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Contributors

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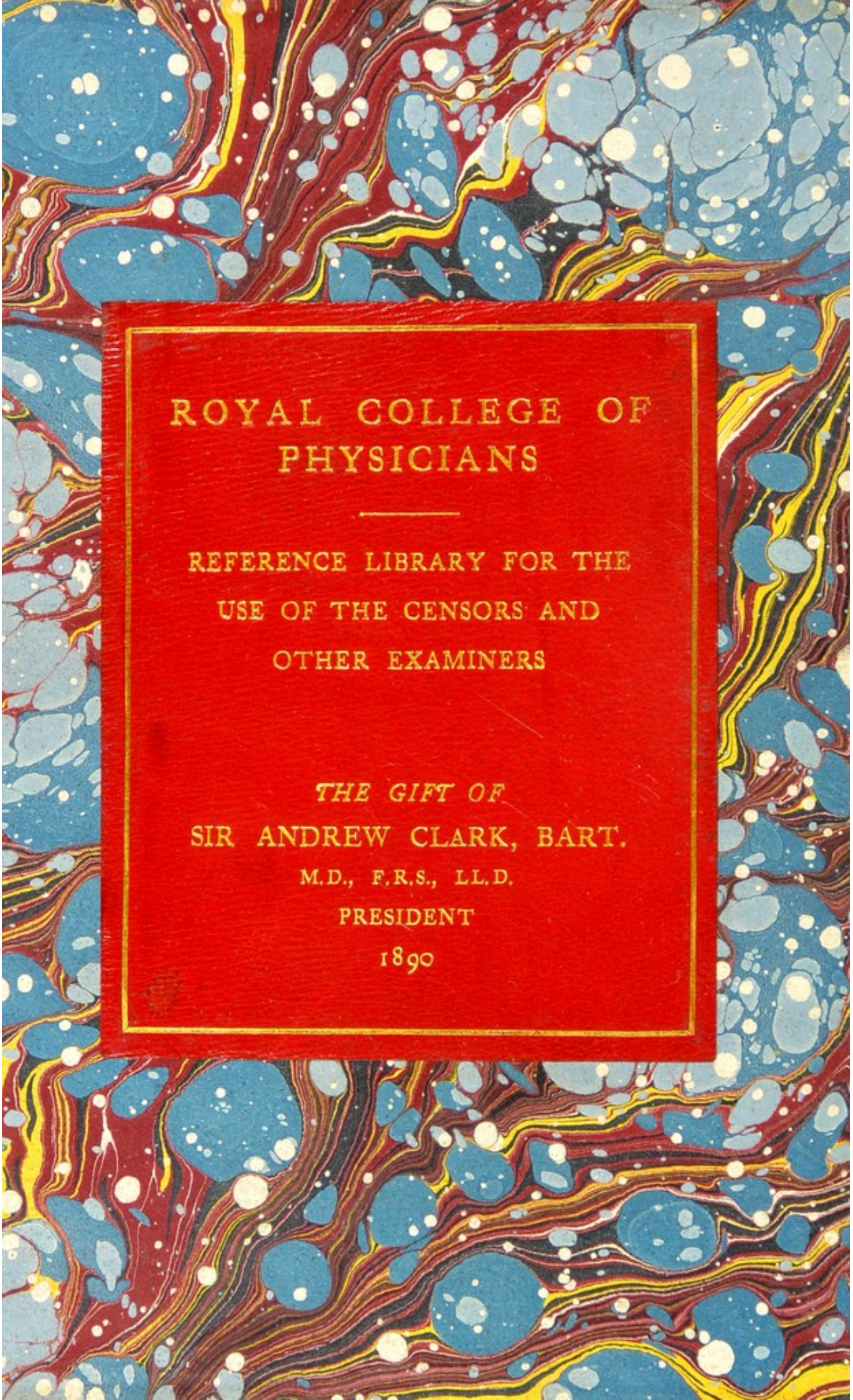
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A TEXT BOOK

OF

PHYSIOLOGY

BY

JOHN GRAY M'KENDRICK, M.D., LL.D., F.R.S.,

PROFESSOR OF THE INSTITUTES OF MEDICINE IN THE UNIVERSITY OF GLASGOW,
FELLOW OF THE ROYAL COLLEGE OF PHYSICIANS OF EDINBURGH.

INCLUDING

HISTOLOGY BY PHILIPP STÖHR, M.D.,
OF THE UNIVERSITY OF WÜRTZBURG.

IN TWO VOLUMES.

VOL. I.—GENERAL PHYSIOLOGY.

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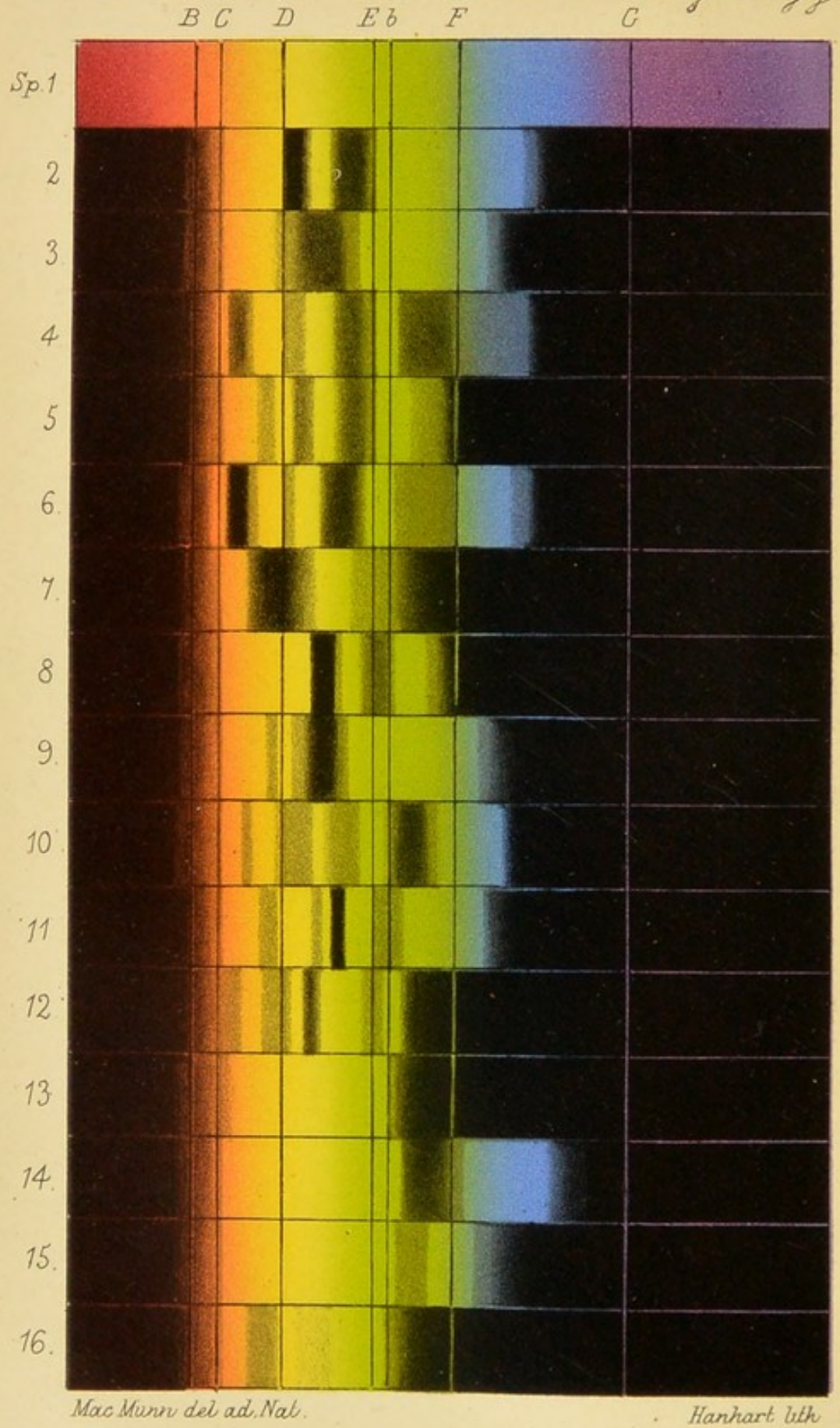

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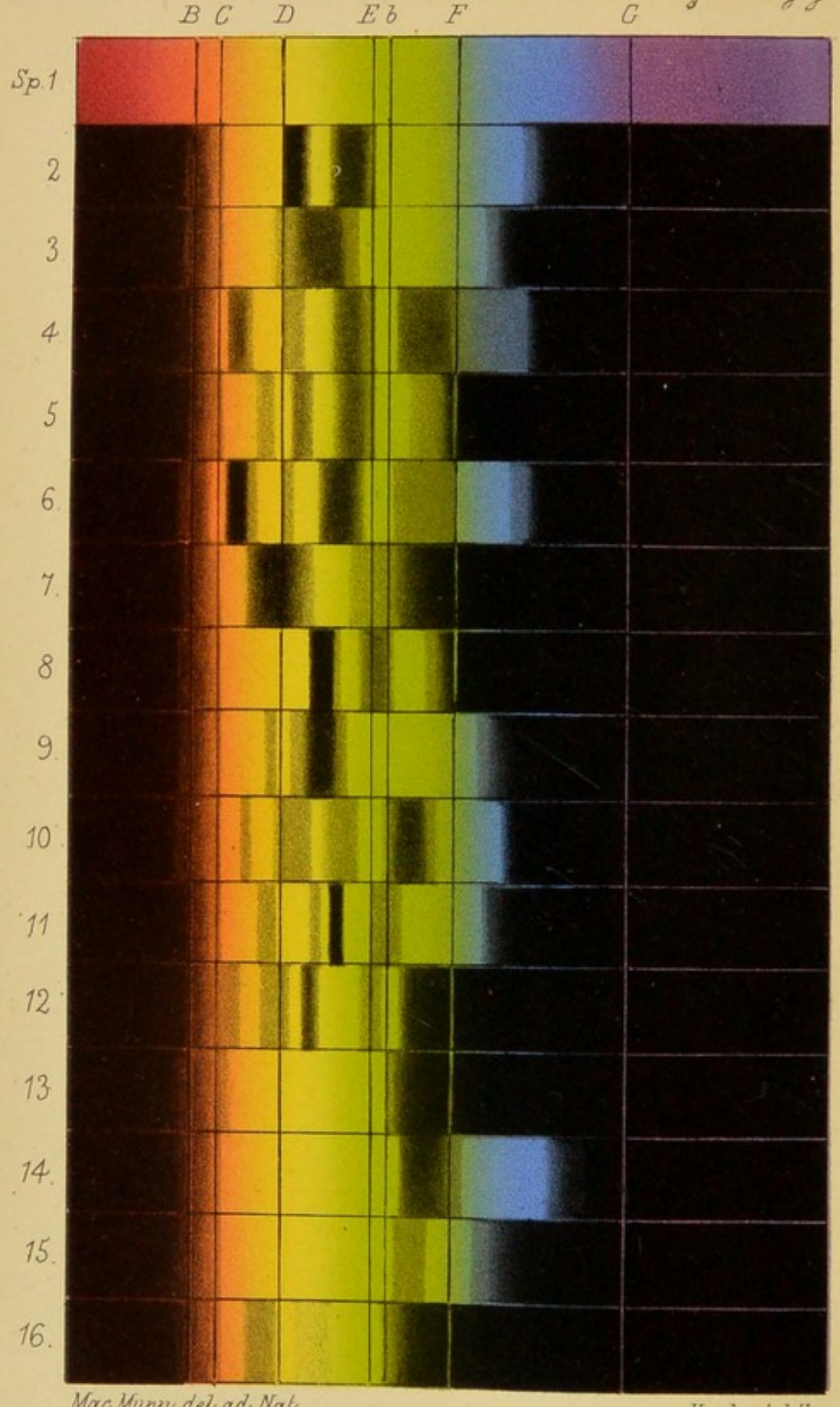

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EXPLANATION OF THE PLATE OF SPECTRA.

(FRONTISPIECE.)

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2. Spectrum of *oxy-hæmoglobin* in a moderately dilute solution, p. 121.

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4. Spectrum of *methæmoglobin*, got by adding a solution of potassium permanganate to a solution of sheep's blood in water, p. 125.

5. Spectrum of *alkaline methæmoglobin*, got by treating the last solution with a little ammonia, p. 126.

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These spectra were mapped by means of a Sorby's microspectroscope. In the chemical spectroscope the bands are more hazy and some of the faint ones difficult to see. The source of light was an Argand gas burner.

GENERAL PHYSIOLOGY:

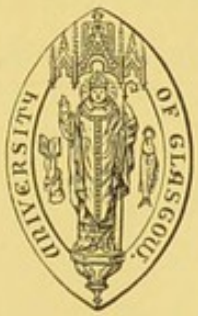
INCLUDING

THE CHEMISTRY AND HISTOLOGY OF THE TISSUES AND THE PHYSIOLOGY OF MUSCLE.

BY

JOHN GRAY M'KENDRICK, M.D., LL.D., F.R.S.,

PROFESSOR OF THE INSTITUTES OF MEDICINE IN THE UNIVERSITY OF GLASGOW,
FELLOW OF THE ROYAL COLLEGE OF PHYSICIANS OF EDINBURGH.



