hexose. The egg-covering of Loligo consists also of a glyco-proteid.

An aminated sugar, after the type of glucosamin, was isolated by v. Fürth[1] from the ground-substance of the gelatinous sponge Chondrosia reniformis.

III. THE PHOSPHO-GLYCO-PROTEIDS

These substances contain phosphorus, and only resemble the mucins and mucoids in their carbohydrate-content. Of the two substances described by Hammarsten under the above name, one, namely, the ichthulin, has already been described amongst the nucleo-albumins on p. 405, while the second, or 'helico-proteid,' shall be discussed here, as there is no other place to put it into.

Hammarsten[2] has found in the serous gland of the vineyard snail Helix pomatia, a proteid having the composition:

C 46·99 H 6·78 N 6·08 S 0·62 P 0·47 Fe.

It differs considerably from all other known albumins. Its solution is of a whitish opalescent colour. It is not coagulated by boiling, while

[1] O. v. Fürth, *Hofmeister's Beitr.* **1**. 252 (1901).

[2] O. Hammarsten, *Pflüger's Archiv*, **36**. 373 (1885).

it is precipitated by acetic acid from a salt-free solution. Nitric acid and hydrochloric acid precipitate; if in excess they redissolve the precipitate. It is further precipitated by alum, copper-sulphate, tannin, and iodide of mercury + potassium iodide, but not by mercuric chloride or by ferrocyanic acid. It gives the reactions of Millon, Adamkiewiez, and the xanthoproteic reaction. Pepsin hydrochloric acid precipitates a nuclein or pseudo-nuclein. Xanthin-bases are not known. When boiled with hydrochloric acid or potash solution there are formed an albuminate, albumoses, and a higher carbohydrate, the 'sinistrin.' This compound, as the name implies, is lævo-rotatory; it does not ferment, does not reduce, and does not give the iodine-reaction. It is not attacked by ptyalin, but is converted by boiling acids into a reducing, dextro-rotatory carbohydrate. These reactions make it impossible to regard helico-proteid as a simple nucleo-proteid.

CHAPTER XI

THE ALBUMINOIDS

THIS group comprises a series of albuminous substances, which form the supporting structures of animals, or the 'connective tissues' of the histologist. They do not form a part of the cell, but are structures which have been secreted by the cells, the latter during the formation of the supporting tissues becoming included in the secretions. Albuminoids are absent in the nutritive fluids of animals, such as the blood and the lymph. (Glutolin is dealt with on p. 567.)

The term albuminoid is thus an anatomical one, and comprises chemically most divergent substances. Gelatine besides heteroalbumose, is the only pure 'anti-albumin,' for tyrosin and tryptophane are absent, while glycocoll and bases are present in large quantities. Keratin contains more cystin than does any other albumin, and therefore more sulphur; it also contains much tyrosin; elastin is so poor in bases as to resemble some plant-albumins. Fibroin is composed of more than 50 per cent of alanin and glycocoll. Amyloid ought, perhaps, to be classed amongst the proteids, because of its large amount of chondro-sulphuric acid. In days to come, when the chemical classification of albumins is more advanced, albuminoids will be brought under quite different headings, for the present arrangement serves simply as a makeshift.

Albuminoids are as much albumins as are the soluble albumins,[1] and it is arbitrary to still classify them as 'substances resembling albumins,' for the differences between gelatine and keratin and the albumins are by no means greater than are those between the albumins and casein or globin.

Chemically, albuminoids are albumins, for they are split up by acids or ferment into albumoses, peptones, and amino-acids; with halogens they give rise to substitution-products; they form salts; they

[1] A. Kossel, *Ber. d. deutsch. chem. Ges.* **34**. III. 3214 (1901).

have the same percentage composition, and give the same colour-reactions as do other albumins.

It must, however, be admitted that the anatomical relationship conditions a series of chemical peculiarities which are common to all albuminoids. As their function is to act as supporting structures and coverings to the body, and to impart to the living organo-plasm shape and adhesiveness, they all possess the physical property of great firmness. Thus the extraordinary hardness of the bones of vertebrates, or of the shells of molluscs, or of other integumental structures acting as a protection to many lower animals, is due to albuminoids forming an organic ground-matrix, which subsequently becomes impregnated with mineral matter. In other cases we have to deal with unyielding tissues possessed of great toughness as in tendons, or with elastic bands as in the ligamentum nuchæ, or with loosely arranged tissues possessing a certain degree of toughness, as in the areolar tissues. In this last case, however, the looseness is simply due to a looser arrangement of the same white fibrous tissue as is met with in tendons. "The essential feature of all connective tissues is that they must be completely insoluble in all animal juices" (Cohnheim). The author must differ from Cohnheim, as he has ample experimental evidence showing this view to be incorrect, for the connective tissues all over the body and including the bones are diminished during inanition, *i.e.* are partly converted into 'circulating proteid.'

All albuminoids are quite insoluble in water and in salt solutions, and are also hardly soluble in dilute acids or alkalies. To get them, therefore, into solution one requires to use means by which their fundamental rigid character is destroyed, and this cannot be done without at the same time destroying, or at least chemically altering these albuminoids. As a chemical substance cannot be properly examined except it be in solution, it follows that it is impossible to examine albuminoids in their natural state, and that in getting them into solution we have to subject them to a good many changes. For these reasons it is even more difficult to isolate albuminoids and to define their chemical individualities, their properties, and compositions than it is in the case of the cell-albumins, for these occur, in the cell-plasm, at least in a semi-fluid state. Most of the investigations into the albuminoids date back a long time or are quite recent. The middle period of investigations into the chemistry of albumins being especially concerned with the study of the solubilities of albumins took no notice of the albuminoids.

A comparison of the more physical properties of the native, firm albuminoids, with those of the colloidal albumins is not well possible.

The only exception to this rule is the most readily soluble of all the albuminoids, namely collagen. Collagen on being boiled for a short time gives rise to glue (glutin or gelatine), which possesses the remarkable property of being fluid when heated, and of 'setting' into a firm jelly on being cooled; if this collagen is split up into its dissociation-products it no longer 'sets,' and this is analogous to the change in solubility which natural albumins undergo on becoming denaturalised. It is also not possible to speak of the molecular weights when dealing with solid bodies (Cohnheim).

Generally speaking, albuminoids are sharply marked off from the rest of the albuminous bodies; they only show certain transitions to the mucins, such as the chondro-mucoid, but the resemblance is not a chemical one, but rather depends on both substances being combined in the animal body for a common function. To separate off the albuminoids from a number of non-albuminous supporting structures found amongst the lower animals is much more difficult. Chitin we know not to be an albuminous compound, but to consist essentially of a nitrogenous carbohydrate, a derivative of glucosamin, as has been shown by Ledderhose,[1] but where to place 'hyaline' and compounds related to it is not so easy to decide, and amongst lower animals Krukenberg[2] has described a number of substances, to which he assigns a place intermediate between albumins and carbohydrates. Whether we are dealing here with mixtures of albuminoids and chitin, or with a splitting off of carbohydrates from glyco-proteids, or with genuine transition-compounds, it is impossible to say. Amyloid, which Hammarsten[3] classifies amongst the glyco-proteids, belongs rather to the albuminoids, if we judge it by its whole habitat, its firmness, and its very great insolubility.

The typical albuminoids are represented by gelatine, keratin, elastin, fibroin, spongin, conchiolin. In addition to these a number of bodies exist which belong anatomically to the supporting structures or connective tissues, but which differ from the true connective tissues in not yielding gelatine, in not resembling keratin or elastin, and also in offering great resistance to the digestive enzymes. They have been discovered by Mörner in the refractive media of the eye; by Hammarsten and his pupils in muscle and in other situations, and are in the meantime called 'albumoids'; in this book they are described collectively in a special chapter. Siegfried's reticulin belongs

[1] G. Ledderhose, *Zeitschr. f. physiol. Chem.* **2**. 213 (1878).

[2] F. C. W. Krukenberg, partly abstracted in the *Grundzüge einer vergleichenden Physiologie der tierischen Gerüstsubstanzen*, Heidelberg, 1885.

[3] O. Hammarsten, *Lehrbuch der physiol. Chem.*, 4. Aufl. 1899, p. 47.

to these albumoids. The numerous substances described by Krukenberg, and called onuphin, spirographin, neossin, etc., are not dealt with. It is very doubtful as to whether these substances are separate individuals, and Krukenberg's descriptions are so contradictory and so much permeated by theoretical speculations that it is quite impossible to make use of them (Cohnheim).

There is one other property peculiar to albuminoids which is also conditioned by their anatomical character, and which makes the description of albuminoids difficult—namely, their 'aging.' According to Cohnheim cells are constantly renewed by their metabolism and do not age. While thus cell-albumins and the soluble albumins remain the same during the whole life of the animal, the ground-matrix of connective tissues alters in a remarkable way with age; it increases in amount, becomes firmer and harder. This is specially distinct in the connective tissue proper; while young connective tissue consists essentially of cells with only a little, soft ground-matrix, it forms in older animals and also in scar tissues a coarse, tough, firm mass, which has hardly a single feature in common with the young tissue. Amongst other albuminoids analogous changes are met with, so amongst the supporting structures and shells of invertebrates. The same tissue which while young is soft and pliable becomes with advancing age, especially if lime is deposited, as hard as stone. It is unknown to what extent the mature organs differ chemically from the young tissue as regards the albuminoid radical, for the percentage composition shows no distinct alterations, but naturally the readiness with which a tissue passes into solution diminishes as the tissue becomes harder; collagen and elastin get to resemble keratin, while their dissociation-products, their reactions, and their composition remain the same as of old. A great many of the contradictions of authors may be explained by taking into account the age-differences of albuminoids (Cohnheim).

The author wishes to point out that it is a great mistake to assume that cells do not age, and that their metabolism keeps them eternally young. If this were the case we ought, according to Cohnheim, only to die because our blood-vessels and other connective tissues have become old. That aging affects all the cells of the body has been established by the author long ago,[1] and holds good for both vegetable and animal cells.[2] The chief feature of advancing age is a diminution in the nuclear activity, *i.e.* of that very factor which normally

[1] Mann, *Report of British Assoc. for Advanc. of Science*, Edinburgh, 1892, p. 735.

[2] L. H. Huie, *La Cellule*, II. 83 (1895), (cells of Lilium Martagon). Hodge and Mann (nerve-cells).

brings about the synthesis of the simple amino-acids into the higher compounds according to the author's view. The cell bodies show with advancing age a diminishing affinity for basic dyes and a great diminution in the size of the nucleoli. As shown above, Cohnheim assumes that all albuminoids undergo a spontaneous change, quite irrespective of cell activity, but this again does not bear out the author's histological experience. Whenever a change occurs, *e.g.* in cartilage, it is always in connection with and in close proximity to the nucleus. The hardening of connective tissues, brought about by a deposit of lime-salts, depends primarily on the excretion of phosphoric acid by the nucleus or the synthesis of chondro-sulphuric acid in the immediate neighbourhood of the nucleus, and therefore depends on the cell. When again elastin during advancing age is changed into elacin (Unna), when all its staining reactions become altered, we may assume a spontaneous change of elastin into elacin, but with equal right we may hold that with advancing age certain substances circulating in the body are deposited in the elastin and thereby change its staining reactions. To compare immature growing connective tissue with fully formed and aged tissue, as Cohnheim has done, is hardly permissible, for judging by micro-chemical data, there is no difference between 'young' (but not that *in statu formandi*) and 'adult' white fibrous tissue. Experimental investigations have further shown, as already pointed out, that the white fibrous tissue is drawn upon during states of inanition, *i.e.* that it is constantly changing within certain limits.