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IN · INTERPRETANDA · SAGACISSIMO

IOANNI · BVRDON · SANDERSON

QVI · LARGIS · INGENII · LVMINIBVS

QVODCVNQVE · ATTIGIT · ILLVSTRAVIT

SANO · IDEM · IVDICIO · ATQVE · AEQVO

SOLIDA · SEMPER · VANIS · PRAETVLIT · VTILIBVS · HONESTA

ATQVE · OMNIA · POSTHABVIT · VERITATI

HOC · MVNVS · QVALECVNQVE

D. D.

IOANNES · G · M'KENDRICK

221 r

ERRATA.

Page 55, line 8 from bottom, for Amines *read* Amides.

Page 225, line 1 from top, for expression *read* extrusion.

Page 310, line 4 from bottom, before Marshall *insert* Melland and.

PREFACE.

ALTHOUGH this work has been modelled to some extent on my "Outlines of Physiology," now out of print, it is essentially a new book, and as it aims at giving a more detailed account of the subject, the title of "Outlines of Physiology" has been abandoned for that of a "Text Book of Physiology." I have not endeavoured to give anything like an encyclopædic account of the modern researches that have built up the physiological science of the present time, but rather to weave into a consecutive narrative the main facts and principles of physiology, as these present themselves to my mind, after nearly eighteen years of experience as a teacher of the science. Probably no science is advancing with greater rapidity, if one may form an opinion from the large number of original papers that are announced, month by month, and from the large number of investigators in the physiological laboratories of the Continent, of America, and of our own country. Thus it is year by year becoming more difficult for a teacher to keep abreast of the wave of progress, and still more difficult to assimilate the new facts and to incorporate them with his previous knowledge. Each teacher will also view the subject more especially according to his predilections for the morphological, the chemical, or the physical side of the science, and it is not easy in the preparation of such a work as this to be impartial and to present the science in all its completeness to the reader.

An explanation may be expected as to my object in introducing another large work on physiology when in recent years several excellent and voluminous treatises have issued from the press and have met with

great acceptance. My chief reason is to have it in my power to place in the hands of my students a text book appropriate to the course of instruction in physiology, which it is my duty to give annually in the University of Glasgow. The present work contains most of the theoretical teaching given to the student, and I hope that with it in his hands, it will be possible for me to do more and more in the way of demonstration and of practical instruction. At the same time, I am not without the hope that the nature of the book may be such as to commend it to a wider circle of readers, more especially to members of the medical profession who desire to become acquainted not only with the facts but also with the methods of physiology.

The general plan of the book is to give an account both of methods and of results, and to introduce illustrations as far as possible from the best sources. No expense has been spared to obtain good illustrations, not merely diagrams, but illustrations that will give the reader a fair idea of the appearance of the thing represented. The reader is recommended carefully to peruse the descriptions of the various figures, as, in these, information is often given which is not contained in the text.

The work is primarily divided into two parts. First, that relating to the general physiology of the tissues, which forms the subject of the first volume; and, second, that relating to the special physiology of organs, to be discussed in the second volume. This arrangement is simple and comprehensive. After an introductory section dealing with general notions as to living matter, more especially with reference to the great doctrines regarding energy, which form the basis of modern science, I proceed to discuss the nature and properties of the chemical substances found in the body and the nature of the chemical reactions with which the phenomena of life are associated. In this section I have introduced a chapter explaining to the physiological student the views he should hold as to the true value of chemical formulæ. This chapter, in the preparation of which I received valuable assistance from Professor W. Dittmar, F.R.S., I introduced after much consideration, and because I know that in the minds of most students there is not a little confusion regarding this matter. No doubt such a chapter would be more appropriate in a strictly chemical work, but my experience as a teacher

assures me that it will not be without its value even in a physiological text book, did it serve no other purpose than to show how little we yet know of the molecular structure of organic chemical substances. In the preparation of this section I derived much assistance from Dr. Arthur Gamgee's *Physiological Chemistry* and from Beaunis' *Physiologie Humaine*.

I venture to direct the attention of the reader to the chapter on pigments in which these interesting substances are more fully discussed than has yet been attempted in any text book of physiology. The writings of Dr. C. A. MacMunn of Wolverhampton have largely assisted me in writing this chapter. Dr. MacMunn, as is well known, has made this subject his own special field of research, and from his stores of knowledge he gave me the valuable measurements of wave-lengths. He also kindly prepared the chart of spectra which forms the frontispiece of this volume, and he added further to my indebtedness by reading the proof sheets of the chapter on pigments and by furnishing me with many valuable suggestions. I consider myself fortunate in having secured Dr. MacMunn's co-operation, and I offer him my most cordial thanks.

The next section deals with the physiology of the tissues. Here I was met by a great difficulty. In my previous work scarcely any description of the microscopical structure of the tissues or organs, or what is termed histology, was introduced, and the omission was regarded by critics as diminishing the value of the book. My own feeling was in favour of omitting the discussion of histology from a physiological text book, but after consulting several physiologists, in whose judgment I place great confidence, and after taking into account the wishes of my students, I found that the balance of opinion was in favour of introducing histology. At the same time, I am bound to admit that several physiologists gave good reasons for omitting histology. Having resolved to introduce this aspect of physiological science, the next difficulty was whether I was prepared to face the trouble and cost of preparing a new set of histological woodcuts. After some consideration, I solved the problem by purchasing from Mr. Gustav Fischer, the well-known publisher in Jena, a set of electrotypes of the woodcuts in Professor Philipp Stöhr's *Lehrbuch der Histologie*, published last year, and also the right of translating the work, and of incorporating it with my

book. This arrangement was effected with the cordial consent of the author, Dr. Stöhr, whose name I therefore place on the title page. Dr. Stöhr's work commended itself to me on account of the excellence of its descriptions, the fidelity of its illustrations,—which are not diagrams but drawings of real preparations,—and the value of its practical directions I have therefore made free use of it, introducing special descriptions and illustrations, where these seemed to be needful, and I have done this the more readily as the directions given by Dr. Stöhr are substantially those followed from year to year in the practical classes held during the summer session in the University of Glasgow. Having Dr. Stöhr's work at my command has also led me to introduce more in the way of practical directions as to histological methods than I might otherwise have done, so that the present volume will be a guide to the student during the practical work of summer, and at the same time serve as a systematic text book. One unique feature of Dr. Stöhr's work is that he gives an account of the method by which each preparation figured was prepared. These descriptions of methods I have relegated to an appendix and numbered consecutively so as to admit of ready reference.

Before taking up the physiology of the tissues, I have discussed their origin in the light of recent investigations regarding the phenomena of fecundation and the minute structure of cells and of nuclei. This is a novel arrangement, so far as recent systematic works are concerned, but it has commended itself to me as one leading to a philosophical view of the whole subject. The questions as to the origin of the tissues and the mysterious way by which they are impressed with hereditary characters are of profound importance and have a bearing not only on physiological but on pathological theories. The portion dealing with the theories of heredity has been read by Professor E. Ray Lankester, F.R.S., who, while not agreeing with me in my general conclusion, has given me valuable suggestions and criticisms. The whole of this section has also been read by Mr. J. H. Fullarton, M.A., B.Sc.

Section III. deals with the contractile tissues, the study of which requires physical appliances. I still hold that without burdening a student by requiring of him a knowledge of complicated apparatus, it is possible to give him, shortly, such information regarding methods as will enable him to take an intelligent view of results. Instead, however,

of introducing a description of apparatus in the parts describing results, I have discussed apparatus and methods as far as possible in separate chapters, so that the reader may pass over these if he chooses. The importance of the uses of electricity in practical medicine and surgery appears to me to justify the account given of electrical apparatus, for assistance in the preparation of which I am indebted to Mr. Thomas Gray, of the Physical Laboratory of this University, and an accomplished electrician; and the immense value of the graphic method in all sciences dealing with movements also warranted me in giving a brief account of its chief instruments. As to the introduction of physical questions into a text book of physiology, a good deal can be said on both sides. No doubt if students of medicine and practitioners read good text books on general physics, or the excellent works on physiological physics that have appeared in recent years, there would be little or no necessity for introducing these matters into a text book of physiology, except in so far as all physiological questions ultimately resolve themselves into physical problems. But the fact is that the teacher has usually to deal with students who know little or nothing of physics. The examination in mechanics required for the registration of a medical student is of no use, but is just sufficient to worry him and exhaust his energies without conferring any real benefit in the shape of a knowledge of the principles of physical science. Had he a course of instruction in general physics before beginning the study of medicine, matters would be on a different footing, but that is not at present available. Consequently the teacher need not lecture on the appearance of a muscle as seen with polarized light without, in the first instance, stating the more elementary facts regarding polarized light, and without explaining the construction of a polarizing apparatus. Again, before beginning the discussion of muscle in which the physiological teacher must make use of electricity, he is obliged to give some explanation of the nature of voltaic cells, of induction coils, and of the general facts regarding resistance, and of the detection and measurement of currents. For these reasons I have introduced certain details as to physics just as I am obliged to introduce them in my lectures. At the same time, I admit that this is so far a provisional arrangement, and if the time arrives when the student, beginning the study of physiology, has already been well grounded in even elementary physics, it may be abandoned. I have in the meantime chosen the mode of teaching

which experience has shown me to be the most useful under the circumstances.

The chapter on the electrical fishes is longer than might be expected in a general treatise on physiology, but I have thought it right to enter into details regarding these remarkable animals, as their physiology has an important bearing on questions as to the functions of nerve and muscle. I am much indebted to Professor Burdon-Sanderson for aiding me with a description and with drawings of the electric organ of the skate, in the investigation of which he and Mr. Francis Gotch, M.A., have been recently engaged and the results of which have not been published. I have also to thank Mr. Gotch for looking over the proof sheets of this chapter and for valuable suggestions.

In the preparation of the entire volume, I have gratefully to acknowledge the valuable services of my assistants, Dr. J. M'Gregor-Robertson, M.A., and of Dr. William Snodgrass, M.A. Dr. Snodgrass has read all the proof sheets twice over, and Dr. M'Gregor-Robertson has read the final revise. Both have given me great help and excellent suggestions. Mr. J. T. Bottomley, M.A., F.R.S., and Mr. Magnus Maclean, M.A., have also aided me in the discussion of various physical questions.

I have also to thank Professor Rutherford, F.R.S., Professor Marey, the Cambridge Scientific Instrument Company, Messrs. Churchill, Blackie & Son, A. & C. Black, Cassell & Co., Collins & Sons, Vieweg & Son of Brunswick, Fischer of Jena, Hahn of Hanover, Carl Ricker of St. Petersburg, Vogel of Leipsig, and Masson of Paris, who have favoured me with electrotypes of illustrations for this work. The sources of all the illustrations are given in the list of woodcuts.

I am indebted for the index to my pupil, Mr. Alexander Farquharson.

The second volume is ready for the press and will be published without delay.

With these introductory remarks, I submit the work to the reader, in the hope that he may find it a guide to the study of physiology and possibly a stimulus to further research.

JOHN G. M'KENDRICK.

University of Glasgow,
May, 1888.

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TEXT BOOK OF PHYSIOLOGY.

SECTION I.

GENERAL INTRODUCTION.

CHAP. I.—NATURE AND OBJECTS OF PHYSIOLOGY.

Physiology is the science which treats of the phenomena normally occurring in living things. It is divided into Vegetable and Animal Physiology, according as the life-history of plant or of animal is made the subject of consideration. Human Physiology, a department of the science which forms the special subject of this treatise, concerns itself with the phenomena occurring in man.

The physiology of man, or indeed of any living being, cannot be studied with success if we regard merely the changes happening in the body of an individual, or even if we attend only to the phenomena of the species as a whole; for much of our knowledge depends on the study of operations occurring in a great variety of living beings.

When we contemplate any living being, we observe, first, a peculiar physical structure or conformation of the solid parts; second, a determinate chemical composition of its several solid and fluid parts; and, third, the occurrence of certain chemical, physical, and vital changes.

In the physical structure of the body, certain parts may be readily isolated, and these may each perform a special office in the economy. Such parts are termed *organs*, and the office which each organ performs is called its *function*. *Life*, so far as the individual is concerned, may be said to be "the sum of the several functions performed by the various organs, or the active state resulting from their concurrent exercise." (Allen Thomson.)

The body may be studied both in the dead and in the living condition. In the dead state its structure and composition are the subjects of anatomical and of chemical research The living state

is more especially the province of the physiologist, it being his duty to study the functions, or the various phenomena manifested by the organs individually, and as part of a system, during life.

The economy of the human body is one of great complexity, and in its study the physiologist finds it necessary to extend his investigations over a wide field. His information is derived partly from observation and experiments made directly upon man himself, both in the healthy and in the diseased conditions, but very largely also from investigations into the functions performed by the organs of other animals. Many of the facts of physiology with which we are at present acquainted have been derived from observations and experiments made on the humbler animals, but the apparently identical nature of many vital actions, more especially as manifested by the elementary tissues and in the simpler organs, and the general plan of structure on which organs are formed throughout the range of the animal kingdom, warrant us in applying these facts generally to the elucidation of the functions of the human body.

Physiology derives its facts from the observation of the phenomena of living beings, made with scientific accuracy, and aided by deductions from facts belonging to other branches of science, more especially anatomy, chemistry, and physics. A general acquaintance, therefore, with these sciences is of paramount importance to anyone about to enter upon the study of physiology.

The student ought, in the first instance, to be acquainted with the structure of the human body and of its various organs, and he should also be familiar with the general plan of structure met with in the great general subdivisions of the animal kingdom. Such anatomical knowledge (*Human and Comparative Anatomy*) must be obtained by dissection and by a careful comparison of organs in different groups of animals.

During the past forty years, much knowledge has been acquired regarding the minute structure of the various tissues forming the body. This constitutes the department of science called *Histology*. As many functions are performed by elements of the body which are of microscopic size, it is evident that we must be accurately acquainted with the anatomical structure of these elements. Such knowledge, therefore, belongs equally to the anatomist and to the physiologist. The anatomist must describe the form, size, and general relations of these minute parts, while the physiologist directs his attention specially to the phenomena they manifest while alive. Both are engaged in the study of the same textural elements, but from different points of view—the one *morphological*, the other *physio-*

logical. Here, as in the study of parts visible to the naked eye, knowledge of structure and of function are really, in the language of Goodsir, different aspects of the same truth.

To acquire a wide knowledge of the functions of the body, it is not sufficient to study it, even as regards its structure, only in its full-grown or mature condition. It is necessary to examine it at various stages of its growth, and to trace minutely the first formation and early development of the embryo, the origin of the primary tissues, the formation of the more complicated organs and systems of organs, and the process of growth of the fœtus and child. This research is designated by the name of *Embryology*, and in recent times it has furnished important information regarding the history and bearing of vital phenomena. Embryology may be conveniently divided into two sections: (1) The history of the development of any single organism from the ovum onwards (*Ontogeny*); and (2) the history of the evolution of the group or race from lower to higher forms (*Phylogeny*). Thus comparative embryology has both an anatomical and a physiological side; on the anatomical it is the basis of *morphology*, or all that relates to organic form, and on the physiological it gives a solution of many of the problems of function.

From the facts and laws of *Chemistry* the physiologist derives information regarding the chemical composition of the solid textures, as well as of the various secreted and circulating fluids, and of the variations to which their composition is subject in different conditions of the body. *Physiological chemistry* has as its ultimate aim the correct statement and explanation of the chemical processes occurring in the body, and it is generally regarded as the most difficult department of the parent science.

The laws of *Physics* are applied by the physiologist to the investigation of all the obvious motions of solids or fluids occurring in the animal organism and also to the explanation of those molecular changes of which a physical explanation can be offered. A knowledge of physics is indispensable in examining the functions of such organs of special sense as the eye and ear, and in studying the influence of light, heat, and electricity, in modifying vital phenomena.

It may further be remarked, that while the researches of the physiologist are directed strictly to establishing the knowledge of the functions of the living economy in a state of health, he not unfrequently receives assistance from the pathologist (*Pathology*), who having his attention called to the organic and functional changes that occur under the influence of different forms of disease, takes advantage as it were of so many experiments made to his hand by nature, and

is thus better able to discriminate the share which each particular condition has in the production of these complex results.

The physiological study of the functions of the human body depends then on the following means of investigation, viz.: (1) The observation of the phenomena of the body in a healthy state; (2) The occasional comparison of the healthy state with morbid conditions of the economy; (3) The experimental variation in man or animals of the conditions in which vital operations take place; (4) The anatomical examination, by dissection and microscopic research, of the structure of the organized parts of the body at various stages of its growth or in the fully-formed state, or as found in a simpler condition in the bodies of the lower animals or even in plants; (5) The chemical analysis of the solids and fluids, and of the substances which are taken into or are ejected from the system, and the history of the chemical changes which occur in the living body; and (6) the application of the facts and principles of physics where these are admissible.

As the object of physiology may be said to be "to ascertain the conditions of body and mind which are necessary to life and health," so the most important practical end which the human physiologist has in view is to furnish data for guiding the physician in detecting diseases with readiness, in distinguishing their different kinds with accuracy, in endeavouring to preserve health, and when disease shall have occurred to allay suffering and to restore the natural condition. A knowledge of the conditions of health must precede the study of disease, and a rational system of surgical and medical treatment has its foundation in an acquaintance with the principles of physiology. But, apart from its practical aspect, physiology considered as a pure science, presents an attractive field of study, taking a wide range over the domain of nature and bringing the mind into the contemplation of some of the most profound problems and of some of the most sublime truths.

Chap. II.—MATTER AND ENERGY.

The permanence of matter and the permanence of energy are two great generalizations which form the groundwork of physical science, and they must be recognized in dealing with the phenomena of the living body just as clearly as in considering the facts of chemistry and physics.

1. **Matter.**—In its passage through the living body matter cannot be created or destroyed; if it apparently disappears it is only transformed into another condition. It need hardly be said that chemistry

only became an exact science when the permanence of matter was recognized. No portion of matter, however minute, can either come into existence or go out of existence in any chemical operation. The study of the chemical transformations in or by a living body is an important branch of physiology, and it is becoming more and more the aim of physiologists to obtain quantitative as well as qualitative results, feeling assured that as they succeed in doing so, will they bring their science nearer to the platform of physics and of chemistry. Measuring and weighing in units of length, time, volume, and mass are operations as important in physiology as in physics.

As the metric system is now in use over the greater part of Europe and is spreading in America, it should be uniformly adopted in Great Britain, at all events in scientific literature, and I would strongly recommend the student to become familiar with it. To assist him, I give the following short *résumé* for reference :—

1. *Length.*—One metre is equal to 39·371 inches, 1 foot is equal to 30·48 centimetres, and 1 inch is equal to 25·4 millimetres. It may be noted that in the metric system the Latin prefixes denote division and the Greek prefixes denote multiplication. Thus decimetre, centimetre, millimetre, and micrometre denote the tenth, the hundredth, the thousandth, and the millionth of a metre respectively; and decametre, hectometre, and kilometre denote ten, a hundred, and a thousand metres. These measures may also be squared or cubed. In Fig. 1, 4 inches are placed alongside of 1 decimetre.

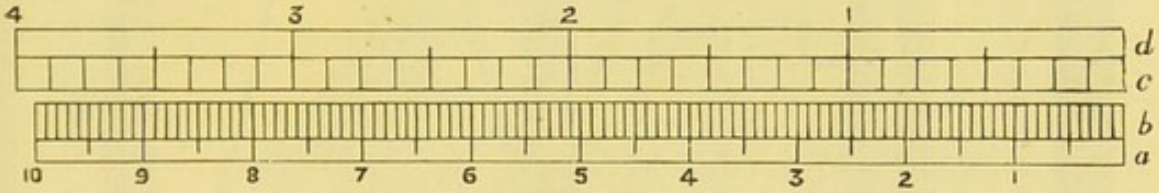


FIG. 1.—Comparison of a decimetre with 4 inches: *a*, centimetres; *b*, millimetres; *c*, $\frac{1}{8}$ths of an inch; *d*, inches.

The micron, μ, the $\frac{1}{1000}$th of a millimetre = ·001 mm. and is now used in the measurement of objects of microscopic size: thus, the average diameter of the red blood corpuscles in man is 77 μ or ·077 mm.

2. *Time.*—The unit of time is the mean solar second. In many physiological observations, fractions of a second, hundredths, or even thousandths, must be accurately determined by means of specially designed chronographs. (See Graphic Method.)

3. *Mass and Weight.*—In the metric system the unit of mass is 1 gramme, which is the mass (or weight) of a cubic centimetre of water

Fig. 2.—Square Centimetre.

$\frac{1}{1}$

Fig. 3.—Cubic Centimetre.

$\frac{1}{1}$

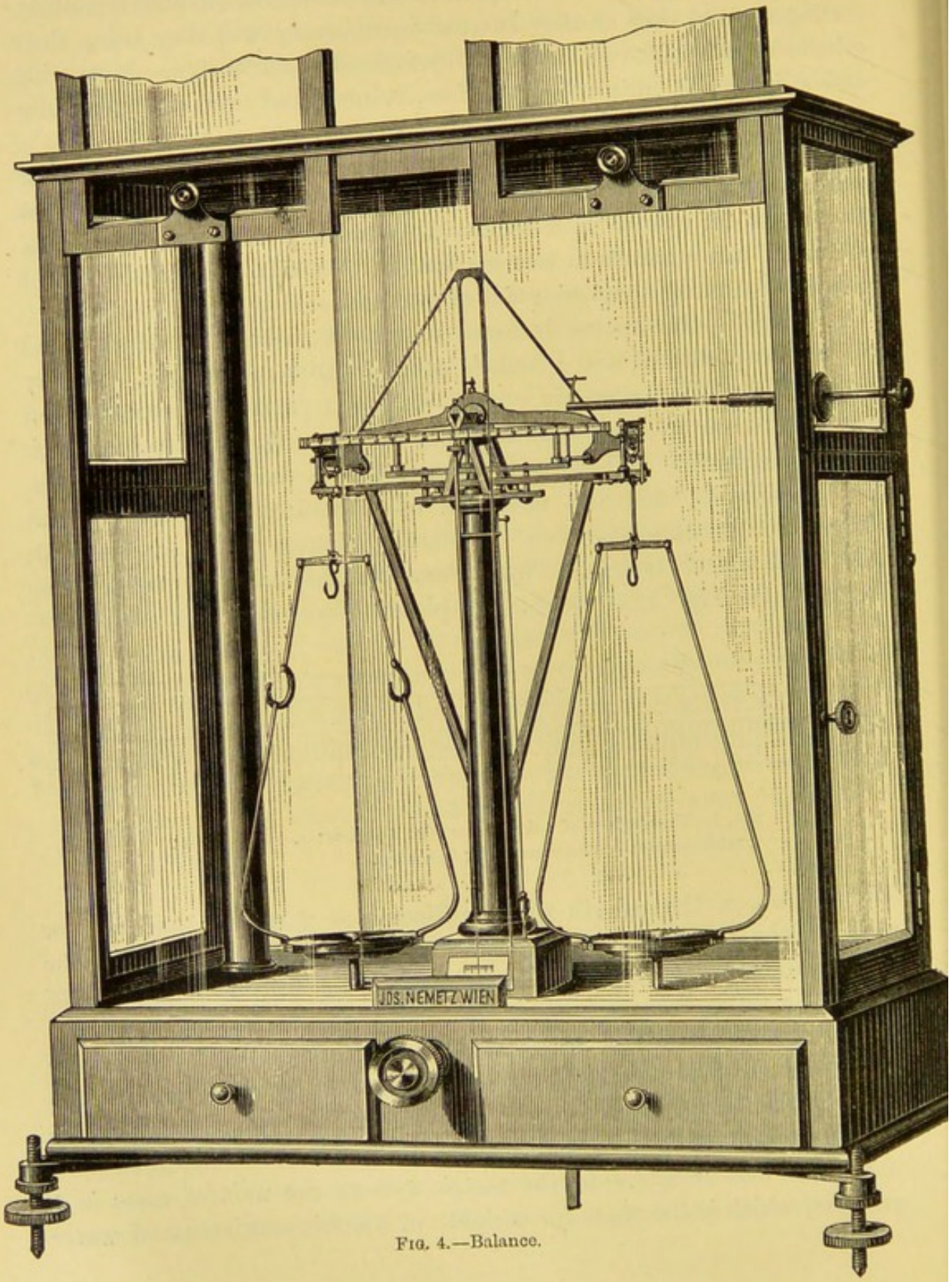


Fig. 4.—Balance.

at its temperature of maximum density, 4° C. (See Figs. 2 and 3.) One gramme is equal to 15·4321 grains avoirdupois. It is also convenient to remember that if we compare imperial units (Avoirdupois) with the metric system, 1 grain = $\frac{1}{7000}$ pound = ·0648 gramme ; 1 dram = $\frac{1}{256}$ pound ; 1 ounce (16 drams) = $\frac{1}{16}$th pound = 28·35 grammes ; 1 pound (lb.) = ·4536 kilog. In Troy weight, 1 ounce = 480 grains = 31·10 grammes, and in Apothecaries' weight, 1 scruple = 20 grains, 1 drachm = 3 scruples, and 1 ounce = 8 drachms. In weighing, a good balance is indispensable. (See Fig. 4.) It consists of a light rigid beam resting on a fixed horizontal axis at its centre. A scale-pan is hung at each end of the beam, one to receive the standard weight and the other to receive the body to be weighed. When there are no weights in the scale-pans, or when these contain equal weights, the beam is horizontal. Both the beam and the scale-pans are suspended on agate knife-edges working on agate planes. A good balance, loaded with a kilogramme in each pan, will turn with ·0007 gramme, or about $\frac{1}{1400000}$th of the weight in either pan. The weights are kept in a suitable box, as seen in Fig. 5.

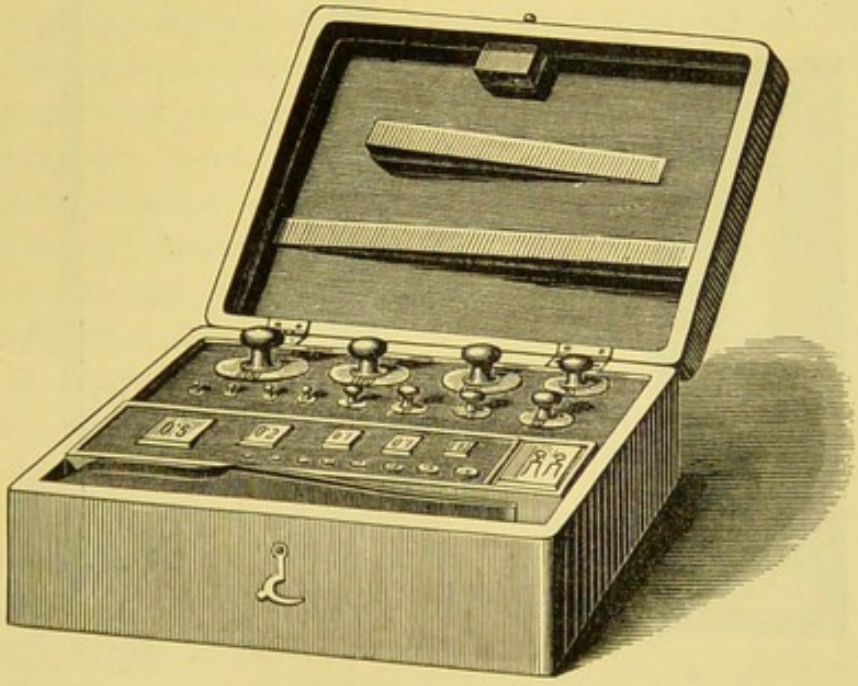


Fig. 5.—Box of Weights.