1. Collagen. Gelatine

Collagen.—The fibrils of ordinary white fibrous tissue, the ground substance of bone and of cartilage, are composed of 'glue-yielding tissue' or collagen. When collagen is treated with boiling water it passes into solution, and to this dissolved collagen the terms gelatine, glutin, or glue are given. The most important property of gelatine consists in it turning into a jelly at room-temperature, becoming fluid on being heated, and again solidifying on cooling, and so on.

Collagen is but little understood for the reasons given above. Kühne and Ewald[1] have shown that collagen of the ordinary connective tissue is very easily attacked by pepsin + hydrochloric acid, while it is not attacked by tryptic digestion. Connective tissue may, therefore, be obtained in a pure state by removing all other albuminous sub-

[1] A. Ewald and W. Kühne, *Verhandl. d. naturh.-med. Vereines Heidelberg*, New Series I. S. 451 (1876); A. Ewald, *Zeitschr. f. Biol.* **26**. 1 (1890).

stances by trypsin. While normal white fibrous tissue is not attacked by trypsin, it is readily digested if it be allowed to swell in acids, and if it be made to contract again by being heated up to 70° in water. If the contraction is not allowed to take place, the connective-tissue fibrils remain unaltered on being treated with trypsin. If white fibrous tissue, made digestible by being boiled with water, is acted upon by chrome-compounds, and is then exposed to light, it becomes indigestible for both peptic and tryptic digestion. The insolubility of chrome-gelatine induced by exposure to light is made use of in the carbon-process of photography.

In ordinary tendons, but also in yellow tendons, such as the ligamentum nuchæ, white fibrous tissue occurs as fibrils along with yellow fibrous tissue, composed of elastin, and along with mucin. The behaviour of these collagen-fibrils towards digestive enzymes has been very thoroughly investigated by Ewald,[1] who found them to react as does ordinary white fibrous tissue, but the collagens of different animals differed considerably as regarded digestibility. Different varieties of collagen will be mentioned later.

Collagen gives rise to gelatine under different kinds of treatment, but most readily on being boiled with acids, although prolonged boiling with water will also render collagen soluble. The time required for changing collagen into gelatine varies greatly according to the collagen under examination.

Mörner[2] found the collagen of fish-scales much more readily soluble than that of the ordinary connective tissues of cartilage and bone. Sadikoff[3] could obtain gelatine from the cartilage of the trachea of the ox or the nasal cartilages of the pig by mere heating on a water-bath, while the ear-cartilage of the pig required to be heated to 110°.

Hofmeister[4] believes the conversion of collagen into gelatine to depend on a hydrolytic dissociation.

Gelatine.—Glue or gelatine in a dry purified state forms a colourless, amorphous powder, but the commercial gelatine occurs in the shape of glassy plates containing water. Gelatine has been frequently investigated because it is so readily accessible, but the analyses of different observers do not agree very closely. The probable reason for this divergence is that the collagen-fibrils always occur mixed up with other tissue constituents, which are also rendered soluble when white fibrous tissue is boiled, and it is very difficult to

[1] A. Ewald, *Zeitschr. f. Biologie*, **26**. 1 (1890).
[2] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **24**. 125 (1897).
[3] W. S. Sadikoff, *ibid.* **39**. 411 (1903).
[4] F. Hofmeister, *ibid.* **2**. 299 (1878).

get rid of these admixtures. van Name,[1] Mörner,[2] Sadikoff,[3] and others have attempted to overcome this difficulty by treating white fibrous tissue with dilute alkalies or with trypsin, which, as already stated, does not attack collagen, but the results have not been quite satisfactory, as the danger of altering the collagen into glutin by a too vigorous manipulation is very great, especially as collagen is converted by boiling water into gelatine, and the latter, if the reaction be not exactly neutral, into its dissociation-products. It follows that the slightest difference in the preliminary manipulation, in the duration or the intensity of boiling, and in the reaction, must of necessity make the final products differ from one another. Sadikoff's[3] recent publications show again that at present it is impossible to prepare glutins which are chemically pure and which agree with one another in all their properties. The investigation of the dissociation-products, or the study of the chemical configuration of gelatine, is, however, scarcely affected by these small differences, although the latter make themselves felt in the analytical numbers and when studying the reactions of the gelatines.

For the reasons just stated, only a few of the analyses of gelatine are given in the following figures :—

	C	H	N	S	O	
Commercial gelatine . .	49·38	6·8	17·97	0·7	25·13	Chittenden.[4]
,, ,, . .	49·09	6·76	17·68	...	...	Faust.[5]
,, ,, . .	51·45	7·08	18·18	0·46	...	Sadikoff.[3]
Gelatine from tendons .	50·11	6·56	17·81	0·256	25·24	van Name.[1]
,, ,, ,, . .	50·9	7·18	18·32	...	...	Scherer.[6]
,, ,, ,, . .	50·9	6·8	18·59	0·53	...	Sadikoff.[3]
Fish glue (gelatine from the swimming bladder of the sturgeon)	49·9	6·73	17·95	...	...	v. Goudoever.[7]
	50·0	6·9	18·79	...	...	Scherer.[6]
	48·69	6·76	17·68	...	...	Faust.[5]
Nasal cartilage of pig .	50·33	6·98	17·77	0·59	...	Sadikoff.[3]

While the nitrogen-percentage is high, the carbon-content is relatively low, and this low percentage is also the reason why gelatine

[1] W. G. van Name, *Journ. of experiment. Med.* **2**. 117 (according to *Maly's Jahresber. f. Tierchem.* **27**. 34 (1897).

[2] C. T. Mörner, "Glutin," *Zeitschr. f. physiol. Chem.* **28**. 471 (1898); "Fish-scales," *ibid.* **24**. 125 (1897); "Cornea," *ibid.* **18**. 213 (1893); "Tracheal cartilage," *Skandinav. Arch. f. Physiol.* **1**. 210 (1889).

[3] W. S. Sadikoff, *Zeitschr. f. physiol. Chem.* **39**. 396 and 411 (1903).

[4] R. H. Chittenden and F. P. Solley, *Journ. of Physiol.* **12**. 23 (1891).

[5] E. S. Faust, *Arch. f. experiment. Path. u. Pharm.* **41**. 309 (1898).

[6] J. Scherer, *Liebig's Annalen*, **40**. 1 (1841).

[7] S. C. v. Goudoever, *ibid.* **45**. 62 (1843).

possesses a low heat value, for the latter, according to Berthelot and Stohmann,[1] is 500-700 calories smaller than in the case of most other albuminous substances.

The study of the dissociation-products of gelatine dates back to 1820, when Braconnot[2] showed that gelatine is rich in 'glycocoll' or 'sweat-glue.' This compound amounts, according to E. Fischer,[3] to 16·5 per cent. In addition, gelatine contains large amounts of glutaminic acid, pyrrolidin- and oxy-pyrrolidin carboxylic acids, and also arginin and lysin.[4] It is poor, however, in histidin, and of the three aromatic compounds it possesses only phenylalanin, as both tyrosin and tryptophane are absent. The amount of phenylalanin is without doubt much higher[5] than the figures of Fischer lead one to suppose. The absence of Millon's reaction was already noticed by the older investigators; later on Maly[6] and Nencki[7] missed the derivatives of tyrosin and also indol; that, notwithstanding this fact, aromatic compounds were found was one of the first indications of the occurrence of phenylalanin in albumins.

Maly[8] by oxidising gelatine with potassium permanganate + caustic potash obtained the oxyprot-sulphonic acids discussed on p. 237.

On oxidising gelatine by means of permanganates, especially by calcium permanganate[9] in boiling solutions, Kutscher and Zickgraf,[10] and Zickgraf[11] obtained a substance which, according to Seemann,[12] is oxaluramid or oxalan $C_3H_5N_3O_3 = NH_2 \cdot CO \cdot NH \cdot C_2O_2 \cdot NH_2$ (oxaluric acid or $C_3H_4N_2O_4 = NH_2 \cdot CO \cdot NH \cdot C_2O_2 \cdot OH$); Kutscher and Schenck,[13] in addition to oxalan, observed also ammonium oxaminate,

[1] F. Stohmann and H. Langbein, *Journ. f. prakt. Chem.* [2] **44**. 336 (1891).

[2] H. Braconnot, *Ann. de Chim. et de Phys.* **13**. 113 (1820).

[3] E. Fischer, P. A. Levene, and R. H. Aders, *Zeitschr. f. physiol. Chem.* **35**. 70 (1902).

[4] A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900); W. Hausmann, *ibid.* **27**. 95 (1899).

[5] K. Spiro, *Hofmeister's Beiträge*, **1**. 347 (1901); V. Ducceschi, *ibid.* **1**. 339 (1901); R. Maly, *Monatshefte f. Chem.* **10**. 26 (1889); M. Nencki, *Ber. d. deutsch. chem. Ges.* **7**. II. 1593 (1874); L. Selitrenny, *Monatshefte f. Chemie*, **10**. 908 (1889).

[6] R. Maly, *ibid.* **10**. 26 (1889).

[7] M. Nencki, *Ber. d. deutsch. chem. Ges.* **7**. II. 1593 (1874); L. Selitrenny, *Monats. f. Chem.* **10**. 908 (1889).

[8] R. Maly, *ibid.* **10**. 26 (1889).

[9] The permanganates of the alkaline earths (viz. barium permanganate) were first used by Steudel, *Zeit. f. physiol. Chem.* **32**. 241 (1901).

[10] Kutscher and Zickgraf, *Sitzb. d. kgl. preuss. Akad. d. Wiss.* May 28, 1903.

[11] G. Zickgraf, *Die Oxydation des Leims mit Permanganaten.* Inaugural Dissertation, Marburg, 1904; and also in *Zeitsch. f. physiol. Chem.* **41**. 259 (1904).

[12] J. Seemann, *Zentralbl. f. Physiol.* **18**. 285 (1904).

[13] Kutscher and Martin Schenck, *Ber. d. deutsch. chem. Ges.* **37**. 2928 (1904).

$C_2O_3NH_2NH_4$, and suggest that the presence of glycocoll in any albuminous compound may readily be determined by oxidising the albumin with boiling calcium permanganate, according to Steudel's method, and then isolating the ammonium oxaminate, for glycocoll gives rise to oxaminic acid, $NH_2 . C_2O_2 . OH$, when it is oxidised with permanganates. The search for oxaminic acid was suggested by the previous paper of Ehrmann, who, working in Hofmeister's Laboratory, put forward the view that on the strength of Hofmeister's theory as to how amino-acids are linked together, there are formed, by hydrolysis, oxamid $\rightleftarrows$ oxaminic acid $\rightleftarrows$ oxalic acid $\rightleftarrows$ ammonia (see p. 243). Seemann found amongst the oxidation products of gelatine, in addition to oxalan and calcium and ammonium oxalate, the following ether-soluble acids: oxalic-, succinic-, benzoic-, formic-, acetic- and butyric-acids, further benzaldehyde, and perhaps also propionic- and valerianic acids.

Zickgraf,[1] in support of Kossel's view that the biuret-reaction depends on a special way in which arginin-molecules are linked to one another and to the other complexes of the albumin molecule, found on oxidising gelatine with permanganates that the biuret reaction disappears at that time when the largest amount of guanidin is found amongst the dissociation-products. As guanidin is derived from arginin, he reasons that some connection must exist between the disappearance of the biuret reaction and the maximal yield of guanidin. That this view is not tenable seems to follow from the researches of von Fürth.[2]

Heating gelatine for four days under pressure gives in the main only rise to albumoses, according to Nasse[3] and Framm.[4]

The absence of tyrosin and tryptophane amongst the dissociation-products and the high percentage of glycocoll show that gelatine belongs exclusively to the antigroup of the albumin-molecule (see index under antigroup). It behaves also towards enzymes exactly like a hetero-albumose. Trypsin, according to the older observations of Nencki,[5] Tatarinoff,[6] Kühne,[7] and Reich-Herzberge,[8] gives rise to no

[1] G. Zickgraf, Inaugural Dissertation. Marburg, 1904; *Zeitschr. f. physiol. Chem.* **41**. 259 (1904).

[2] Otto v. Fürth, *Hofmeister's Beiträge*, **6**. 296 (1905).

[3] O. Nasse and A. Krüger, *Naturf. Gesellsch. zu Rostock, Rostocker Ztg.* 1889, Nr. 105 (reprint).

[4] F. Framm, *Pflüger's Archiv f. d. ges. Physiol.* **68**. 144 (1897).

[5] M. Nencki, *Ber. d. deutsch. chem. Ges.* **7**. II. 1593 (1874); L. Selitrenny, *Monats. f. Chem.* **10**. 908 (1889).

[6] P. Tatarinoff, *Zentralbl. f. die medizin. Wissensch.* 1877, p. 275.

[7] W. Kühne, *Verh. d. Heidelberger Naturhist.-med. Verein*, N.F. I. p. 194 (1876).

[8] F. Reich-Herzberge, *Zeitschr. f. physiol. Chem.* **34**. 119 (1901).

crystalline dissociation-products, or only to traces. In their place are found not only antipeptones, which Krüger[1] has described and from which Siegfried first isolated kyrin (p. 200), but according to Chittenden and Solley[2] and Klug[3] also albumoses, which as a rule are very quickly destroyed.

On subjecting gelatine to tryptic digestion for ten months Levene[4] obtained in the main only albumoses and peptones, and noticed the peculiar fact that the primary dissociation-compounds of gelatine, namely the gelatoses, contain more glycocoll than does the original gelatine, but that the secondary products, namely the peptones, contain again less glycocoll than the gelatoses. An explanation was found, for the gelatoses in becoming peptones split off glycocoll, which, along with traces of leucin, represents nearly the whole of the mono-amino-acid-fraction liberated by digestive enzymes. From 1500 grams of gelatine, after ten months' digestion, Levene obtained 40 grams of glycocoll-ester, a small amount of leucin, traces of phenylalanin, glutaminic and aspartic acids, but large amounts of ammonia. From the phosphotungstic acid precipitate a copper salt of the racemic pyrrolidin-carboxylic acid was obtained.

Peptic digestion, according to Scheermesser,[5] proceeds likewise slowly, and gives rise to peptones only at a late period; 'anti-albumid' is, however, formed abundantly.[6] On combining tryptic digestion with putrefaction Nencki[7] obtained similarly only small quantities of leucin and glycocoll; most of the products gave the biuret reaction.

Scheermesser in his last paper describes a new pepsin-glutin peptone in which arginin, lysin, glutaminic acid and glycocoll were present while histidin was certainly absent.

The percentage distribution of nitrogen in the pepsin-glutin-peptone molecule, taking the mean of two experiments is as follows:—

[1] T. R. Krüger, *Zeitschr. f. physiol. Chem.* **38**. 320 (1903).

[2] R. H. Chittenden and F. P Solley, *Journ. of Physiology*, **12**. 23 (1891).

[3] F. Klug, *Pflüger's Arch. f. d. ges. Physiol.* **48**. 100 (1891).

[4] P. A. Levene, *Zeitschr. f. physiol. Chem.* **41**. 8 and 99 (1904).

[5] W. Scheermesser, *ibid.* **37**. 363 (1903); *Philosophical Dissertation*, Leipzig, 1903; *Zeitschr. f. physiol. Chem.* **41**. 68 (1904).

[6] R. H. Chittenden and F. P. Solley, *Journ. of Physiol.* **12**. 23 (1891); F. Klug, *Pflüger's Arch. f. d. ges. Physiol.* **48**. 100 (1891).

[7] M. Nencki, *Ber. d. deutsch. chem. Ges.* **7**. II. 1593 (1874).

[TABLE

Amid-N.	Di-amino-N.		Mono-amino-N.	Total N instead of 100 per cent.
0	24·96 of which		70·03 of which	94·99
	Arginin.	Lysin.	Glutaminic Acid.	
	15·24	9·37	10·67	

The nitrogen-determinations by Kjeldahl's method give lower values than those by Dumas' method, according to Sadikoff.[1] If, however, gelatine is treated with hydrochloric acid, its nitrogen-content becomes lowered, and the difference in the results of the two methods disappears. Whether the phenomenon is to be explained as depending on a dissociation caused by HCl or on the removal of an impurity is an open question.

The action of the proteolytic ferments of the liver on gelatine has been studied by Arnheim.[2] The amount of the non-coagulable, by zinc sulphate not-precipitable nitrogen, was in all gelatine experiments much greater than in the control experiments, owing to the gelatine having been converted partly into mono-amino acids,[3] and partly into peptone and di-amino acids, which could be precipitated by phosphotungstic acid.

The formation of a reducing substance on dissociating-gelatine has only been observed by Sadikoff[1] in the case of gelatine prepared from cartilage, and is probably due to contamination by remnants of chondro-mucoid or chondro-sulphuric acid. The only sulphur-containing dissociation-product obtained so far by Horbaczewski[4] is sulphuretted hydrogen.

Gelatine gives a well-marked, violet biuret reaction, while the reaction of Millon and the xantho-proteic reactions, judging by the dissociation-products, ought to be absent. As a matter of fact, one may always observe, according to van Name[5] and Mörner,[6] a very slight pink coloration on boiling gelatine with Millon's reagent, and

[1] W. S. Sadikoff, *Zeitschr. f. physiol. Chem.* **39**. 411 (1903).

[2] J. Arnheim, *Zeitschr. f. physiol. Chem.* **40**. 234 (1903).

[3] Gum arabic, dextrose, dextrin, and lactose increased the autolysis of the liver substance, while the chlorides of sodium, potassium, and ammonium were without any influence.

[4] J. Horbaczewski, *Sitzungsber. d. Wiener Akad.* **80**. math.-naturw. Kl. II. June (1879).

[5] W. G. van Name, *Journ. of Experiment. Med.* **2**. 117 (according to *Maly's Jahresber. f. Tierchem.* **27**. 34) (1897).

[6] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **28**. 471 (1899).

according to Klug[1] and Hofmeister[2] a feeble yellow colour after treatment with nitric acid. Both these reactions must be caused by the presence of foreign substances. The lead sulphide reaction is given by commercial gelatine, but is absent in purified gelatine, according to Mörner.[3] The reaction of Molisch has been observed by Klug[1] and Hofmeister,[2] while that of Adamkiewicz is absent, owing to gelatine not containing any tryptophane.

The precipitation-reactions of non-dissociated gelatine have been described by Klug,[1] Mörner,[3] and others, while those for the glutoses have been investigated by Klug[1] and Hofmeister.[2] Gelatin is not precipitated by nitric acid or other mineral acids, nor on being acidified with acetic or hydrochloric acid. Neither do neutral lead acetate, silver nitrate, copper sulphate, ferric chloride, or alum, precipitate. On the other hand, the chlorides of gold and platinum and stannous chloride give precipitates, which are soluble at the boiling temperature and which return on cooling. Mercuric nitrate and basic lead acetate cause a precipitate, as does also mercuric chloride in the presence of HCl or of neutral salts. The alkaloidal reagents, taken as a whole, also cause precipitation ; the phospho-molybdic acid precipitate is permanent when heated, while the precipitates formed by picric acid, tannic acid, chromic acid, mercury + potassium iodides + HCl are dissolved by heating, and return on cooling. Bromine- and chlorine water and potassium iodide also precipitate. With tannic acid salt-free gelatine gives no precipitate, and in this resembles salt-free albumin-solutions ; on the addition of salt, however, a precipitate is formed.[4] The same holds good for alcohol. Till a short time ago it was considered characteristic of solutions of gelatine that, in contrast to other albumins, they gave no precipitate with potassium ferrocyanide + acetic acid,[5] but Mörner[4] has shown that under 30° C. it is possible to obtain a precipitate with very dilute solutions, but that the precipitate is dissolved by both an excess of gelatine or of ferrocyanic acid, and also that the presence of salts, organic acids, or bases or urea prevents the formation of a precipitate. Gelatoses never give a precipitate. The behaviour of gelatine towards neutral salts has been investigated but little ; it is precipitated by not quite saturated

[1] F. Klug, *Pflüger's Arch. f. d. ges. Physiol.* **48**. 100 (1891).

[2] F. Hofmeister, *Zeitschr. f. physiol. Chem.* **2**. 299 (1878).

[3] C. T. Mörner, *ibid.* **28**. 471 (1899) ; see also *ibid.* **18**. 213 (1893) and *Skandinav. Archiv. f. Physiol.* **1**. 210 (1889).

[4] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **28**. 471 (1899) ; see also *ibid.* **18**. 213 (1893) and *Skandinav. Arch. f. Physiol.* **1**. 210 (1889) ; H. Weiske, *Zeitschr. f. physiol. Chem.* **7**. 460 (1883).

[5] J. Müller, *Liebig's Annalen*, **21**. 277 (1837).

ammonium sulphate solutions, and according to Mörner also by sodium sulphate.

Gelatine and the gelatoses form, as do other albumins, salts with acids and bases; the salts of gelatoses and gelatin-peptones with HCl have been investigated by Paal.[1] Gelatine is, however, essentially acid in character, for according to Hofmeister,[2] Tatarinoff,[3] and Nasse[4] it possesses, when pure, an acid reaction and dissociates carbonates. Hofmeister[2] has analysed the platinum and copper salts of the gelatoses, without, however, obtaining constant values. Nasse[4] has investigated the barium salts, and has found the amount of barium, *i.e.* the basic equivalent, to rise in passing from gelatin to gelatin-peptones. Halogen-derivatives of gelatine are unknown.