4. *Volume.*— The unit of volume in the metric system is 1 cubic centimetre. In this connection it may be noted that 1 pint = ·568 litre (the litre being the capacity of 1 cubic decimetre) ; 1 quart = 1·136 litre ; 1 gallon = 4·544 litres ; 1 litre = ·22 gallon ; 1 fluid ounce (in sale of drugs) = 1·8 cubic inch, or ·0284 litre ; 1 cubic foot = ·02831 cubic metre ; and 1 cubic metre = 1·308 cubic yard.

The volume of fluids is measured by flasks having a capacity when filled up to a mark on the neck of from 100 to 2,000 cubic centimetres (Fig. 6); by graduated cylindrical glasses, as shown in Fig. 7; or by graduated pipettes, as in Figs. 8 and 9.

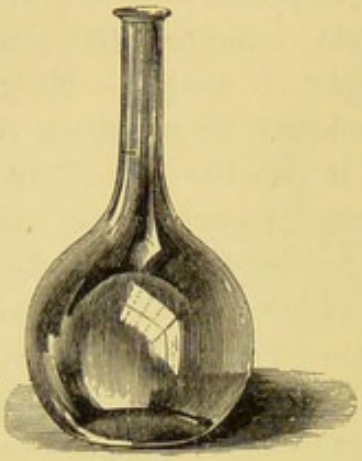


FIG. 6.—Litre flask, ⅛ nat. size.

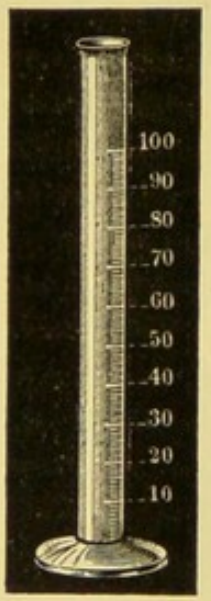


FIG. 7.—Flask, ⅛ nat. size, graduated for 100 cb. cm.

FIG. 8.—Pipette for 10 cb. cm., ⅛ nat. size.

FIG. 9.—Pipette, for 25 cb. cm., ⅛ nat. size.

The knowledge of the physiologist regarding the *molecular phenomena* occurring in living things is still so imperfect that he has no occasion to follow the physicist in his speculations as to the ultimate nature of matter. The atomic theory of Democritus and Leucippus "glorified," as Professor Tait writes, "in the grand poem of Lucretius," Newton's conception of hard, finite atoms, the substitution by Boscovitch for

the material atom of a mathematical point, "towards or from which certain forces tend," or the splendid vortex-atom hypothesis of Sir William Thomson, in which "matter, such as we perceive it, is merely the rotating parts of a fluid which fills all space," throws no light at present on vital phenomena, except in so far as these can be brought within the domain of physical science. It is possible, however, to conceive that certain vital phenomena may be due, in their essence, to special properties inherent in the constitution of matter, but the physiologist is still far from the discussion of such questions. He finds that living matter is composed of certain solid, liquid, or gaseous constituents, and that it is the assemblage of these in varying proportions that gives to living matter its peculiar properties. The phenomena of life are never manifested by solid, liquid, or gaseous matter individually, but only when matter is in a peculiar colloidal condition or mass which, when death occurs, will split up into solid, liquid, and gaseous substances. At the same time, it is becoming more and more evident that vital actions such as growth, secretion, and the contraction of living muscle, depend on molecular, it may be, atomic changes occurring in living matter, changes as much beyond direct inspection as the molecular movements in a conductor of electricity, or in the isomeric transformation of cyanate of ammonia into urea.

2. **Energy.**—Physiology, in common with the science of physics and of chemistry, has received a strong impetus towards exactitude and comprehensiveness from modern generalizations regarding energy. The term energy must be clearly distinguished from the term force, and before entering into a consideration of energy we must understand clearly what is meant by the terms force and work. The notion of force is suggested by the sensation of pressure or of resistance experienced when we move a body with the hand or foot, as in lifting a weight from the floor to the table, but it will be observed that this is merely a sensation or feeling due to what is termed the muscular sense and that we must distinguish between the sensation and the cause of the sensation. Suppose the muscular sense perverted by disease, the weight might still be lifted from the floor to the table, but our notions of the force would be quite different. There is no proof then of the objective reality of force. But the weight has been lifted from the floor to the table and there has been a transference of what is called energy from one portion of matter (the muscles of the arm and body) to another (the weight resting on the table). "Whenever such a transference takes place, there is a relative motion of the portions of matter concerned, and the so-called force in any direction is merely the rate of transference, or of transformation, of energy per

unit of length for displacement in that direction." (Tait, *Recent Advances in Physical Science*, p. 16.) Force, then, is rate of transformation of energy in time. The British unit of force is the "poundal," defined as that force which applied to one pound of matter for one second generates in it a velocity of one foot per second. In the centimetre-gramme-second system (C.G.S.), the unit of force is the "dyne," similarly defined as that force which, acting on one gramme for one second, generates in it a velocity of one centimetre per second.

The next conception it is important clearly to grasp is that of *Work*. Work is done when resistance is overcome, and the quantity of work done is measured by the product of the resisting force and the distance through which it is overcome, in the direction in which the force acts. The British absolute unit of work is the "foot-poundal," which is the work done by a poundal acting through a foot. The absolute unit of work on the metrical system is the "erg," which is the work done by a dyne acting through a centimetre. Again, work may be measured in gravitation-units—the British being the "foot-pound," which is the work required to overcome a force equal to the weight of a pound through the space of a foot; the unit in the metrical system being the "centimetre-gramme," defined as that required to overcome a force equal to the weight of one gramme acting through the space of one centimetre. In the study of the work done by a contracting muscle, the unit usually employed is the "millimetre-gramme," similarly defined.

We are now in a position to understand what is meant by saying that *Energy* is the power or capacity of doing work. When a weight is lifted from the floor to the table, work is done in overcoming the attraction between the earth and the weight, and the energy of the system—the earth and the weight—is increased. The weight on the table is now in a position to do work; for example, it might, by proper arrangements, be allowed to descend slowly, as the weight of a clock, and do work in overcoming the friction of the wheels, the resistance of the air to the motion of the pendulum, and in giving rise to the waves of sound by which we might hear the ticking of the clock. Before it begins to descend, the weight possesses *Potential Energy*, or energy of position. A bent bow, a coiled mainspring of a watch, a head of water, are familiar instances of this kind of energy. Another mode of energy is the energy of motion or *Kinetic* energy. Thus, suppose a certain amount of work is done in lifting a weight, the weight acquires energy of position (potential); if it be now allowed to fall to the ground its energy of position is gradually transformed into energy of motion (kinetic), and it can be shown that "the amount of potential energy lost

in every stage of the operation is precisely equal to the amount of kinetic energy gained." (Tait, *op. cit.* p. 19.) It follows from this that energy, like matter, can neither be created nor destroyed. It may dis appear, but there has only been a transference from one material thing, or system of material things, to another. It may suddenly appear, but it has not originated *de novo:* it has appeared at the cost of potential energy somewhere else. Further, it is known that when a transformation of energy takes place "there is always a tendency to pass, at least in part, from a higher or more easily transformable to a lower or less easily transformable form." (Tait, *op. cit.* p. 20.)

The doctrine of the *Conservation of Energy* asserts, in the language of Clerk Maxwell, "The total energy of any material system is a quantity which can neither be increased nor diminished by any action between the parts of the system, though it may be transformed into any of the forms of which energy is susceptible." (Clerk Maxwell's *Matter and Motion*, p. 60; also proved by Von Helmholtz.) This great doctrine was enunciated after much speculation, calculation, and experiment, with regard to the nature of heat. So long ago as the beginning of the 18th century Locke wrote the following passage: "Heat is a very brisk agitation of the insensible parts of the object, which produces in us that sensation from which we denominate the object hot; so what in our sensation is heat, in the object is nothing but motion." (John Locke, *Natural Philosophy*, 1706.) Professor Tait shows (*op. cit.* Lecture II.) that Sir Isaac Newton was in possession of many of the facts of the conservation and transformation of energy. Count Rumford demonstrated "that the great quantity of heat excited by the boring of cannon could not be ascribed to a change taking place in the calorific capacity of the metal, and he therefore concluded that the motion of the borer was communicated to the particles of the metal, thus producing the phenomena of heat. 'It appears to me,' he remarks, 'extremely difficult, if not quite impossible, to form any distinct idea of anything capable of being excited and communicated in the manner the heat was excited and communicated in these experiments, except it be motion.'" Sir Humphry Davy, sometime after the date 1799, showed that by rubbing two pieces of ice against one another in the vacuum of an air pump, part of the ice was melted, although the temperature of the receiver was kept below the freezing point, and he inferred that "the immediate cause of the phenomena of heat is motion, and the laws of its communication are precisely the same as the laws of the communication of motion." (Davy's *Elements of Chemical Philosophy*, p. 94.) In 1834, Faraday discovered important relations existing between magnetism, electricity, and light. In 1840, Joule showed, "1st, that the heat evolved by any voltaic pair is proportional, *cæteris paribus*,

to its intensity or electro-motive force; and, 2nd, that the heat evolved by the combustion of a body is proportional to the intensity of its affinity for oxygen," thus establishing relations between heat and chemical affinity. Similarly, in 1843, Joule showed relations between magneto-electricity and heat; and in 1844, he proved "that the heat absorbed and evolved by the rarefaction and condensation of air is proportional to the force evolved and absorbed in those operations." In 1842, Mayer announced that he had by agitating water raised its temperature from 12° C. to 13° C., without however indicating the force employed; and, about the same date, and founding largely on physiological considerations regarding animal heat and the heat of the blood, Mayer attempted to calculate the mechanical equivalent of heat. This important determination was finally experimentally made by Joule in 1845 and 1847, when he employed a paddle-wheel to produce fluid friction, and obtained the equivalent from the agitation of water, sperm oil, and mercury. (See Joule's *Scientific Papers*, vol. I., p. 298).

Heat then being a kind of energy, it can be measured in terms of the unit of energy, as, for example, by the foot pound, the centimetre-gramme or erg, or it may be expressed in thermal units. The thermal unit is the quantity of heat required to raise the temperature of unit mass of water unit degree between the limits of 0° C. and 40° C. Thus the British thermal unit is the "pound-degree," that is the heat required to raise a pound avoirdupois of water from 60° F. to 61° F., and the French thermal unit is the "calorie" or kilogramme-degree, the heat required to raise a kilogramme of water from 15° C. to 16° C. This statement enables one to understand what is meant by the dynamical equivalent of heat, or the value in terms of a unit of work of a unit of heat. Joule found by experiment, as already indicated, that 772·55 foot-pounds of work are required to raise 1 pound of water from 60° F. to 61° F., the intensity of gravity being that of the sea-level at Greenwich, or 1389·26 foot-pounds are required to warm 1 pound of water from 0° C. to 1° C. Reduce 1389·26 feet to metres and we have 423·437 metres. The work done therefore is equivalent to (approximately) 424 kilogrammetres, if the unit of heat be the calorie above defined. To convert kilogrammetres into calories divide by 424, and to convert calories into kilogrammetres multiply by 424. The dynamical equivalent, or Joule, denoted by J, may be stated thus: The same energy which elevates 424 kilogrammes 1 metre high will, as heat, raise 1 kilogramme of water 1° C., or a weight of 424 kilogrammes, if allowed to fall from a height of 1 metre, would, by its concussion, produce as much heat as would raise the temperature of 1 kilogramme of water 1° C. Difficulty of experimentation has hitherto prevented the accurate determination of

the mechanical equivalent of light, electricity, or magnetism; but there is sufficient evidence that these also are modes of energy, and that they may be therefore transformed or transmuted, just as the energy of molar motion can become the energy of molecular motion or heat.

Consult Von Helmholtz' Lectures on Scientific Subjects, No. 7; Prof. P. G. Tait's Properties of Matter; Prof. Tait's Recent Advances in Physical Science; Joule's Scientific Papers; Grove—The Correlation of Physical Forces; also, Address on Continuity.

Chap. III.—GENERAL PRINCIPLES OF BIOLOGY.

Before entering upon the study of the functions of a specific organism, it will be instructive to consider some of the general characteristics of living things, and to endeavour to grasp some of the conditions—chemical, physical, and vital—which govern the origin, development, life-history, and death of all living beings. This preliminary discussion of the General Principles of Biology prepares the way for the more detailed treatment of the Physiology of Man. The leading characteristics of living beings or of living matter may be considered under the following heads:—

1. **Physical Structure.**—Solid and fluid matters invariably co-exist in living bodies: the solids contain fluids in their cavities or interspaces. No living matter ever assumes a crystalline form, but crystals may be embedded in it. The colloidal state, in which matter is soft, diffluent, and readily permeated by water, oxygen, and the crystalloids, is so characteristic of living stuff that Graham, its first investigator, termed it the dynamical state of matter. The colloidal condition, however, is not peculiar to living stuff, inasmuch as silicic acid and peroxide of iron may assume this state. All the solid parts of living matter are more or less moist; many have the character of softness, and they readily absorb water, becoming swollen by imbibition. When water is absorbed by starch there is an evolution of heat. This property of swelling up by absorption of water has led to the conception that the molecular structure of such substances as the walls of cells, and starch grains, may consist of very minute solid particles (*micellæ* of Nægeli), each particle being surrounded by a layer of fluid. According to this view, in a perfectly dry organic substance the micellæ would be in contact on all sides, and when it absorbed water the water would penetrate between the micellæ, forcing them asunder against the opposing action of cohesion. Another view is that of Strasburger, which appears to explain the phenomena of imbibition more satisfactorily. Rejecting the micellar theory of Nægeli, he

supposes that "the force which binds together the molecules is of a chemical as opposed to a physical nature; that they are held together not by cohesion, but by chemical affinity: he regards them as being linked together, probably by means of multivalent atoms, into molecular networks, the water present being retained in the meshes by intermolecular capillarity." (Vines' *Physiology of Plants*, p. 33.) This theory assumes that living colloidal matter consists of a distensible molecular network, capable of absorbing water until the capillary attraction is greater than the chemical affinity by which the molecules are bound together, and when this limit is passed the molecular structure is destroyed. In the case of protoplasm,[1] it may be supposed that the molecules are in a state of incessant change, and that the absorption and the liberation of water play an important part in vital phenomena.

2. **Chemical Composition**—Of the sixty-five or sixty-eight elements, not more than eighteen or twenty have been found in living things. Chief among these are oxygen, hydrogen, nitrogen, and carbon. It is interesting to note that the first three can only pass into the solid state under enormous pressures and at a low temperature, an indication of their great molecular mobility. Oxygen enters into combination with hydrogen, carbon, and even with nitrogen, an element remarkable for its chemical indifference. Hydrogen and carbon are chemically indifferent to other substances at ordinary temperatures. Carbon is allotropic, appearing in the unlike forms of charcoal, graphite, and the diamond. Along with these there are associated, of the non-metals—sulphur, phosphorus, and chlorine; of the alkalies—sodium and potassium; of the alkaline earths—calcium and magnesium; and of the metals—iron. In addition, there are minute quantities of silicon and fluorine, and there may be iodine, bromine, manganese, aluminium, copper, and lead in the tissues of particular plants and animals. It is interesting to notice that if we take the classification of the elements generally accepted by chemists we find that organic matter is built up of representatives of the hydrogen group—hydrogen (1), chlorine (35·37), and bromine (79·75); of the iodine group—iodine (126·53) and fluorine (19); of the oxygen group—oxygen (15·96), sulphur (31·98); of the nitrogen group—nitrogen (14·01), phosphorus (30·96); of the carbon group—carbon (11·97), silicon (28); of the sodium group of metals—sodium (22·99), potassium (39·04); of the calcium group of metals, calcium (39·9); of the magnesium group of

[1] *Protoplasm.* πρῶτος, first, πλάσμα, anything formed. A term first used by Von Mohl to describe the mucilaginous granular contents of the vegetable cell; now applied to the simplest form of matter constituting the physical basis of life.

metals, magnesium (23·94); and of the iron group of metals, iron (55·9)—all the other groups being unrepresented. Excluding the metals of the alkalies and alkaline earths, iron is the only metal essential to some of the higher forms of living animal matter. Of the fifteen non-metals, nine are represented, whilst of the fifty-three metals we find only five representatives, excluding those of rare and probably of accidental occurrence. Further, if we consider that the earth's solid crust consists chiefly of oxygen, silicon, aluminium, iron, calcium, magnesium, sodium, and potassium, and that water consists of oxygen and hydrogen, we see that carbon and nitrogen in particular are characteristic of living matter, and it may further be stated that no living matter can exist without the presence of the four, oxygen, hydrogen, carbon, and nitrogen. Their compounds form, from the chemical point of view, the basis of life.

The elements are so combined in living matter as to constitute compound bodies which may be separated as such by chemical processes. Such compounds are termed *proximate principles.* For example, phosphate of lime is a proximate constituent of bone, but phosphoric acid and oxide of calcium are not proximate constituents; in bone they do not exist individually, but united to form the salt, phosphate of lime. Chief among proximate substances is water, which as a rule forms more than three-fourths of the weight of a living body. An organism may, however, contain almost no water and still have the potentiality of life, as has been shown in the case of dried seeds and of dried rotifers and infusoria. In the process of freezing, also, water may separate from the organic stuff and become ice, and yet on careful thawing it may be taken up again, and the structure may return to its former condition. In these instances, the molecular changes on which life depends, and for the play of which the presence of water is necessary, have been arrested, and it is only on the return of water that life is renewed.

Certain proximate principles are the same as those met with in the crust of the earth or in the water of oceans and rivers, such as chlorides of sodium and potassium, sulphates of soda and potash, phosphates of soda, potash, lime, and magnesia, and fluoride of calcium. The proximate substances more characteristic of the tissues of plants and of animals are compounds formed of carbon, hydrogen, and oxygen, such as starches, sugars, fats; or formed of carbon, hydrogen, oxygen, nitrogen, and sulphur or phosphorus, such as albumin, glutin, or lecithin. Compounds formed of three elements have, especially in the dry condition, a considerable amount of chemical stability. Thus dry starch will not readily decompose. On the other hand, compounds con-

taining four or more elements are much less stable. They tend easily to disintegration. They exist only within a limited range of temperature and pressure, and under the action of bodies in a more active molecular state (ferments) the complex organic molecule falls to pieces, not usually resolving itself into its elements, but into simpler groups of elements. Thus a molecule of albumin may split up into simpler molecules of leucin, tyrosin, and other less complex bodies, such as water and urea. This molecular instability, probably, is one of the conditions of vitality, and as it is always most highly manifested by the compounds containing nitrogen, it has been conjectured that it may be due to the weak affinities of nitrogen-molecules for the other simpler molecules existing in organic matter. For example, two well-known explosives are gun cotton and nitroglycerine. Gun cotton is cellulose, $C_6H_{10}O_5$, in which 3 atoms of hydrogen are replaced by 3 atoms of a stable nitrogen compound, NO_2—thus : $C_6H_7(NO_2)_3O_5$. Again, nitroglycerine is glycerine, $C_3H_8O_3$, in which 3 atoms of hydrogen are replaced by 3 atoms of the same compound, NO_2, thus : $C_3H_5(NO_2)_3O_3$. Another example is picric acid : first one atom of hydrogen in benzol, C_6H_6, is replaced by an atom of hydroxyl, HO, thus : $C_6H_5.HO$, or phenol ; then 3 atoms of the hydrogen in phenol are replaced by 3 atoms of NO_2, forming picric acid, thus : $C_6H_2(NO_2)_3HO$. These compounds, especially the former two, are remarkable for chemical instability, and they offer an analogy to the constitution of living stuff. A sudden change of temperature, a vibration, a concussion, may cause the molecule to fly to pieces, and the fragments are of course simpler in structure. We shall see that all vital actions are characterized and accompanied by the appearance of chemical substances much simpler in composition than the substance which was the seat of these actions.

A living body is continually undergoing a series of chemical changes of composition and decomposition, as a result of which there is an incessant renovation of the molecules of the organism. Chemical changes are a necessary condition of the action of living matter, or it may be said that the living state is always associated with chemical change; part of the living matter dies, is decomposed, or rather its decomposition is its death, and the dead matter is then thrown out of the organism. New matter is added from without, and thus there is a perpetual exchange between the organic and the inorganic worlds, which may be termed the *circulation of matter*.

3. **Organic Form and Mode of Growth.** Living bodies are *organized*, that is, they are composed of dissimilar or distinct parts arranged in a certain order, and each part performs a determinate office

or function in connection with the maintenance of the life of the whole. There is reason to believe that this is true even of the apparently simple structure of an amœba, as new modes of investigation reveal details of structure which no doubt have a special physiological significance. The external form of living beings is consistent with a certain morphological type. At the beginning of existence the typical form is nearly spherical; afterwards, in the process of growth, a form is developed peculiar to the species. The spherical form is not only characteristic of the organism at the commencement of life—the egg or ovum, Fig. 10—but is seen also in the primitive elements which compose the developed organism. (See cells.) Organized matter may be composed of molecules, granules, cells, fibres, membranes, and tubes.

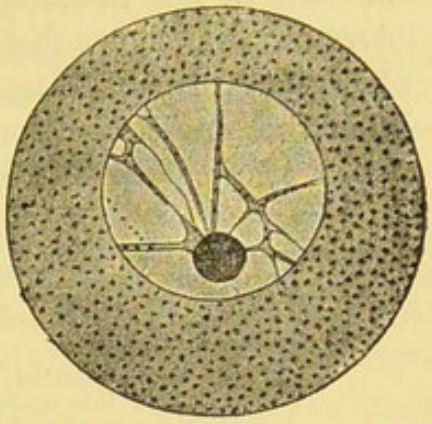


FIG. 10. Ovum from an Echinoderm

The mode in which new particles of matter enter a living organism furnishes also a distinctive character. A crystal grows by new molecules of similar composition to itself being applied directly to its surface, whilst a living thing absorbs the dead matter into its own substance and converts what was previously dead into living matter like itself. In other words, dead bodies increase in size by apposition, living bodies grow by metabolism[1] and intussusception.[2]

The manner in which the growth of crystalline forms may be modified by various physical conditions so as even to simulate organic forms has been the subject of various exhaustive inquiries, which have thrown light on the mode of origin of concretions, on the formation of shell, and on the deposition of earthy matter in soft tissues. In 1857, George Rainey showed that certain crystalline matters, when deposited in viscous or gummy solutions, assume globular and cell-like forms.[3] His method consisted in saturating a solution of gum arabic with carbonate of potash (specific gravity 1·4068), placing the fluid in a small wide-mouthed bottle, so as to fill it half full, and introducing into the bottle two clean

[1] *Metabolism*, from μεταβολη, change; the power living matter possesses of changing other matters brought under its influence. First used by Schwann.

[2] *Intussusception*. *Intus*, within; *suscipio*, I receive. The absorption of matters into the substance of living material.

[3] *On the Mode of Formation of Shells of Animals, of Bone, and of several other Structures by a Process of Molecular Coalescence*. By Geo. Rainey, M.R.C.S. Lond., 1858. See also *Quart. Jour. of Micros. Science*, 1858, and *On the Influence of Colloids on Crystalline Form and Cohesion*, by William Miller Ord, M.D., etc. Lond., 1879.

slides of glass, leaning against each other at the top. The upper part of the bottle was then filled by gently pouring in a solution of gum arabic in water, of a specific gravity of 1·0844, and the mouth of the bottle was covered with a sheet of paper. The whole was laid aside for a period of three weeks or a month. The slides were then removed and were found to be coated with a deposit of carbonate of lime. The carbonate, however, instead of being deposited in a crystalline form was in the condition of fine molecules, and by examining such slides at various intervals it was shown that these molecules coalesce so as to form spheres, which in turn may coalesce to form larger ones. The perfect spheres exhibit both radial and concentric markings, and in polarized light show a well marked cross. (See Figs. 11 and 12.) The presence of the

FIG. 11.—Precipitation of Carbonate of Lime, showing the crystalline form passing into globular forms. (*a*) Crystalline form; (*b*) angles of crystals rounded off; (*c*) ovoid forms; (*d*) coalescence of ovoid forms.

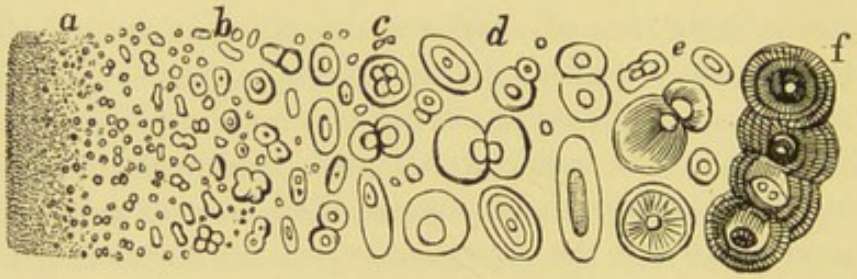


FIG. 12.—Precipitation of Carbonate of Lime from a Viscous Solution on a slide of glass, showing (beginning on the left hand) (*a*) finely molecular forms, then globular forms (*b*); and these forming larger and larger bodies by molecular coalescence (*c*, *d*.) On the right hand, large bodies showing concentric circles and radiating lines (*f*).

colloidal gum, according to Rainey, "annuls the polarity of the crystal, and allows the molecules of the crystal to obey simple laws of common and mutual attraction." This aggregating of the molecules he terms "molecular coalescence." His words are:

"When the molecules of pure carbonate of lime, that is carbonate of lime uncombined with a viscid substance, come into existence they immediately commence arranging themselves in straight lines, and thus when collected together form rectilineal figures or crystals; but when the impure carbonate, that is carbonate combined with a viscid substance, comes into existence, under similar circumstances, its molecules assume a curvilinear disposition, and hence become collected into globules." (Rainey, *op. cit.* p. 31.)

Further, if the spheres thus formed are plunged into solutions of gum of different specific gravity, the spheres break up along the radial lines and crumble into molecules. This is "molecular disintegration." Rainey showed that in the soft parts under the shell of the young

lobster, crab, or shrimp, bodies exactly like those thus artificially produced may be found. I possess a number of Rainey's original preparations, and after the lapse of thirty years they show all the gradations of form depicted in Figs. 11 and 12.