Gelatine is insoluble in cold water, but swells up in it; it is also, generally speaking, insoluble in salt-solutions, acids, and alkalies, but is readily converted into a soluble modification,[5] and then differs somewhat from the original gelatine. In hot water gelatine is very readily soluble and, according to the amount of water present, on cooling gives rise either to a nearly solid mass, as employed for carpentering purposes, or to a thin trembling jelly, as used for culinary ends. The temperature for setting and the melting-points of gelatine solutions have been recently reinvestigated by Pauli.[6] Pure gelatine, according to its concentration, sets between 18° and 25°, and when heated melts between 26° and 29°. The melting and setting temperatures are, however, modified by salts and by organic crystalloids.[7] Sulphates, citrates, tartrates, acetates, glycerin, and sugar raise the temperature at which gelatine sets, while chlorides, chlorates, nitrates, bromides, iodides, alcohol, and urea diminish the same. Mörner[8] has shown that the presence of salts is not essential for the gelatinisation of gelatine, for it sets in the absence of salts. By stronger concentrations of salts gelatine is precipitated and gelatinisation is prevented.[8] Pauli and Rona have also investigated this question.

The power of gelatinising is only possessed by unaltered gelatine, but not by its dissociation-products: the gelatoses or glutoses or the

[1] C. Paal, *Ber. d. deutsch. chem. Ges.* **25**. 1202 (1892).

[2] F. Hofmeister, *ibid.* **2**. 299 (1878).

[3] P. Tatarinoff, *Zentralbl. f. d. med. Wissensch.* 1877, p. 275.

[4] O. Nasse, *Naturf. Gesellschaft zu Rostock, Rostocker Ztg.* 1889, No. 105.

[5] W. S. Sadikoff, *Zeitschr. f. physiol. Chem.* **39**. 411 (1903).

[6] W. Pauli and P. Rona, *Hofmeister's Beitr.* **2**. 1 (1902). Here the former papers of Pauli are abstracted and also the other literature referred to. See also Pascheles (or Pauli), *Pflüger's Archiv*, **71**. 333 (1898).

[7] Levites, *Journ. d. russ. phys. chem. Ges.* **34**. 110 and 439; W. S. Sadikoff, *Zeitschr. f. physiol. Chem.* **39**. 396 and 411; **41**. 15 (1904).

[8] C. T. Mörner, *ibid.* **28**. 471 (1899).

glutin-peptones. If, therefore, a gelatine-solution is acted upon by any means by which albumins are converted into albumoses, it at once loses its power of solidifying. This occurs, for example, if gelatine is boiled at ordinary or at increased pressure with pure water—as a rule, it must be admitted that the reaction is not exactly neutral, and therefore usually the action of dilute acids or of dilute alkalies comes also into play—or on boiling with acids or alkalies, or as the result of peptic and tryptic digestion, and of putrefaction. All the processes first convert gelatine into gelatoses, and the latter into further dissociation-products.[1] But before the dissociation, just alluded to, has occurred, there is a certain stage during which the gelatine has only lost its power of gelatinisation, being otherwise unaltered, and during this stage it may be compared with ordinary albumins which have become denaturalised without having as yet undergone a further dissociation. Kept at body temperature gelatine-solutions lose their power of gelatinising as a rule fairly quickly, sometimes in one to two days.[2]

Injected subcutaneously, and even more so when injected intravenously, gelatine gives rise to certain symptoms of poisoning, apart from acting on the blood. Whether these effects depend upon impurities or whether they are due to the gelatine itself is as yet uncertain.[3]

Different kinds of Collagen

The gelatine described above may be obtained from connective tissues or tendons, but the collagen in the cornea and the sclerotic coat of the eye, discovered by Morochowetz,[4] and more carefully examined by Mörner,[5] and fish-gelatine, prepared from fish-scales by Weiske [6] and Mörner,[7] do not differ from the ordinary tendon-gelatine. The dried corneal tissue contains 80 per cent, and that of the sclerotic 87 per cent of collagen, according to Mörner. The remainder is represented by mucoid. The lens and the other parts of the eye contain no collagen. Fish scales are composed of 20 per cent of ichthylepiden (see p. 578), and 80 per cent of collagen, which is remarkable as the collagen is very readily converted into gelatine.

[1] F. Hofmeister, *Zeitschr. physiol. Chem.* **2**. 299 (1878).

[2] C. T. Mörner, *ibid.* **28**. 471 (1899) ; A. Dastre et N. Floresco, *Arch. de Physiol. normale et path.* **27**. 701 (1895).

[3] Compare different abstracts in the *Zentrabl. f. d. Grenzgebiete von Med. u. Chir.* 1902 to 1904 ; H. Kaposi, *Heidelberger Habilitationsschrift*, 1904.

[4] L. Morochowetz, *Verhandl. des naturh.-med. Vereins zu Heidelberg*, N.F. I., p. 480, (1876).

[5] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **18**. 213 (1893).

[6] H. Weiske, *ibid.* **7**. 466 (1883).

[7] C. T. Mörner, *ibid.* **24**. 125 (1897).

Gelatine from Cartilage.—Formerly it used to be supposed that cartilage was composed of a uniform substance which was called chondrigen, and that the latter when boiled gave rise to chondrin or cartilage-glue, but Morochowetz showed that chondrin is a mixture of gelatine and mucin, and Krukenberg confirmed this view. The most exact investigation into the chemistry of cartilage has been made by Mörner, according to whom cartilage contains the following substances:—

1. Chondromucoid and its dissociation-product:
2. Chondroitin-sulphuric acid. This 'chondro-sulphuric' acid occurs normally in cartilage in small quantities.
3. Collagen.
4. An albumoid in old but not in young cartilage.

The framework of old cartilage consists of albumoid + collagen, while the enclosed 'chondrin-balls' are composed of collagen + mucoid. These two substances have distinct staining reactions and are histologically readily recognisable.

On treating cartilage with dilute acids at 40° C., a mixture of gelatine + chondro-sulphuric acid is obtained, while boiling in a Papin's pot yields a compound consisting of gelatin + mucoid + chondro-sulphuric acid. This compound resembles in its reactions the 'chondrin' of the older observers, and differs from ordinary gelatine in not being precipitated by tannin, because the chondro-sulphuric acid prevents the precipitation of the gelatine moiety. The mucoid is described on p. 542, and the albumoid on p. 577. The glutin of cartilage has been investigated in addition to Mörner by Lönnberg,[1] who also examined fish-cartilage, and by Sadikoff.[2] To prepare this gelatine requires very intense boiling with water. The reducing power and the feeble pentose reaction noticed by Sadikoff are probably due to impurities.

Collagen from Bone or 'Ossein.'—The ground substance of bone consists for the greater part of collagen + lime-salts. It contains in addition an albumoid[3] (p. 577), and a mucoid,[4] resembling chondromucoid (p. 577). Keratin or other albumins are absent.[5] Bone-gelatine has been investigated by Weiske;[6] it gives the ordinary gelrtine reactions, and is not readily formed from the collagen.

[1] J. Lönnberg, *Maly's Jahresbericht*, **19**. 325 (1889).

[2] W. S. Sadikoff, *Zeitschr. f. physiol. Chem.* **39**. 411 (1903).

[3] P. B. Hawk and W. J. Gies, *Amer. Journ. of Physiol.* **7**. 340 (1902).

[4] L. Morochowetz, *Verhandl. d. Heidelberger naturh.-med. Vereins*, N.F. I. p. 480, (1876); C. T. Mörner, *Zeitschr. f. physiol. Chem.* **23**. 311 (1897); P. B. Hawk and W. J. Gies, *Amer. Journ. of Physiol.* **5**. 387 (1901).

[5] H. Smith, *Zeitschr. f. Biol.* **19**. 469 (1883).

[6] H. Weiske, *Zeitschr. f. physiol. Chem.* **7**. 460 (1883).

'Ossein,' *i.e.* decalcified bone, is as readily dissolved by pepsin as are other kinds of gelatine.

Collagen of Invertebrates. — *Silk Glue.* — Hoppe-Seyler[1] has found gelatine-yielding tissues amongst the invertebrates in the cephalopods : Octopus and Sepiola. Silk-glue is discussed on p. 571.

Glutolin.—Under this name Faust[2] has described an albuminous substance which he found in blood-serum, and which he considers to be the mother substance of gelatine because of its low sulphur content and the feeble Millon's reaction it gives. The possibility of this substance being a transformation-product of one of the serum-albumins is not excluded.

2. Keratin

Keratin is the chief constituent of the horny substances found in mammals and birds. It occurs in the stratum corneum of the epidermis, in hairs, nails, hoofs, horns, and in feathers. It is also found in the egg-shells of birds,[3] and of the Echidna aculeata[4] (a mammal), of crocodiles[4] and snails,[5] in the cocoons of leeches,[6] and probably in many other tissues amongst invertebrates. (Compare the articles by Neumeister[4] and Sukatschoff[6]). Neurokeratin is met with in the medullary sheath of medullated nerves.

Keratin is the most insoluble of all the albuminoids, being quite insoluble in water, dilute acids, and alkalies ; according to Smith[7] even 10 per cent KOH dissolves it only with the help of heat ; in the cold a 20 per cent caustic soda solution is required to render it soluble, and this procedure of course decomposes keratin. The digestive enzymes have also the greatest difficulty in rendering it soluble.

An exact study of keratin, for the reasons just given, is impossible ; on the other hand, its insolubility in acids, alkalies, pepsin, and trysin, makes it possible to prepare keratin in a fairly pure state, as all other albuminous substances may be removed by treatment with the reagents just mentioned.[8]

Notwithstanding this the analyses given by Kühne and Chittenden,[8]

[1] Hoppe-Seyler, *Med.-chem. Untersuch.* p. 586 (1871).

[2] E. S. Faust, *Arch. f. exper. Path. u. Pharm.* **41**. 309 (1898).

[3] V. Lindwall, Hammarsten's abstract of the Swedish original in *Maly's Jahresber. f. Tierchem.* **11**. 38 (1881).

[4] R. Neumeister, *Zeitschr. f. Biol.* **31**. 413 (1895).

[5] W. Engel, *ibid.* **27**. 374 (1890) ; **28**. 345 (1891).

[6] B. Sukatschoff, *Zeitschr. f. wissensch. Zool.* **56**. 377 (1899).

[7] H. Smith, *ibid.* **19**. 469 (1883).

[8] W. Kühne and R. H. Chittenden, *ibid.* **26**. 291 (1890).

Lindwall,[1] van Laer,[2] Horbaczewski,[3] Bibra,[4] Suter,[5] and Mohr[6] differ greatly from one another on all points except as regards the high sulphur content, which has already been alluded to on p. 73.

Its dissociation-products are enumerated on p. 73, under Nos. 25 to 27. Keratins are remarkable for the high percentage of tyrosin, and in particular of cystin, a compound which was prepared for the first time in a pure state from keratin by Mörner;[7] from human hair he obtained 14 per cent.[8] Other sulphur-containing dissociation-products have also been found in keratin, for on dissociating keratin, sulphuretted hydrogen and methyl mercaptane are obtained[9] (see index).