Professor Harting, of Utrecht, investigated this matter independently of, and even prior to, the appearance of Rainey's papers. He obtained similar results by allowing carbonate of lime to separate out of an albuminous or colloid fluid.

"He states that the albumin contained in the calcosphœrites [globular bodies of Rainey] undergoes a chemical change bringing it near to chitin, and says that the same is effected in albumin by chloride of calcium. To this modification he gives the name calcoglobulin. He finds in his experiments explanations of the structure of the shells of Lamellibranchiata and some Gasteropoda, of the calcareous plates of the bones of Sepia, of the shells of Foraminifera and loculi of Bryozoa, the spicules and sclerites of Alcyonaria; and states that the last mentioned forms are produced when cartilage, previously impregnated with calcium chloride, is placed in a solution of potassium carbonate mixed with a little sodium phosphate." (Ord, *op. cit.* p. 7.)

Dr. Ord has modified Rainey's method with important results bearing on the formation of bone and especially on the crystalline forms assumed by various urinary sediments.

Another striking example of the production of organic-like forms from dead matter is the formation of processes from myelin or protagon by the action of water.[1] Beat up the yolk of an egg in 30 cc. of absolute alcohol, boil for one or two minutes, and then filter on a flat plate. In the small amount of filtrate obtained a gelatinous yellow stuff appears. This is impure protagon or myelin. Allow the alcohol to evaporate. Place a small portion of protagon on a slide, lay a cover-glass gently over it, place it on the stage of the microscope, and focus so as to see the edge of the mass. This will be seen to be amorphous. Then allow a drop of water to pass below the cover-glass and immediately beautiful snake-like forms will shoot out, bend, curl, and assume grotesque forms such as are seen in Fig. 13. Montgomery showed that by acting on protagon with water, albumin, or glycerine, forms may be obtained simulating organic fibres, varicose nerve fibres, the broken down matter of the spinal cord or brain, and even cell-like bodies.

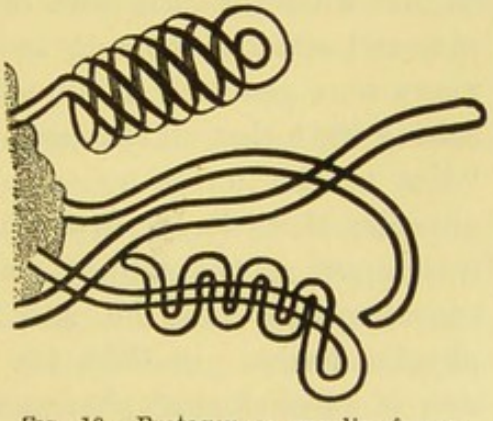


FIG. 13.—Protagon or myelin forms, curved and spiral. Processes shooting out from a mass of protagon on addition of water.

[1] Montgomery, *On the Formation of so-called Cells.* Lond., 1867.

All of these observations indicate that forms similar to those produced in and by living matter may be artificially produced in dead matter, and that molecular processes of coalescence, disintegration, and imbibition, may play an important part in the formation of shell, in the development of the curious spicules of sponges and synaptidæ, in the deposition of calcareous matter in tendon, in the walls of vessels, and in fibrous membranes (phenomena familiar to the pathologist), in the formation of the hard tissues of teeth, and in the calcification of cartilage, one of the early stages of ossification.

4. **Dynamical Characters.**—We have already seen that the various forms of physical activity, heat, light, electricity, motion, chemical affinity, are all related and convertible so that they are now regarded as but various modes of one energy, potential or kinetic. But plants and animals exhibit activities peculiar to themselves, such as those of assimilation and growth, irritability, contractility, reproduction, and nervous actions, which are all manifestations of energy. Is this the same energy as is active in the world of dead matter, or have living things an energy of their own? This is a question of profound importance. The older physiologists thought that the energy of living things was an entity or principle derived from the germ, and quite different in kind from the various forces of the outer world. This energy was described under such terms as vital force, life, the vital principle, and it was supposed to exert power not only over the matter introduced into the body, but also over the outer forces acting on the body. Further, instead of this vital force being correlated to the outer forces, it was supposed to be in some way opposed to them, so that when the vital force ceased to act, the natural forces came into play and acted on the body as if it were composed of dead matter. Such views were generally held until 1845, when Mayer first broke ground by setting forth that all change in the living organism, animal or vegetable, "lies in the forces acting on it from without." There can be no question that Mayer first directed the attention of physiologists to this aspect of the subject, and that it was largely from physiological considerations that he was led to his speculations regarding the physical forces. In 1850, Dr. W. B. Carpenter applied to the explanation of physiological phenomena the doctrine of the correlation of the physical forces, and showed that the vital forces were generated in living bodies by the transformation of light, heat, and chemical action, and that the energy thus derived from the outer world was given back to it again as heat and motion, and to a less degree in most animals as electricity and light. In 1859, these views were supported and extended in a modified form by Professor Joseph

Le Conte, who argued that the chemical forces, set free in the plant and animal, were transformed into vital force, and that by this action matter was raised from the plane of dead matter to the higher plane of living matter. Both of these writers, however, laid special stress on the view that the medium of this transformation of physical into vital force was organized or living matter. This is clearly shown in the words of Dr. Carpenter:

"It is the speciality of the material substratum thus furnishing the medium or instrument of the metamorphosis which establishes, and must ever maintain, a well-marked boundary line between physical and vital forces. Starting from the abstract notion of force as emanating at once from the Divine Will, we might say that this force, operating through inorganic matter, manifests itself as electricity, magnetism, light, heat, chemical affinity, and mechanical motion; but that when directed through organized structures it affects the operations of growth, development, and chemico-vital transformations and the like, and is further metamorphosed, through the instrumentality of the structures thus generated, into nervous agency and muscular power." (Carpenter, *Trans. of Royal Society*, 1850, p. 752.)

A careful study of the properties of protoplasm, more especially in the vegetable cell, and of the action on it of physical forces, such as those of heat and light, and the influence of the teaching of Herbert Spencer in his *Principles of Biology*, have led to the general adoption of these views; and the principle of the conservation of energy, as affecting the interactions of the forces manifested by dead and living matter, is now, to the physiological inquirer, what it is, in the language of Clerk Maxwell, "to the physical inquirer, a principle on which he may hang every known law relating to physical actions, and by which he may be put in the way to discover the relations of such actions in new branches of science." (Clerk Maxwell, *Matter and Motion*, p. 60.)

Let us now discuss how these principles apply to the phenomena of plant and animal life.

Plants containing chlorophyll require comparatively simple chemical substances as food. All plants absorb oxygen from the air, and all green plants, under the influence of sunlight, absorb carbonic acid. This carbonic acid is decomposed, the carbon being retained in the cell, whilst oxygen is liberated. Further, nitrogen exists in the juices of plants, but there is no evidence that it is directly used by the living matter. Plants may also absorb a small amount of ammonia from the air, and they obtain water chiefly from the soil. The roots obtain from the soil, or from the water in which the plant grows, ammonia and its salts (the nitrate, sulphate, and phosphate), nitrates of soda, potash, lime, and magnesia, chlorides of the alkalies and

alkaline earths, sulphates of potash and lime, phosphates of soda, potash, ammonia, lime, and magnesia, and iron in the form of a salt. From the substances thus derived from the air and the soil, the plant obtains the elements necessary for its existence, namely, carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium, calcium, magnesium, and iron, and in some plants, chlorine. The first four—carbon, hydrogen, oxygen, and nitrogen—are absolutely necessary for those metabolic processes on which the life of the plant depends, whilst the others apparently assist in the performance of these processes. In addition, plants may contain silica. The carbon is obtained by plants containing chlorophyll by the decomposition of carbonic acid under the influence of light, but plants destitute of chlorophyll (fungi) cannot thus decompose carbonic acid, and they obtain the requisite carbon from organic matter in which carbon is united to hydrogen. Hydrogen is obtained from water, oxygen from the air, and nitrogen from ammonia and its salts by plants of lower organization, and from nitrates by plants of a higher kind. The sulphur necessary for building up proteid matter is yielded by sulphates; the phosphorus which exists in chlorophyll and protoplasm, by phosphates; and the bases, potassium, calcium, and magnesium, by various salts. Although iron does not exist in chlorophyll, it appears to be necessary for its formation. From these simple materials, the protoplasm of the plant builds up more complex bodies, some non-nitrogenous and others nitrogenous. This process of building up may go along with the reverse process of pulling down; or, in other words, synthetic and analytic changes may go on, even simultaneously. The new matter supplied by the food may be, by these processes of constructive or destructive metabolism, divided into three portions—one part by constructive metabolism is used for building up the tissues of the plant (growth); a second portion, also by constructive metabolism, is built up into certain bodies which are stored up in the tissues of the plant, constituting reserve material; whilst the third portion, by a process of destructive metabolism, may be decomposed in the tissues of the plant, never entering into the composition of these tissues, and the products of decomposition are excreted as useless. In plants, synthetic are carried on to a much greater extent than analytic processes; synthesis indeed is characteristic of the lives of all plants containing chlorophyll.

By such processes the plant builds up either non-nitrogenous or nitrogenous substances. The formation of non-nitrogenous organic substances, such as starch, is known to be directly connected with the action of chlorophyll, under the stimulus of light. In green

vegetable cells, the protoplasm contains small corpuscles, the chlorophyll corpuscles, and it has been shown that these consist of a kind of matrix permeated by the chlorophyll, probably dissolved in an oil. The green matter of chlorophyll, called by Hoppe-Seyler chlorophyllan, has a formula of $C_{19}H_{22}N_2O_3$(P?), and is believed to be a lecithin compound containing phosphorus. It is important to note that it is a highly complex body, formed originally from the protoplasm of the cell. Vegetable cells containing chlorophyll, under the influence of sunlight, as already said, decompose carbonic acid and liberate oxygen. This process is not to be confounded with the respiration of the protoplasm of the cell, which consists in the absorption of oxygen and the liberation of carbonic acid; but is the first step in the constructive metabolism by which the chlorophyll builds up starch. Without light no starch is formed, nor, on the other hand, can it be formed without carbonic acid, even when the chlorophyll is exposed to light. The rapidity of the process in certain plants is very remarkable. Thus "Kraus found that starch grains made their appearance in the chlorophyll corpuscles of Spyrogyra within five minutes after exposure to bright sunlight, within two hours in diffuse daylight; in Funaria they made their appearance after two hours' exposure to sunlight, and after six hours' exposure to diffuse daylight." (Vines, *Physiology of Plants*, p. 147.)

It is probable that chlorophyll-corpuscles form starch by the union of carbonic acid and water to produce formic aldehyde, which, by polymerization,[1] becomes starch, thus: $CO_2 + H_2O = CH_2O$ (formic aldehyde) $+ O_2$; then $6CH_2O = C_6H_{12}O_6$; and $C_6H_{12}O_6$ (grape sugar) $- H_2O = C_6H_{10}O_5$ (starch). But the starch is really formed by the dissociation of the protoplasm under the action of the chlorophyll, so that the process may be, first, the formation of formic aldehyde, then the building up of the protoplasm, and finally a dissociation of the protoplasm, one of the products being starch. In addition to starch, sugars, fats, cellulose, and organic non-nitrogenous acids may be formed. In a similar manner, nitrogenous bodies may be built up, although physiological chemists have not advanced so far in this direction. It has been supposed that nitrates and sulphates absorbed as food may be decomposed by organic acids (oxalic acid) and that the nitric and sulphuric acids thus set free may combine with a non-nitrogenous organic substance, such as formic aldehyde. Again, bodies of the nature of amides (that is ammonias in which hydrogen is replaced by the radicle of an organic acid), such as asparagin,

[1] Polymeric is a term given by Berzelius to organic compounds possessing the same composition but differing in molecular weight.

leucin, etc., may be formed either as steps towards the building up of protoplasm or as products in its destructive metabolism. For our present purpose it is sufficient to note that nitrogenous matters may also be formed synthetically.

Now arises the question as to the source of the energy that enables the protoplasm of the plant-cell containing chlorophyll to perform these remarkable synthetic processes. There can be no doubt that the energy is the radiant energy of light, more especially of the red and blue rays absorbed by chlorophyll. It has been ascertained that a solution of chlorophyll presents certain absorption bands when examined with the spectroscope. It is the light of just those parts of the spectrum that is most active in the decomposition of carbonic acid by green plants, and it is remarkable that the maximum amount of absorption of light by chlorophyll occurs at that part of the spectrum where, according to Langley, of America, we have the maximum of energy. Further, Engelmann has shown that bacteria, placed along with a filament of *Cladophora* in the solar spectrum under the microscope, collect round the filament in those regions that coincide with the absorption bands of chlorophyll, and that this is an indication of oxygen being evolved from the chlorophyll corpuscles in the Cladophora, more especially where they are exposed to rays of the spectrum at the junction of the orange and the red and also at the blue, just beyond the line F. Light has been found to have an influence even on the absorption of food materials by the roots. The initiation of these changes is due to heat, a somewhat elevated temperature being essential to the active life of all plants; but in the later stages, the heat, as a source of energy, is, relatively to the light, of less importance. The importance of light to the plant can hardly be overstated. Keep a green plant in darkness and, although it may appear to grow, it loses weight by the exhalation of carbonic acid and watery vapour, and if kept too long in darkness it will die. Under the influence of light, assimilation will go on and the plant will gain in weight. The true respiration of the plant does not seem to be affected by light. Thus a plant containing chlorophyll lives on simple food elements and, under the influence of light, builds these up into more complex substances. In doing this, the kinetic energy of light is stored up and becomes potential. Such products as starch, gums, glutin, cellulose, may be regarded as matter containing stores of energy. They may be decomposed by various chemical operations, or they may be completely oxidized and converted into carbonic acid and water, but in these oxidations heat would again become kinetic and could be employed to do work. Thus the furnace of a steam-engine might be

fed with starch or vegetable oil and the energy liberated from these might to a certain extent be converted into motion. The green plant then, from the dynamical point of view, is storing up energy. On the other hand, plants destitute of chlorophyll cannot live on such simple materials as can green plants, nor can they obtain energy directly from the radiant energy of the sun. Like animals, they require food containing complex organic matters representing potential energy. Both classes of plants, however, expend energy, either as growth, as movement, as heat (more especially during germination and at certain stages of the reproductive process), or, to a small extent, as light or electricity. The green plant stores more energy than it expends: it is, as already explained, more concerned in building up complex substances than in decomposing these into simpler ones, and hence the life and growth of such a plant is practically unlimited. Thus, immense accumulations of energy have been made and are being daily made by the vegetable world, and the energy of the sun's rays is being stored up, to be liberated again as heat in combustion, or as heat or motion in the bodies of living animals.

We have now to consider the question from the point of view of the animal kingdom. Some of the lowest forms of animals originate by a fission or division of the parent organism, but, above this low level of existence all originate in a cell, the ovum, or egg. After fecundation, this structure passes through numerous stages of development until the body of the animal is formed. During this process of growth the animal requires food, and, like the plant, it also requires a supply of energy to carry on the internal changes in its body, possibly to move its body from place to place and to supply energy for an expenditure going on in the form of heat, and to a smaller degree, of electricity and of light. In the first instance, energy as heat is required to start the processes of assimilation and of growth. The development of all ova requires certain favourable conditions as regards temperature; but, like the plant, neither the ovum nor the adult animal form receives in the form of heat more than a small fraction of the energy it needs. Heat in both instances initiates or provides suitable conditions for the changes in the body peculiar to life, and it will presently be seen that the production of heat, and thus of the condition favourable to living actions, is one of the invariable accompaniments of such actions. Food is now supplied. With the exception of oxygen, no matter can be used by an animal in the form of an element, nor can an animal live on the comparatively simple compounds that, as we have seen, constitute the food of plants

Animals obtain oxygen from the air, or, in the case of aquatic organisms, from the oxygen dissolved in the water. The food-stuffs, with the exception of a few simple compounds such as water, common salt, etc., are derived either from the bodies of other animals or from plants, and a little consideration will show that all food-stuffs must ultimately have been derived from plants. Substances, then, formed in plants constitute the food of animals. These food-stuffs have, as already explained, been constructed by the plant and they represent energy in the potential condition. The substances essential to the formation of a diet for an animal derived directly from plants consist of (1) albuminous or proteid matters, as represented by glutin or legumin; (2) fatty matters, such as olive oil; (3) carbo-hydrates, such as starch, sugar, gum; (4) mineral matters—phosphates, sulphates, chlorides of the alkalies and alkaline earths, and iron; and (5) water. All of these, after undergoing various changes, chemical and physical, are ultimately assimilated by the living matter of the animal's body and pass through metabolism of the most complicated kind. Thus, part of the matter may, by constructive metabolism, be built up into the tissues of the animal, bone and muscle and nerve and the tissues of the various organs; another portion may also, after constructive metabolism, be stored up as reserve material in the form of fat and glycogen; whilst a third portion may, by destructive metabolism, be split up into simpler substances which may be excreted without having entered into the composition of the body. Most, if not all, of these operations are intimately connected with oxidations. Oxygen is absorbed in respiration and, directly or indirectly, it combines with the matter in the food-stuffs and simpler products are formed. It is not improbable that, in the first instance, constructive metabolism occurs by which the oxygen, proteids, fats, carbo-hydrates, salts, and water are built up into the highly complex matter, living protoplasm, and that thus there is, for a short period, a still further conversion of energy into the potential condition even in the animal. This condition, however, does not last. Animal protoplasm is characterized by molecular instability: its large and complex molecule can exist only in very limited conditions, and a sudden change in these conditions, or what is termed a stimulus, causes it to break up into simpler bodies. Thus energy is liberated and appears in the kinetic form as heat, and probably as motion. The decomposition, in the first instance, of living protoplasm is like a molecular explosion, shattering the complex molecule into fragments. These fragments, however, may still be complex, and the process may be repeated, simpler bodies being formed, and ultimately we find that the matter thrown out of the body as useless is carbonic

acid, water, a nitrogenous substance called urea (two molecules of ammonia in which two atoms of hydrogen are replaced by CO), and saline matters. These substances represent only a small amount of energy, and it will be observed that they are essentially the same as the simple substances constituting the food of the plant. Thus the complex bodies built up by the plant may become for a time part of the still more complex living matter of the animal, and then, by oxidations, this complex living matter is decomposed into simpler matters. In the latter operation, energy is set free and appears in the kinetic form as heat, motion, electricity, light, and sound. Thus the body of animals produces heat; there are movements of the whole body, as in locomotion, or of portions of it, as in the respiratory movements or the beating of the heart; and to a smaller extent, electricity (especially in electric fishes), light, and sound may be manifested. Energy becomes kinetic chiefly as heat and motion, and even motion (internal and external) is ultimately resolved into heat.

Contrast now the plant and the animal—the plant transforms kinetic into potential energy, the animal transforms potential into kinetic energy. But neither the plant nor the animal is wholly concerned in the one operation. The plant also converts potential into kinetic energy, but relatively to a small extent; whilst the animal also converts kinetic into potential, but relatively to a small extent. It will be observed, finally, that whilst the plant furnishes potential energy to the animal, and the animal liberates this as kinetic energy, the kinetic energy of the animal is not re-transformed into the potential energy of the plant. The plant must therefore always receive new supplies of energy from the solar rays.

The following illustration will still further elucidate this interesting subject. It is well known that muscles and nerves may exhibit some of their vital properties after the death of the animal and that this is especially the case with the so-called cold-blooded animals such as the frog. Suppose a preparation is made consisting of the gastrocnemius muscle of the frog with sciatic nerve attached, as in Fig. 14. This preparation is connected with an instrument termed a myograph, one form of which is shown in Fig. 15. The upper end of the femur is secured by the clamp *C*, sliding on the pillar *B*, and the tendo Achilles (*J* in Fig. 14) is attached by a hook to

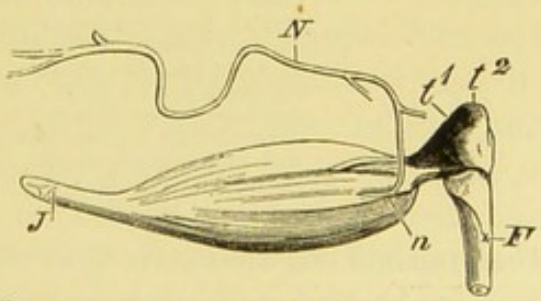


FIG. 14.—Nerve-muscle Preparation. *F*, femur; *N*, nerve; *J*, aperture for hook.

the horizontal lever *EE*, which carries a marker *J*; this marker is brought into contact with *G*, a smoked glass plate that can be moved horizontally in sliding grooves. The nerve is stretched over wires coming from a battery or induction coil, so that it may be irritated by an electric current. When the nerve is irritated the muscle contracts, lifts up the lever *EE*, and the marker *J* draws a vertical line on the plate *G*. The plate is

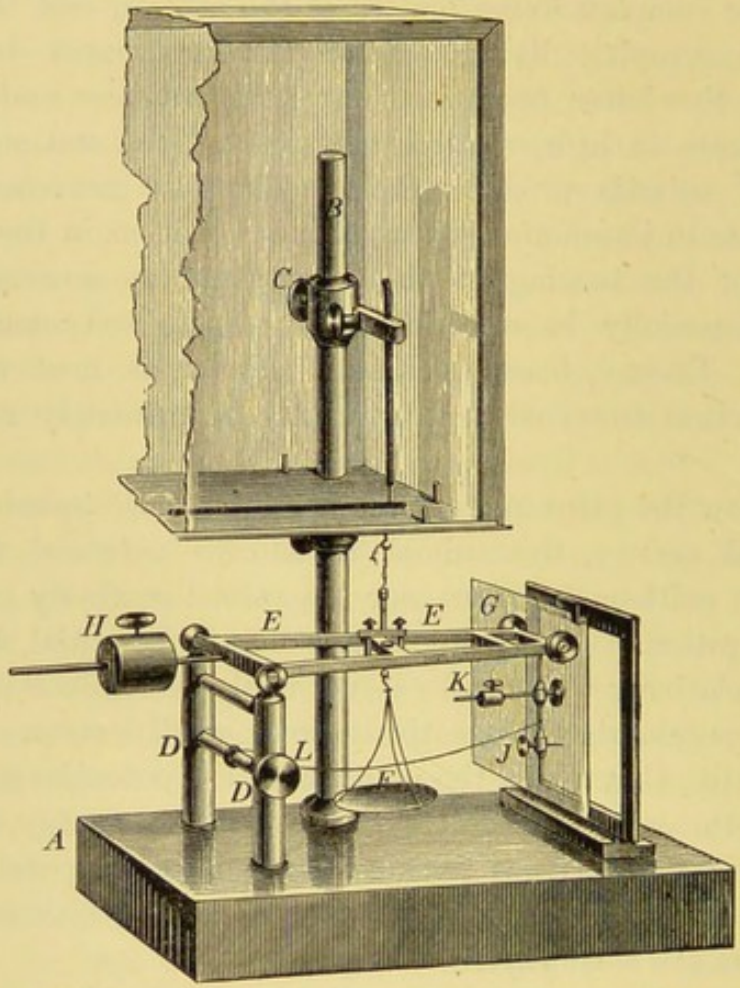


FIG. 15.—Myograph, an instrument for recording the contraction of a muscle. *A*, wooden stand; *B*, vertical brass pillar; *C*, sliding forceps or clamp for holding upper end of femur of nerve-muscle preparation; *D*, *D*, short vertical pillars on the top of which the lever *EE* works; *F*, scale pan for weight; *J*, marker; *G*, glass plate; *K*, counterpoise to keep *J* in contact with *G*; *H*, counterpoise to lever *EE*. The nerve-muscle preparation is covered with a glass shade, to the walls of which pieces of wet blotting paper are attached, thus forming a moist chamber to preserve the nerve from drying.

then pushed on, the nerve again stimulated, and thus a series of vertical lines is obtained, indicating the work done by the muscle in lifting a weight placed on the scale pan *F*. (See Fig. 16.) It can thus be shown that the muscle does work in lifting the weight, and by a refined method of inquiry it can also be demonstrated that with each muscular contraction heat is produced. Energy thus becomes kinetic as heat and motion. This energy is not derived from the battery used in irritating the nerve.

The electricity is used merely as a stimulus to the nerve. When the nerve is irritated a molecular change is generated in the nerve; this travels down the nerve with a certain velocity (nerve-current), and when

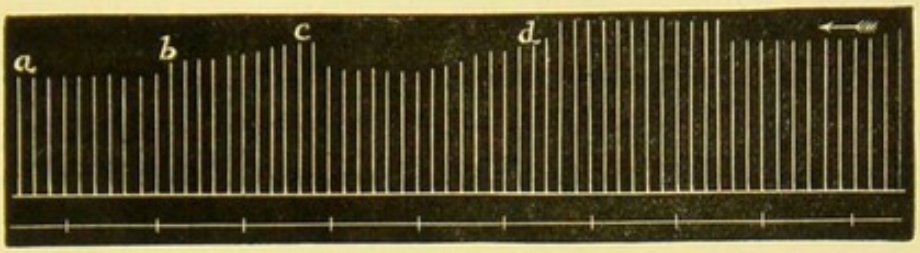


FIG. 16.—Examples of tracings taken with myograph. Plate moving in direction of arrow.

it reaches the muscle it sets up molecular changes in it which result in a contraction, attended by the phenomena of heat and motion. Nor is there any quantitative relation between the strength of the electric current employed in irritating the nerve and the amount of work done by the contracting muscle. A very feeble current would be quite sufficient, just as the pull of a hair-trigger would be enough to set free the energy from a charge of gunpowder in a rifle. The energy then that has become kinetic as heat and motion was stored up in the muscle. Further, it might be shown that the contraction of the muscle is associated with chemical changes in it, the splitting up of its complex protoplasm into simpler bodies. Thus energy is liberated in accordance with the principles already explained. This energy, potential in the muscle substance, must have been derived from the food of the frog, and if we trace back the history of this food we find it came originally from plants, and that the plants formed it from simple materials, in the processes transforming the energy of light into potential energy. Finally, at one end of the chain we have matter in the form of simple substances along with the kinetic energy of light, and at the other we have again matter in the comparatively simple conditions of carbonic acid, water, and nitrogenous substances, and kinetic energy as heat and motion. This experiment also shows that muscle substance may be regarded as a magazine or store of energy, which may be liberated by the molecular action of the nerve. We shall find many examples of this important relation between nerve and muscle and between the external modes of energy and the action of living matter. Just as nerve energy liberates the energy of the muscle, so light and sound liberate the energies of the sense organs on which they act, and these again liberate energies in the nerve centres, which in turn liberate the energy stored up in muscle or gland.

5. **Evolutional History of Living Beings.**—The evolution of a living being is determinate: it has a commencement, an existence, and an

end; it passes through different phases, which succeed regularly and in a certain order. The same statements may be made, with a certain amount of truth, regarding a crystal, but its life history is distinguished from that of a living animal by the absence of waste and of repair, and by its mode of growth, already referred to.