Ammonia is as a rule also obtained in large amounts;[10] amongst the bases, arginin has been observed, while the others have not yet been looked for. A carbohydrate was neither found by Kühne and Ewald[11] nor by Neumeister.[12] When keratin is treated with alkalies or with superheated water, albuminates, albumoses, and peptones are formed, according to Lindwall[1] and Bauer.[9]

As can be judged by its composition, keratin gives the following reactions typical of albumin: an intense Millon's reaction and equally intense xanthoproteic and lead-sulphide tests. Whenever, therefore, a substance gives the just-mentioned tests very strongly, and is at the same time insoluble in acids and alkalies and resists trypsin and pepsin, we know we are dealing with keratin. Very finely divided, *i.e.* very young, keratin is digested, however, by pepsin, according to Kühne[13] and Neumeister[12] (Cohnheim); young keratin differs chemically from adult keratin (the author).

Neuro-keratin was discovered by Kühne and Ewald, and more carefully investigated by Kühne and Chittenden; it is even more resistant to the action of alkalies than is the keratin of the skin, and

[1] V. Lindwall, Hammarsten's abstract of the Swedish original in *Maly's Jahresber. f. Tierchem.* **11**. 38 (1881).

[2] J. F. J. van Laer, *Liebig's Annalen*, **45**. 147 (1843).

[3] J. Horbaczewski, *Sitzungsber. d. Wiener Akad.* **80**. math.-nat. Kl. II. (1879). (Reprint).

[4] v. Bibra, *Liebig's Annalen*, **96**. 289 (1855).

[5] F. Suter, *Zeitschr. f. physiol. Chem.* **20**. 564 (1895).

[6] P. Mohr, *ibid.* **20**. 400 (1894).

[7] K. A. H. Mörner, *ibid.* **28**. 595 (1899).

[8] K. A. H. Mörner, *ibid.* **34**. 207 (1901).

[9] R. Bauer, *ibid.* **35**. 343 (1902).

[10] J. Horbaczewski, *Wiener Akad.* **80**. math.-nat. Kl. II. (1879).

[11] A. Ewald and W. Kühne, *Verhandl. d. naturh.-med. Vereins Heidelberg*, New Series I. p. 457 (1876).

[12] R. Neumeister, *Zeitschr. f. Biol.* **31**. 413 (1895).

[13] W. Kühne, *Untersuch. an dem Heidelberg. physiol. Institut*, I. p. 219 (1877).

it forms a portion of the medullary sheath of nerves in vertebrate animals, and is found therefore abundantly in the brain, spinal cord, and peripheral nerves; it also occurs in the retina; in the central nervous system it amounts to from 15-20 per cent of the dry residue after the myelin-substances have been removed, and according to Chevalier 0·3 per cent of the fresh sciatic nerve. In the ventral ganglionated cord of the lobster neuro-keratin is replaced by chitin according to Kühne and Chittenden.

Analysis shows it to contain a remarkably high carbon percentage and a somewhat lower sulphur-content than that possessed by other keratins. On being dissociated it yields leucin and tyrosin.

To the keratins belongs also, according to Drechsel,[1] the gorgonin which forms the ground matrix of the axial skeleton of the coral Gorgonia Cavolinii. According to Drechsel and Henze,[2] it contains no glycocoll, but histidin, lysin, arginin, leucin, ammonia, sulphuretted hydrogen, and very large amounts of tyrosin. It also contains iodine, and therefore belongs to the naturally occurring iodo-albumins (see p. 230). It differs from spongin, which it otherwise resembles, in containing tyrosin. A more detailed account of allied bodies is given when dealing with spongin. Mendel[3] has found an iodo-keratin also in other corals. The framework of the tropical horny sponges, which Hundeshagen[4] has called iodo-spongin, is probably also a keratin judging by its tyrosin-content.

3. Elastin

Elastin arranged into fibres forms 'elastic tissue,' which may occur as a thick, coarse strand as in the yellow neck-band or ligamentum nuchæ of the ox, or arranged as a membrane as in the fasciæ, and in the walls of the aorta, or as fibres more or less freely intermingled with white fibrous tissue (as in skin, the ordinary connective tissues, in the smaller blood-vessels, and in tendon), or with cartilage, as *e.g.* in the arytenoid cartilages and many ear-cartilages. It also forms, according to Neumeister,[5] the organic ground substance of the egg-shells in some reptiles and fishes. The elastin of the eggs of the grass-snake, which has been investigated by Hilger[6] and Engel,[7] is

[1] E. Drechsel, *Zeitschr. f. Biol.* **33**. 85 (II. to IV.) (1896).

[2] M. Henze, *Zeitschr. f. physiol. Chem.* **38**. 60 (1903).

[3] L. B. Mendel, *Amer. Journ. of Physiol.* **4**. 243 (1900).

[4] F. Hundeshagen, *Zeitschr. f. angew. Chem.* 1895, p. 473 (according to the *Chem. Zentralbl.* 1895, II. p. 570).

[5] R. Neumeister, *Zeitschr. f. Biol.* **31**. 413 (1895).

[6] Hilger, *Ber. d. deutsch. chem. Ges.* **6**. I. 166 (1873).

[7] W. Engel, *Zeitschr. f. Biol.* **27**. 374 (1890).

an elastin if judged by its composition and its properties, but is almost as insoluble as is keratin, and has therefore been called kerato-elastin by Neumeister.

Ordinary elastin is not much more soluble than is keratin; it is not attacked by 5 per cent KOH in the cold, and hardly by hot 1 per cent KOH. It is, however, digested by both pepsin + hydrochloric acid and by trypsin, and is converted, although slowly, into albumoses. To obtain it in a pure form, the other constituents with which it occurs together are removed by alternate treatment with acids and alkalies.

Chittenden and Hart[1] found the percentage-composition of the elastin of the ligamentum nuchæ of the ox to be:

C 54·08 H 7·2 N 16·85 S 0·3.

The analysis of Horbaczewski[2] and Zoja[3] for the same elastin, and those of Schwarz[4] and Bergh[5] for the aorta-elastin, agree well with one another, for all give a high carbon and a low sulphur percentage. All observers agree in finding large amounts of leucin (see below). Horbaczewski found 0·7 per cent ammonia, 0·25 per cent tyrosin, glycocoll, and amino-valerianic acid; Schwarz obtained 0·34 per cent tyrosin, and Kossel and Kutscher 0·3 per cent arginin. The latest analysis by Abderhalden and Schittenhelm[6] gives for 400 grammes of elastin these figures:—

	Grammes.	Per Cent.		Grammes.	Per Cent.
Glycocoll . .	103·0	25·75	α-Pyrrolidin-carboxylic acid	6·97	1·74
Leucin . .	85·5	21·38	Amino-valerianic acid .	4	1
Alanin . .	26·3	6·58	Glutaminic acid . .	3·04	0·76
Phenylalanin .	15·55	3·89	Aspartic acid . . .	?	?
			Total . .		61

Elastin is amongst all albuminous substances the one poorest in tyrosin and arginin. The indol-derivatives are completely absent on subjecting elastin to bacterial digestion, according to Wälchli[7] and Zoja.[8] Engel[9] found that elastin, which had been rendered soluble

[1] R. H. Chittenden and A. S. Hart, *Zeitschr. f. Biol.* **25**. 368 (1889).

[2] J. Horbaczewski, *Zeitschr. f. physiol. Chem.* **6**. 330 (1882); *Monatsh. f. Chem.* **6**. 639 (1885). [3] L. Zoja, *Zeitschr. f. physiol. Chem.* **23**. 236 (1897).

[4] H. Schwarz, *ibid.* **18**. 487 (1893).

[5] Ebbe Bergh, *ibid.* **25**. 337 (1898).

[6] Abderhalden and Schittenhelm, *ibid.* **41**. 293 (1904).

[7] G. Wälchli, *Journ. f. prakt. Chem.* [2] **17**. 71 (1878).

[8] L. Zoja, *Zeitschr. f. physiol. Chem.* **23**. 236 (1897).

[9] W. Engel, *Zeitschr. f. Biol.* **27**. 374 (1897).

with alkali, gave all the colour tests excepting the lead sulphide reaction. Superheated steam, according to Horbaczewski [1] and Schwarz,[2] and peptic and tryptic digestion, according to Horbaczewski [1] and Chittenden and Hart,[3] give rise to albumoses.

Elastic tissue is, however, so slowly acted upon by pepsin that Stirling in 1873, while working in Ludwig's laboratory, optically isolated the elastic fibres in the skin by digesting the latter with artificial gastric juice, which renders all other elements, excepting nerves and nuclei, indistinct.[4] Subsequently Ewald and Kühne [5] treated various organs with slightly alkaline glycerine extract of the pancreas. The results obtained by them are given in the author's *Physiological Histology*, p. 153. Ewald [6] has subsequently extended his research. He found elastin to be very slowly soluble in both pepsin and trypsin ; and that it was more readily acted upon by digestive enzymes if it had previously been boiled or had been acted upon by acids or by alcohol. Barium tetroxide renders fibrils indigestible for pepsin, but more readily digestible for trypsin, while chromic acid, if light has access, produces exactly the opposite effect.

4. Fibroin and Silk Glue

Natural silk consists of delicate fibres composed of a core of fibroin surrounded by an envelope of a glue-like substance. Raw silk contains therefore, apart from salts, a mixture of fibroin and silk-glue or sericin. Both these bodies have been investigated by Mulder,[7] Städeler,[8] Cramer,[9] Weyl,[10] Vignon,[11] Wetzel,[12] and E. Fischer and Skita.[13] Lombardy silk yields about 70 per cent of fibroin and 30 per cent of glue. Even technically purified silk contains still about 5 per cent of glue.[13]

Fibroin is insoluble even in superheated water, in dilute acids and

[1] J. Horbaczewski, *Zeitschr. f. physiol. Chem.* **6**. 330 (1882) ; *Monatsh. f. Chem.* **6**. 639 (1885).

[2] H. Schwarz, *ibid.* **18**. 487 (1893).

[3] R. H. Chittenden and A. S. Hart, *Zeitschr. f. Biol.* **25**. 368 (1889).

[4] W. Stirling, *Ber. Verh. d. königl. Sächs. Gesellsch. d. Wissensch.* Leipzig, **26**. 221 (1874) ; and in *Journ. of Anat. and Physiol.* (1875).

[5] A. Ewald and W. Kühne, 'Die Verdauung als histologische Methode,' *Verh. naturhist. Vers.* Heidelberg (N.F.), **1**. 451.

[6] A. Ewald, *Zeitschr. f. Biol.* **26**. 1 (1890).

[7] According to E. Fischer and Skita.

[8] G. Städeler, *Liebig's Annalen*, **111**. 12 (1859).

[9] E. Cramer, *Journ. f. prakt. Chem.* **96**. 76 (1865).

[10] Th. Weyl, *Ber. d. deutsch. chem. Gesellsch.* **21**. II. 1407 ; **21**. II. 1529 (1888).

[11] L. Vignon, *Compt. rend.* **115**. 613 (1892).

[12] G. Wetzel, *Zeitschr. f. physiol. Chem.* **26**. 535 (1899).

[13] E. Fischer and Skita, *ibid.* **33**. 171 (1901) ; **35**. 224 (1902)

alkalies, and therefore a Papin's pot has always been used for separating the two constituents of silk from one another. E. Fischer and Skita have shown that fibroin may be heated for hours to 117-120°, provided care is taken to keep the reaction exactly neutral; in the presence of acids or alkalies it is, however, considerably changed under these conditions.

The analysis of fibroin by Cramer, Weyl, and Vignon show a low carbon percentage and a high nitrogen percentage, namely, 19 per cent; the sulphur has not been determined. The dissociation-products obtained by E. Fischer and Skita have been given on p. 73, No. 29. Fibroin differs in its constitution considerably from other albumins, as it contains more than 50 per cent of glycocoll and tyrosin, and 10 per cent of tyrosin, while leucin is only present in small amounts; glutaminic and aspartic acids are absent. The basic radicals are slight in amount. Fibroin gives the biuret and Millon's reactions,[1] and, according to Krukenberg,[2] also that of Adamkiewicz. It is not attacked by either pepsin or trypsin, according to Weyl, while by strong alkalies and acids it is converted into albumoses or albuminates.

The lids covering the compartments in which wasp-embryos are hatched, Engel[1] has found to give the reactions and to show the solubilities of fibroin, and Schlossberger[3] states that cobwebs are also composed of fibroin.

Silk glue, according to the authors mentioned above, and according to Bondi,[4] resembles ordinary gelatine in its solubility, but it does not gelatinise so readily, and is further precipitated by acids. Its dissociation-products show, however, that it is entirely different from ordinary gelatine (see p. 73, No. 30). Glycocoll, if present at all, only occurs in traces, while tyrosin is very abundant, and serin is also met with in large amounts. Serin was first discovered by Cramer in silk glue. Wetzel obtained the bases in ample amounts. Positive results are obtained with the tests of Millon and Molisch.

5. Spongin, Conchiolin, etc.

Spongin forms the framework of the bath-sponge. It was first investigated by Posselt[5] and Croockewit,[6] and later by Städeler,[7] who

[1] W. Engel, *Zeitschr. f. Biol.* **27**. 374 (1890).
[2] F. C. W. Krukenberg, *ibid.* **22**. 241 (1886).
[3] J. Schlossberger, *Liebig's Annalen*, **110**. 245 (1859)
[4] S. Bondi, *Zeitschr. f. physiol. Chem.* **34**. 481 (1902).
[5] L. Posselt, *Liebig's Annalen*, **45**. 192 (1843).
[6] J. H. Croockewit, *ibid.* **48**. 43 (1843).
[7] G. Städeler, *ibid.* **111**. 12 (1859).

gave the substance its name, by Harnack,[1] and by Kossel and Kutscher.[2] The analyses[3] show a low carbon-content; sulphur is present; the dissociation-products are given on p. 73, No. 33. The basic radicals are present in abundance, and so are leucin, glycocoll, and glutaminic acid. Tyrosin is absent. It dissolves at the ordinary temperature only in very concentrated H_2SO_4 or HCl, more readily in caustic potash and in ammoniated copper oxide, and also in superheated water.[4] The presence of iodine, first observed by Croockewit, is of special interest. Harnack prepared from sponges the iodo-spongin, an abiuretic, iodised dissociation-product (compare p. 234). The supporting frameworks of the tropical and subtropical horny sponges, which contain in older specimens up to 14 per cent of iodine, according to Hundeshagen[5] do not belong to this group, but to the keratins, as they yield an iodised tyrosin, which ordinary spongin probably does not do. A detailed account is still wanting.

Conchiolin forms the organic ground matrix of the shells of molluscs. It was first examined by von Voit,[6] and subsequently by Krukenberg,[7] Engel,[8] and especially Wetzel.[9] It is only slightly soluble in acids and in superheated water, but fairly readily in alkalies, at least as long as it is young, for old conchiolin, according to Voit, is much more insoluble. It gives the biuret- and xanthoproteic tests and Millon's reaction. Its dissociation-products are given on p. 73, No. 31. Conchiolin contains in addition 8·66 per cent of its nitrogen in a basic form (Wetzel). A carbohydrate radical and iodine seem to be absent, while sulphur and, according to Voit, also iron are present, but it does not give the lead-sulphide reaction. In the shells of mussels, conchiolin is arranged in definite lamellæ, which, notwithstanding their different colours, do not show any considerable difference in their chemical constitution. The eggs of snails [Murex] also contain conchiolin (Engel).

Cornein forms the framework of corals; it yields indol, according

[1] E. Harnack, *Zeitschr. f. physiol. Chem.* **24**. 412 (1898).

[2] A. Kossel and F. Kutscher, *ibid.* **31**. 165 (1900).

[3] L. Posselt, *Liebig's Annalen*, **45**. 192 (1843); J. H. Croockewit, *ibid.* **48**. 43 (1843); E. Harnack, *Zeitschr. f. physiol. Chem.* **24**. 412 (1898).

[4] F. C. W. Krukenberg, *Zeitschr. f. Biol.* **22**. 241 (1886).

[5] F. Hundeshagen, *Chem. Zentralbl.* 1895, II. p. 570.

[6] C. Voit, 'Physiologie der Perlmuschel,' *Zeitschr. f. wissenschaftl. Zool.* **10**. 470 (1860).

[7] F. C. W. Krukenberg, *Ber. d. deutsch. chem. Ges.* **18**. I. 989 (1885); *Zeitschr. f. Biol.* **22**. 241 (1886).

[8] W. Engel, *Zeitschr. f. Biol.* **27**. 374 (1890); **28**. 345 (1891).

[9] G. Wetzel, *Zeitschr. f. physiol. Chem.* **26**. 535 (1899); **29**. 386 (1900); *Zentralbl. f. Phys.* **13**. No. 5 (1899).

to Krukenberg.[1] Attention is again drawn to the other compounds described by Krukenberg, which have been mentioned on p. 554, and to the substance forming the casing of Echinococcus, which has been studied by Lücke.[2]

6. Amyloid

Amyloid is generally held to be a pathological product, but Krawkow[3] has shown it to occur normally in healthy aortæ, and occasionally also in old cartilage. Under certain pathological conditions, especially those accompanied by great proteid-disintegration, it is found in enormous quantities. It is found either in the form of the so-called corpora amylacea in the brain and in other places, or as a diffused mass in the parenchyma of the liver, the spleen, the kidney, etc., whenever these organs have undergone an amyloid degeneration. Amyloid was first discovered by Virchow,[4] who originally held it to be a carbohydrate because of its peculiar colour-reactions, and hence gave it its name. That amyloid is an albumin was first shown by Schmidt[5] and Friedreich and Kékulé,[6] and Kühne and Rudneff.[7] Amyloid occurs as shiny, homogeneous masses, which, if they be present in large amount, give to the affected organs a firm, almost wooden consistency, and a peculiar greasy appearance resembling bacon.

The colour-reactions given by amyloid are very characteristic :—

1. With iodine + potassium iodide it is stained a deep reddish-brown mahogany colour, while normal tissues appear pale yellow. If amyloid stained with iodine is subsequently treated with sulphuric acid or with a solution of zinc chloride, its colour is deepened still more, or becomes bright red or violet or more bluish or green; occasionally a violet colour is seen by simple treatment with iodine.

2. With methyl-violet amyloid is stained a beautiful ruby colour or pink or reddish violet, and not blue or bluish violet, as are normal tissues. The colour-reactions of amyloid have been fully dealt with by Lubarsch in the *Encyclopædia of Microscopical Technique*.[8]

Apart from the authors mentioned above, amyloid has also been pre-

[1] F. C. W. Krukenberg, *Ber. d. deutsch. chem. Ges.* **17**. II. 1843 (1884); *Zeitschr. f. Biol.* **22**. 241 (1886).

[2] A. Lücke, *Virchow's Archiv*, **19**. 189 (1860).

[3] N. P. Krawkow, *Arch. f. exper. Path. u. Pharm.* **40**. 195 (1897).

[4] R. Virchow, *Virchow's Arch.* **6**. 135, 268, 416 (1853).

[5] C. Schmidt, *Liebig's Annalen*, **110**. 250 (1859).

[6] N. Friedreich and A. Kékulé, *Virchow's Archiv*, **16**. 50 (1859).

[7] W. Kühne and Rudneff, *ibid.* **33**. 66 (1865).

[8] *Encyklopädie der mikroskopischen Technik*, Urban and Schwarzenberg, Berlin, 1903.

pared by Tschermak,[1] Krawkow,[2] Ludwig,[3] Kostjurin,[4] Modrzejewski,[5] Cohn,[6] and Oddi.[7] They removed the other tissue constituents with boiling water, dilute alkalies and pepsin + hydrochloric acid.

Analyses[8] show a not inconsiderable amount of sulphur. According to Lubarsch (Krawkow) its percentage-composition is—

C	H	N	S	O
48·86–50·38	6·65–7·02	13·79–14·07	2·65–2·89	25·6–28·0.

The dissociation-products are enumerated on p. 75, No. 51; the tyrosin-content is therefore fairly high. Amyloid gives all the colour-reactions of albumins, except the lead-sulphide reaction (Tschermak). Neither Kühne and Rudneff nor Cohn could obtain a carbohydrate, but Oddi found in organs which had undergone the amyloid degeneration chondro-sulphuric acid (see p. 542), which, under normal conditions, is absent except in cartilage. Krawkow has further isolated chondro-sulphuric acid from pure amyloid, but it is more firmly bound up in amyloid than in the chondro-mucoid of cartilage, as it could not be liberated by means of pepsin + hydrochloric acid, which converts the amyloid into albumoses. According to Krawkow the staining reaction with methyl-violet depends on chondro-sulphuric acid, but the latter does not cause the staining with iodine. Amyloid should therefore be classed amongst the glyco-proteids, but, as mentioned above, a carbohydrate-radical has not yet been demonstrated. Cohn has shown that Krawkow's view that amyloid is related to chitin is wrong.

Amyloid is quite insoluble in cold water and in salt-solutions; if boiled for some days with water it is partially dissolved, according to Kühne and Rudneff; while if heated under pressure it dissolves more readily (Tschermak). Coarse amyloid dissolves only with difficulty in acids (Kühne and Rudneff, and Krawkow), while finely divided amyloid dissolves in 4 per cent HCl and also in organic acids (Ludwig and Tschermak). Digestive ferments also only attack very finely divided amyloid readily, while with coarser complexes they have difficulty. Amyloid dissolves readily in alkalies, in baryta water, and in ammonia,

[1] A. Tschermak, *Zeitschr. f. physiol. Chem.* **20**. 343 (1894).
[2] N. P. Krawkow, *Arch. f. exper. Path. u. Pharm.* **40**. 195 (1897).
[3] E. Ludwig, *Wiener med. Jahrbüch.* **82**. 183 (1886).
[4] S. Kostjurin, *ibid.* **82**. 181 (1886).
[5] E. Modrzejewski, *Arch. f. exper. Path. u. Pharm.* **1**. 426 (1873).
[6] R. Cohn, *Zeitschr. f. physiol. Chem.* **22**. 153 (1896).
[7] Ruggero Oddi, *Arch. f. exper. Path. u. Pharm.* **33**. 376 (1893).
[8] A. Tschermak, *Zeitschr. f. physiol. Chem.* **20**. 343 (1894); W. Kühne and Rudneff, *Virchow's Archiv*, **33**. 66 (1865); C. Schmidt, *Liebig's Annalen*, **110**. 250 (1859); N. Friedreich and A. Kékulé, *Virchow's Archiv*, **16**. 50 (1859).

there being formed albuminates, primary and secondary albumoses, and peptones, and these, according to Tschermak, still give the two colour-tests of amyloid, and in some instances even better than the original mother substance.

7. The Albumoids

Under this name, which in reality is a but rarely used synonym for albuminoid, Cohnheim has grouped a number of substances which cannot be put into any of the other groups, and which possess a number of properties in common. They form the membranæ propriæ of many glands, the hyaline membranes, the sarcolemma, surrounding muscle fibres, the firm constituents of the lens, of fish scales, etc. Their number will no doubt be increased before long, and then a further subdivision of this group may be made possible. The chemistry of these substances is practically unknown, because they can be obtained only in very minute quantities.

As regards solubility and digestibility they remind one of the gelatine-yielding tissues, but they completely differ from them in not yielding gelatine. In most respects they resemble coagulated albumin, according to most authors. If albumin is coagulated and is then exposed to dry heat of 115°, it gradually becomes firmer, harder, and less soluble, and finally nearly insoluble and indigestible, according to Kühne and Smith.[1] The albumoids about to be described resemble such a coagulated albumin more or less, although, of course, this statement does not in any way clear up the genetic relationship between the albumoids and albumins. In other respects the albumoids differ so much from one another as to necessitate their separate descriptions.

Sarcolemma.—Chittenden[2] has investigated its behaviour towards digestive enzymes histologically, and has thereby shown that it differs from the soluble muscle-albumins and from collagenous tissue. When fresh it is readily digested by trypsin, but is rendered quite indigestible by treatment with osmium tetroxide, while ordinary white fibrous tissue remains soluble. The grey sheath of medullated nerves or the sheath of Schwann; the membranæ propriæ of the uriniferous tubules, the gastric glands, and the pancreas; the substance forming the lens-capsule and Descemet's membrane in the cornea all behave similarly to the sarcolemma. v. Holmgren[3] has found that after the removal of the soluble constituents of muscle, there remains a fraction which is

[1] H. Smith, *Zeitschr. f. Biol.* **19**. 469 (1893).

[2] R. H. Chittenden, *Untersuch. a. d. Heidelberger physiol. Institut*, **3**. 171 (1879); also H. F. A. Sasse, *ibid.* **2**. 433 (1877).

[3] J. F. v. Holmgren, *Maly's Jahresber. f. Tierchem.* **23**. 360 (1893).

insoluble in water and in salt-solutions, but soluble in alkalies. Whether this substance, coagulating at 60°, is the albumin of the sarcolemma or coagulated myosin is uncertain.

Membranin is the term which Mörner[1] has given to the substance forming Descemet's membrane. He has isolated it and finds it gives the following reactions: It is insoluble in water, in salt-solutions, and in cold, dilute acids and alkalies; it dissolves, however, in boiling water, in acids and alkalies, and forms a gelatinising fluid. It gives a distinct biuret- and lead-sulphide reaction, a feeble furfurol reaction, but very intense Millon's and xanthoproteic reactions. By boiling with acids a reducing substance is split off, which is not due to an admixture of mucin.

Osseo-albumoid is a substance which Hawk and Gies[2] prepared from powdered bone after having removed all soluble albumin, mucoid, and gelatine.

Chondro-albumoid has been prepared in a similar manner from cartilage.[3]

The lens-albumoid was described by Mörner,[4] after Knies[5] had shown that lenses suffering from cataract were not composed of keratin, but of an albumin which was digested by pepsin. This albumoid is insoluble in water and in salt-solutions, with great difficulty soluble in acetic acid and ammonia, but readily soluble in very dilute HCl or KOH. It is precipitated from its solutions on neutralisation and by ferrocyanic acid. It is salted out by completely saturated solutions of sodium chloride and sodium sulphate and by half-saturated solutions of ammonium and magnesium sulphates. Its coagulation-temperature Mörner gives as very low, namely, between 43° and 47°. The albumoid gives the reactions of Millon, Adamkiewicz, and Liebermann, and the lead-sulphide test. A carbohydrate seems to be absent.

The albumoid in conjunction with two globulins, the α- and β-crystallin, builds up the lens fibres. In the lenses of fully-grown cattle examined by Mörner, the albumoid amounts to 48 per cent of the albumins in the lens and to 17 per cent of the fresh lens; its amount varies, however, with age, as it is much more abundant in the inner older portions than in the outer younger ones.

The Chorda Dorsalis Albumoid. — Stenberg[6] has shown that the

[1] C. T. Mörner, *Zeitschrift für physiol. Chemie*, **18**. 233 (1893).

[2] P. B. Hawk and W. J. Gies, *American Journ. of Physiol.* **7**. 340 (1902).

[3] P. B. Hawk and W. J. Gies, *American Journ. of Physiol.* **7**. 340 (1902); C. T. Mörner, *Skandinav. Arch. f. Physiol.* **1**. 210 (1889).

[4] C. T. Mörner, *Zeitschr. f. physiol. Chem.* **18**. 61 (1893).

[5] M. Knies, *Untersuchungen aus dem Physiol. Institut Heidelberg*, **1**. 114 (1877).

[6] S. Stenberg, *Arch. f. Anat. u. Physiol.* Anat. Abteil. 1881, p. 105.

chorda dorsalis of the lamprey is poor in firm constituents and that mucin and collagen are absent; but it contains a body, not readily soluble in acids, and more soluble in alkalies, which can be digested by pepsin and trypsin. Kossel[1] has investigated the chorda dorsalis of the sturgeon, and has found neither gelatine nor mucin nor mucoid, but an albuminous substance which is fairly readily soluble in alkalies, which is precipitated by acids, and readily digested by pepsin.

Ichthylepidin is the name which Mörner[2] gave to a substance he found in fish scales besides collagen. It is insoluble in boiling water, and is also only partly soluble in superheated steam. It is soluble in dilute hot, and in strong cold, acids and alkalies. It is digested by pepsin and by trypsin. It gives the biuret-, xanthoproteic-, lead-sulphide tests and an especially well-marked Millon's reaction, which latter distinguishes it from gelatine. It does not give the Adamkiewicz reaction, nor does it yield a carbohydrate. It amounts to 20 per cent in the scales of the teleostean fishes, and is absent in the Ganoids. Amongst the Teleosts it is only absent in the Schleie.[3] Green and Tower[4] have also studied its distribution.

Keratinoid.—The horny layer of the muscular stomach of birds has been examined by Hedenius,[5] who obtained a substance which was insoluble in water, dilute acids and alkalies, and which could only be dissolved by strong alkalies. Pepsin digests it slowly, while trypsin has no action. Superheated steam does not render it soluble. It contains sulphur, and gives the xanthoproteic-, furfurol-, and Millon's reactions. When dissociated it yields leucin, a little tyrosin, but no reducing substance. It differs from the keratins in possessing less sulphur and less tyrosin.

Reticulin was prepared by Siegfried[6] from the reticular tissue of the mucous membrane of the intestine, where it occurs along with collagen. The mucous membrane of the small intestine of the pig is first thoroughly digested with trypsin + soda, and then with boiling water to remove all traces of gelatine, of the cell constituents, etc. There is left over, finally, a powder, the reticulin, which is insoluble in water, in dilute acids and alkalies, in pepsin and trypsin; it contains large amounts of sulphur, and in addition also phosphorus in an organic form.

[1] A. Kossel, *Zeitschr. f. physiol. Chem.* **15**. 331 (1891).
[2] C. T. Mörner, *ibid.* **24**. 125 (1897).
[3] C. T. Mörner, *ibid.* **37**. 88 (1902).
[4] E. H. Green and R. W. Tower, *ibid.* **35**. 196 (1902).
[5] J. Hedenius, *Skandinav. Arch. f. Physiol.* **3**. 244 (1891).
[6] M. Siegfried, *Sitzungsber. d. sächs. Ges. d. Wissensch.* 1892 (preliminary communication); *Habilitationschrift*, Leipzig, 1892; *Journ. of Physiol.* **28**. 319 (1902).

It gives the biuret-, xanthoproteic-, lead-sulphide tests and the Adamkiewicz reaction, but not that of Millon. When boiled with HCl it yields lysatinin, much amino-valerianic acid, also ammonia and sulphuretted hydrogen, but little or no glutaminic acid, no tyrosin, and no carbohydrate. Boiled with superheated water, or with caustic-soda solution, it changes into albuminates, albumoses, and peptones. Reticulin differs from all other albuminoids in containing phosphorus.

Cuticulæ of Worms.—The cuticula of Lumbricus is, according to Sukatschoff,[1] not chitin, but also an albumoid.

[1] B. Sukatschoff, *Zeitschr. f. wissenschaft. Zool.* **66**. 377 (1899).

CHAPTER XII

MELANINS

THE melanins are dark, black or brown, even reddish-brown pigments, occurring in hairs, the skin, the choroid coat of the eye, and in pigmented new growths which start generally in connection with the skin. As the melanins are built up qualitatively out of the same radicals as are met with in albuminous substances, they are regarded as derivatives of albumins, and are hence included in this book.

It has already been mentioned that a number of the dissociation-products of albumin, *e.g.* glucosamin, tyrosin, tryptophane, and lysin, have a tendency to become converted into darkly coloured compounds of unknown constitution forming the so-called 'humin substances' when they are boiled with acids (Samuely).[1]

Schmiedeberg[2] has drawn attention to the fact that these bodies have a considerable resemblance to the melanins both as regards composition and reactions, and hence has called them melanoidins. Schmiedeberg and Nencki[2] are of the opinion that melanins may be derived from albumins over the humin substances, but especial attention is drawn to the fact that our chemical knowledge regarding these amorphous mixtures of substances is as yet very limited, and that the proof is still outstanding that humin substances and melanins are one and the same. Spiegler,[3] on oxidising melanins with sulphuric acid and potassium bichromate, has isolated a substance which he believes to be methyl-dibutyl-acetic acid.

Melanins differ considerably in their composition according to the source they are derived from. They only agree in possessing a high carbon and low hydrogen percentage. Some contain no sulphur, while others may contain up to 7 and even 10 per cent. The iron-content

[1] Samuely, *Hofmeister's Beiträge*, **1**. 229 (1902).

[2] O. Schmiedeberg, *Archiv f. experiment. Pathol. und Pharm.* **39**. 1 (1897) (see also the index).

[3] M. Nencki, *Berichte d. deutsch. chem. Ges.* **28**. I. 560 (1895); C. Beitler, *ibid.* **31**. II. 1604 (1898).

[4] E. Spiegler, *Hofmeister's Beiträge*, **4**. 40 (1903).

varies also, and melanins free from iron as well as others containing high percentages of iron have been described. A great deal of attention was paid to the iron-radical, because it was hoped to get some information as to whether melanins were derived from the blood pigments. Inasmuch as most cell-albumins and all the nucleo-proteids contain iron, no definite conclusions can be formed regarding this question.

Being amorphous, melanins offer no guarantee of having been freed from all remains of blood pigments, albumin, etc., and therefore the presence of small amounts of iron is of no value in throwing light on their origin; but, on the other hand, two groups of melanins may be distinguished by containing either a very high or a very low sulphur-percentage.

Most of the investigations regarding melanin have been made on tumours. Pigments from cases of human melanotic sarcomata, mostly metastases of the liver, have been investigated, apart from the older inquiries, by Berdez and Nencki,[1] who use the term phymatorhusin; by Mörner,[2] Miura,[3] Brandl and Pfeiffer,[4] Hensen and Nölke,[5] Schmiedeberg,[6] Zdanek and v. Zeynek,[7] and v. Zumbusch,[8] and Wolff.[9] Berdez and Nencki,[10] and Nencki and Sieber,[11] have also analysed the 'hippomelanin,' a pigment they obtained from the melanotic tumours of a white horse.

The black colouring-matter of the hair and the skin of negroes has been studied by Sieber,[12] Nencki and Sieber,[13] and Abel and Davis;[14] that of the choroid by Scherer,[15] Sieber,[12] and Landolt.[16] The melanin from the ink-bag of Sepia has been analysed by Nencki and Sieber.[13] In the following table the analytical numbers which Schmiedeberg gives for melanoidins have been included.

[1] J. Berdez and M. Nencki, *Archiv f. experiment. Pathol. u. Pharm.* **20**. 346 (1885).

[2] K. A. H. Mörner, *Zeitschr. f. physiol. Chem.* **11**. 66 (1886); **12**. 229 (1887).

[3] M. Miura, *Virchow's Archiv*, **107**. 250 (1887).

[4] J. Brandl and L. Pfeiffer, *Zeitschr. f. Biol.* **26**. 348 (1890).

[5] H. Hensen and Nölke, *Deutsches Archiv für klin. Medizin*, **62**. 347 (1899).

[6] O. Schmiedeberg, *Archiv f. experiment. Pathol. und Pharm.* **39**. (1897) (see also the index).

[7] E. Zdanek and R. v. Zeynek, *Zeitschr. f. physiol. Chem.* **36**. 493 (1902).

[8] L. v. Zumbusch, *ibid.* **36**. 510 (1902).

[9] H. Wolff, *Hofmeister's Beiträge*, **5**. 476 (1904).

[10] J. Berdez and M. Nencki, *Arch. f. exper. Path. u. Pharm.* **20**. 346 (1885).

[11] M. Nencki and N. Sieber, *ibid.* **24**. 17 (1888).

[12] N. Sieber, *ibid.* **20**. 362 (1885).

[13] M. Nencki and N. Sieber, *ibid.* **24**. 17 (1888).

[14] Abel and Davis, *Journ. Exper. Med.* **1**. 361 (1896).

[15] J. Scherer, *Liebig's Ann.* **40**. 1 (1841).

[16] H. Landolt, *Zeitschr. f. physiol. Chem.* **28**. 192 (1899); here the older literature.

C.	H.	N.	S.	Fe.		
Per cent.	Per cent.	Per cent.	Per cent.	Per cent.		
53·74	4·22	10·59	10·1	...	Melanotic sarcoma	Berdez and Nencki.[1]
55·76	5·95	12·3	8 to 9	0·06 to 0·2	,,	Mörner.[2]
59·42	6·16	11·16	7·57	...	,,	Hensen and Nölke.[3]
48·95 to 54·93	4·23 to 5·15	12·58 to 13·02	1·92 to 8·23	0·39 to 0·41	,,	Zdanek and v. Zeynek.[4]
54·5	5·06	11·75	2·72	...	,,	Miura.[5]
53·87	4·2	10·56	3·63	0·52	,,	Brandl and Pfeiffer.[6]
54·93	5·11	9·28	2·13	2·7	,,	Schmiedeberg.[7]
51·68	6·46	14·56	1·74	0·47	,,	v. Zumbusch.[8]
53·6	3·88	10·48	2·83	...	Hippomelanin	Berdez and Nencki.[1]
56·14 to 57·6	4·2 to 7·57	8·5 to 11·6	2·1 to 4·1	0	Human hair	Sieber.[9]
58·44	5·55	11·7	3·64	...	Horse hair	Nencki and Sieber.[10]
52·74	3·53	10·51	3·34	...	Hair	Abel and Davis.[11]
51·83	3·86	14·01	3·6	...	Skin of negro	,, ,,
58·2	5·9	13·77	0	...	Choroid coat of the eye	Scherer.[12]
60·34	5·02	10·81	0	...		Sieber.[9]
54·48	5·35	12·65	0	...		Landolt.[13]
56·34	3·61	12·34	0·52	...	Sepia	Nencki and Sieber.[10]
66·27	5·49	5·57	0	...	Melanoidins	Schmiedeberg.[7]
60·34	4·86	8·09	0·95	...		
57·28	5·41	9·34	1·67	Cr = 3·36	Melanins	Wolff.[14]
41·77	4·60	9·85	2·62	O = 26·30		

The melanins are not known to give special reactions; they do not give the reactions of albuminous substances. The dissociation-products have been investigated by Zdanek and v. Zeynek, v. Zumbusch, Nencki and Sieber, and Berdez. Neither bases nor phenol, leucin, tyrosin, nor cystin could be found. Indol and skatol were isolated by Hoppe-Seyler,[15] and Berdez and Nencki, and in addition also formic and succinic acids and nitrites. These derivatives may, however, be due to impurities. Spiegler's results have already been alluded to.

The melanins of tumours, when dry, form black, shiny masses; when

[1] J. Berdez and M. Nencki, *Arch. f. experiment. Path. u. Pharm.* **20**. 346 (1885).
[2] K. A. H. Mörner, *Zeitschr. f. physiol. Chem.* **11**. 66 (1886); **12**. 229 (1887).
[3] H. Hensen and Nölke, *Deutsch. Arch. f. klin. Med.* **62**. 347 (1899).
[4] E. Zdanek and R. v. Zeynek, *Zeitschr. f. physiol. Chem.* **36**. 493 (1902).
[5] M. Miura, *Virchow's Archiv*, **107**. 250 (1887).
[6] J. Brandl and L. Pfeiffer, *Zeitschr. f. Biol.* **26**. 348 (1890).
[7] O. Schmiedeberg, *Arch. f. experiment. Path. u. Pharm.* **39**. 1 (1897).
[8] L. v. Zumbusch, *Zeitschr. f. physiol. Chem.* **36**. 510 (1902).
[9] N. Sieber, *Arch. f. exper. Path. u. Pharm.* **20**. 362 (1885).
[10] M. Nencki and N. Sieber, *ibid.* **24**. 17 (1888).
[11] Abel and Davis, *Journ. Exper. Med.* **1**. 361 (1896).
[12] J. Scherer, *Liebig's Ann.* **40**. 1 (1841).
[13] H. Landolt, *Zeitschr. f. physiol. Chem.* **28**. 192 (1899); here the older literature.
[14] H. Wolff, *Hofmeister's Beitr.* **5**. 476 (1904).
[15] F. Hoppe-Seyler, *ibid.* **15**. 179 (1891).

powdered they appear of a lighter colour, more brown; a preparation of Hensen and Nölke was ochre-coloured. They are insoluble in water, neutral salt-solutions, alcohol, amyl-alcohol, ether, chloroform, benzene, etc., and in dilute acids; in 50 per cent acetic acid they are soluble to a slight extent, and this soluble fraction possesses, according to Mörner, somewhat different properties. In alkalies, ammonia, and the alkali-carbonates, melanins are readily soluble, and form in concentrated solutions black or brown-red, and on dilution yellow-brown solutions. Examined spectroscopically, melanins do not show any distinct bands; there is a uniform darkening commencing at D which becomes absolute at the latest by *b*.

The colouring power of the melanin may be judged by the fact that an average negro, according to Abel and Davis,[1] has in his skin 3·3 grammes of pigmentary granules, of which amount only 1 gramme consists of pigment, the other 2·3 grammes consisting of a colourless substratum and much inorganic matter (Ca, Mg, Fe, silicic-, phosphoric-, and sulphuric-acids). Urin containing 0·1 per cent of melanin has the colour of dark beer (Hensen and Nölke).—Nencki and Berdez have obtained from one liver 300 grams of melanin.

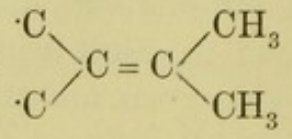
From their alkaline solutions melanins are precipitated by acidification, and likewise by the addition of barium hydrate, lead acetate, and by saturating the solution with magnesium sulphate. To extract the melanin from tumours, the tissues are extracted with water, which converts the pigment into a fine suspension, which latter is readily carried down by precipitating phosphates. The pigment is then purified by repeatedly dissolving it in alkalies, and precipitating it by acids.

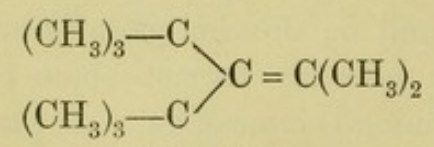
In some of the cases mentioned above, the melanin was also found in the urine, either as such or as 'melanogen,' which by oxidation is converted into the dark melanin. Urine containing melanogen shows the normal colour, but assumes the dark melanin-colour on the addition of nitric acid; potassium bichromate + sulphuric acid; bromine water or ferric chloride. Miura has been in some cases successful in demonstrating melanogen in the urine of rabbits after having injected melanin into the peritoneal cavity.

Wolff obtained two pigments from a melanotic liver, one of which was soluble in soda-solution, while the other one was insoluble in 5 per cent cold soda-solution, but it could be made soluble by heating to 50° or 60°, becoming at the same time denaturalised. The soluble pigment gave lower carbon-values than most of the melanins. From

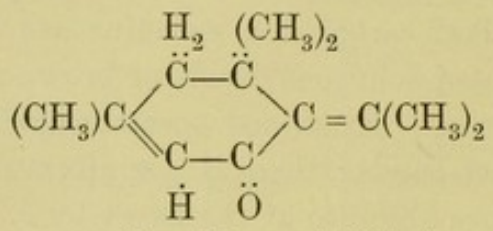
[1] Abel and Davis, *Journ. Exper. Med.* **1.** 361 (1896).

another melanin Wolff obtained 15 per cent of a hydro-aromatic body, which was identical with xyliton, and which agrees with Spiegler's isotributylen in containing the radical





isotributylen



xyliton (Kerp and Müller).

In addition to xyliton was found isovaleronitril to the extent of 2·5 per cent. Another substance of unknown composition was isolated. How the sulphur is linked on in the melanin could not be determined.

Brandl and Pfeiffer and Wolff[1] digest melanotic liver with pepsin + HCl, till albumoses can no longer be demonstrated, and then extract the residue with 8 per cent soda-solution.

The melanins from the hair, the skin, choroid coat of the eye, and the ink-bag of Sepia agree in their properties with the melanins found in tumours. Landolt found indol and ammonia amongst the dissociation-products.

[1] H. Wolff, *Hofmeister's Beiträge*, **5**. 476 (1904).

Schon heute darf man sagen, daß die Betrachtung der Zelle als einer mit chemischen und physikalisch-chemischen Mitteln arbeitenden Maschine nirgends zu Problemen führt, welche die Annahme anderer als bekannter Kräfte unvermeidlich erscheinen ließen, und daß, so weit abzusehen, hier für jene Resignation, die sich einmal in einem „ignorabimus," das andere Mal in vitalistischen Schlußfolgerungen äußert, kein Anlaß vorliegt.

Hofmeister, 1901.

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