Living beings have a certain individuality. Among the higher grades each member has a certain independence, although related to all; but this characteristic may almost disappear in the lower classes of plants and of animals.

All living organisms take their origin in a *germ* which was developed in a parent—that is a previously-existing being having essentially the same structure and properties. Every plant and animal, accordingly, have the power, at one stage of their existence, of producing a germ by which the species may be perpetuated. This germ, which is known as the spore, seed, or egg of plants and animals, is a cell—the simplest form of structure. After its separation from the parent body, it is capable of independent existence, and, under favourable external influences, of growing or developing into a new individual, in most respects similar to that from which it derived its origin.

Living beings have formed a continuous series, from the first appearance of life upon the earth until now. Offspring usually possess more or less of the character of their parents; and they may transmit peculiarities—either acquired by new conditions of existence, or received from their parents—to their descendants. This is known as *heredity*.

The evolution of energy undergoes changes during the life of an organism. Usually the production of energy increases up to a certain maximum, and then slowly declines, so that in the life history of each individual there is a period of *maximum vital activity*. In certain species of animals, however, there are successive phases of repose and of movement, as in the encysted conditions of infusoria, the metamorphoses of insects, and the occurrence of hybernation. In other organisms also, vital phenomena may appear to be entirely suspended under certain conditions. For instance, some of the *rotiferæ* may be kept in a state of dormant vitality for a considerable time by the simple process of drying.

During its life an organism undergoes change of form, increase of its mass, and development of its organization. This development is not, however, continued indefinitely. The organization becomes at last complete for each organism, reproductive functions are exercised, and then gradually the wear and tear become greater than the power of upbuilding, and the organism breaks down. *Death* at last necessarily terminates its evolution. When this occurs, the body is sub-

mitted to the action of external agencies, both physical and chemical, which ultimately reduce it to the simple elements of which it was at first composed.

It is important to remember that an organism is affected during every instant of its life by the medium in which it lives. The medium furnishes the materials requisite for existence. Dead matter is supplied to take the place of living matter, and external modes of energy—such as heat and light—act upon it at the same time. There is thus an action and reaction between the organism and the conditions in which it lives. These conditions may be conveniently summarized by the term *environment*. In this adaptation of relations every living organism has a power of suiting itself to modifications of conditions within certain limits. This power is known as its *variability*, and it is a necessary condition of the existence of every living being.

To recapitulate, the essential characters of a living being are the following:—

1. Molecular complexity; heterogeneity of parts; and chemical instability of the organic compounds forming it.
2. Waste and incessant repair of organic materials.
3. The conversion of kinetic into potential energy as the framework of the body is built up or stores of reserve material are formed.
4. Liberation of kinetic energy in various modes, and, in particular, as mechanical movement, heat, and electricity.
5. Organization, or the adaptation of certain parts of the body to particular functions.
6. A regular evolution from origin to death.
7. Origin from a parent, and the possibility of producing the elements of offspring.
8. A power of variability and of adaptation to external conditions.

6. **Theories of Life.**—Numerous efforts have been made to define life, of which the following are examples:—*Aristotle* says, "Life is the assemblage of the operations of nutrition, growth, and destruction;" *Lamarck* states that "Life, in the parts of the body possessing it, is that state which permits organic movements, and the movements which constitute active life result from the application of a stimulus;" *Bichat* says, "Life is the sum total of the functions which resist death;" *Treviranus* calls it "The constant uniformity of phenomena, with diversity of external influences;" *Laurence* says, "It consists in the assemblage of all the functions or purposes of organized bodies, and in the general result of their exercise;" *Duges* calls it "The special activity of organized bodies;" *Béclard's* definition is "Organization in

action;" and *Herbert Spencer* asserts that "Life is the continual adaptation of internal relations with external relations." It will be observed from the above definitions that authors have felt the necessity of presupposing some organized structure, the existence of which is taken for granted in their definitions. The dictum of Béclard, "Organization in action," is, on the whole, the best.

In the history of physiology several distinct schools of thought have from time to time had the ascendency. Aristotle and Galen laid the foundations of anatomy, and in their physiological speculations they adopted the leading tenet of Hippocrates that there exists a principle which he termed φύσις, nature, to which were ascribed the origination and control of all bodily actions. This intelligent principle directed the operation of other subordinate principles or faculties (δύναμεις), which carried out the various functions. In the middle ages, a school arose, the *iatro-chemists* (ἰατρός, a surgeon or physician,) which ascribed all vital phenomena to the operation of chemical laws, and more especially to fermentations which took place in the blood and other fluids. This school, represented by Paracelsus, Van Helmont, and Mayow, may be regarded as the offspring of the Alchemists. Then came the *iatro-mathematicians*, who attained their greatest celebrity in the 17th century, and who ascribed vital actions to the sizes of particles and pores, the amount of retardation from friction, and other mechanical causes, and who indeed asserted that all the phenomena of life are mechanical. Borelli, Boerhaave, and De Gorter may be taken as representatives of these views. This school was succeeded by the *animists*, founded by Stahl, who reverted essentially to the more ancient doctrine of the existence of a rational principle as the cause of life. The archeus, psyche, anima, soul, mind, were all names given to this principle by writers previous to Stahl, but Stahl especially termed it the *anima*. It was not the soul in the modern sense; it was a mysterious power affecting all vital operations, sometimes consciously, sometimes not. It is remarkable how these old views still exert an influence both on scientific and on popular thought. Crystallized in the form of language, these ideas have passed on from generation to generation, so that such terms as derivation, revulsion, resolution, fermentations, the *vis medicatrix naturæ*, vital force, still may be employed without reference to the philosophical systems that gave them birth. Further, it may be said that even scientific opinion oscillates amongst these contending modes of thought, and that now and again views, physiological or pathological, are enunciated, which are only repetitions, in modern language, of ancient theories. The error in all these schools was to attribute the actions of the living body

solely to chemical, mechanical, or vital causes. Modern thinkers endeavour to study the phenomena as they find them, to explain such of these as can be accounted for by the application of physical and chemical principles, and leave the remainder unexplained. This unexplained residue is termed *vital*, but without any assumption of the existence of a special principle or vital force, and the more refined and accurate our means of research become the more limited is the sphere of the phenomena assigned to vital force.

To obtain a clear view of what is meant by the living state, consider the phenomena manifested by a unicellular organism such as an amœba, or by a living cell, such as a colourless blood corpuscle, or a cell from a gland, or a cell from a nerve centre. These show two or more of the following properties—(1) a power of *assimilation*, that is, of absorbing dead matter and of converting it by metabolism into living matter, or protoplasm; (2) *irritability*, or the property of being affected by a stimulus, such as electricity or light, and of visibly responding to it by *movement;* (3) *secretion*, or the elaboration from protoplasm of new substances, as the formation of the constituents of milk in the cells of the mammary gland; (4) *automatic molecular changes*, leading to the apparently spontaneous liberation of energy, as in the nerve cells of certain nerve centres; and (5) *reproduction*, or the giving off of beings or living things similar to themselves. These properties are termed *vital.* Wherever they are manifested, we say the thing is alive. All dogmatic statements as to the nature of life can only be misleading, inasmuch as in the present state of science our knowledge of vital processes is limited, and it is probable that the molecular changes associated with vital phenomena may never be thoroughly understood. What is termed *vital* may simply be a condition of matter, like the phenomena usually called physical; but even if it were found to be so, life would be none the less mysterious.

Consult HERBERT SPENCER—Principles of Biology, vol. I., part I., chaps. 2, 3, 5, 6; BEAUNIS—Physiologie Humaine, vol. I.; CARPENTER, on the Mutual Relations of the Vital and Physical Forces—Philos. Trans. 1850; also, Quarterly Journal of Science, vol. I., 1864; JOSEPH LE CONTE—The Correlation of Physical, Chemical, and Vital Force, and the Conservation of Force in Vital Phenomena—Philos. Mag., 1860; GROVES—The Correlation of the Physical Forces; VINES—Physiology of Plants, Lectures V., VIII., XIII., and XIV.

SECTION II.

THE CHEMISTRY OF THE BODY.

Chap. I.—THE INORGANIC CONSTITUENTS.

Before considering the structure and functions of the tissues and organs, we ought to become acquainted with the general facts of animal, or as it is sometimes termed physiological chemistry. These facts relate either to the nature of the chemical compounds of which the body is composed or to the chemical processes connected with the phenomena of life. The inquirer, however, soon ascertains that there are special difficulties in the study of this branch of chemistry. In the first place, living matter cannot be analyzed, because in the very processes of analysis to which it is subjected it ceases to be living, and the results obtained show only the constitution of dead matter that once was alive. Thus, suppose a chemist attempted the analysis of a piece of living muscle, the processes to which it was subjected would kill it, and the products of analysis, such as proteid matter, fatty matter, saline matter, and water, would only be the substances existing in dead muscle. Such an analysis would not throw much light on the chemical nature of the living protoplasm on the activity of which the irritability and contractility of the muscle depended. Again, in the second place, the chemical reactions occurring in the body are so modified in amount and character by the nature of the living matter in which they occur as to be obscure and difficult of interpretation, and the most profound acquaintance with organic chemistry and with molecular physics is required for their elucidation. For example, every one is familiar with the well known fact that in respiration oxygen is absorbed by the blood from the air-cells of the lungs whilst carbonic acid is given off by the blood to the air, and yet the exact nature of these interchanges is far from being thoroughly understood. How far these interchanges depend on physical conditions, and how far on the chemical combinations or decompositions occurring in the blood, are questions that must be considered, while the chemical constitution of hæmoglobin, the colouring matter of the blood, and the

chemical constitution of other substances in the blood, require careful investigation. Still, with all its difficulties, animal chemistry is making rapid progress, and, as will be shown in discussing the chemical operations occurring in living matter, it is affording glimpses of the molecular machinery of life.

If a human body were analyzed, the substances obtained would depend upon the methods employed and the extent to which the analysis was pursued. The chemist might resolve it into the elements of which it was composed, or he might only isolate certain compounds formed by these elements. Compounds exist of greater or less complexity. Thus there are bases and acids, and the salts formed by their union. Still more complex bodies exist of the nature of fats and starch-like compounds, whilst there are substances, such as albumin or hæmoglobin, of the highest degree of chemical complexity.

The *elements* found in the body are hydrogen, carbon, nitrogen, oxygen, sulphur, phosphorus, fluorine, chlorine, silicon, sodium, potassium, calcium, magnesium, lithium, iron, and occasionally manganese, copper, and lead. The relative importance of these in organic matter has already been discussed.

The *compounds* formed from these elements are either of the nature of *proximate principles*, or they exist in such principles (see p. 15), and they are primarily divided into (1) *inorganic*, and (2) *organic* compounds. The inorganic comprise such substances as water, common salt, and phosphate of lime; whilst the organic are such as sugar, stearin, and albumin. According to modern views, all the latter fall into the domain of organic chemistry, that branch of the science dealing with carbon and its compounds.

The inorganic compounds consist of water, acids, and salts.

1. **Water**, H_2O, forms about two thirds of the weight of the body, so that a body weighing about 75 kilogrammes contains about 50 kilogrammes of water. The proportion of water to solid matter is greatest in embryonic tissues, and becomes smaller and smaller as life goes on. Thus, according to Bischoff, the body of an infant may contain 664 parts of water to 336 of solid matter, whilst in adult life the proportions may be 585 to 415 per 1000. Beaunis gives the following table, compiled from the analyses of Gorup Besanez and others, showing the quantities of water in some of the tissues, organs, and fluids—

Tissues or Organs.	Water.	Solid Parts.
Enamel,	2	998
Dentine,	100	900
Bone,	486	514
Fat,	299	701
Elastic tissue,	496	504
Cartilage,	550	450
Liver,	693	307
Spinal cord,	697	303
White substance of brain,	700	300
Skin,	720	280
Brain,	750	250
Muscles,	757	243
Spleen,	758	242
Thymus,	770	230
Connective tissue,	796	204
Kidneys,	827	173
Grey substance of brain,	858	142
Vitreous humour,	987	13

Liquids.	Water.	Solid Parts.
Blood,	791	209
Bile,	864	136
Milk,	891	109
Liquor sanguinis,	901	99
Chyle,	928	72
Lymph,	958	42
Serum,	959	41
Gastric juice,	973	27
Intestinal juice,	975	25
Tears,	982	18
Aqueous humour,	986	14
Cerebro-spinal fluid,	988	12
Saliva,	995	5
Sweat,	995	5

An adult introduces into the body daily about 2 litres of water in the form of drinks of various kinds, whilst it may be estimated that about $\frac{1}{2}$ litre is contained in the articles of diet consumed. In addition to this a small amount is probably formed in the body by chemical processes. In ordinary circumstances about 1500 cub. cent. are eliminated daily by the kidneys, 800 to 1000 cub. cent. by the skin and lungs, and 100 cub. cent. by the intestine. There is thus in a state of health an equilibrium established between the amount absorbed and the amount eliminated. If the amount falls below a certain minimum the sensation of thirst referred to the pharynx is experienced, and if the quantity be still further reduced there will be disturbances of the circulation and of respiration, such as irregular action of the heart and difficulty in breathing and even diminution of sensibility and convulsive seizures. Death has been observed to occur in frogs when the loss of water amounted to 30 per cent. of their weight. On the other hand, the excessive absorption of water into the blood and tissues is not harmless. In these circumstances the kidneys secrete a large quantity of urine of low specific gravity and the total elimination of solids in a given time is also increased. Thus the blood and tissues become impoverished as to saline matters. There has also been observed in such cases a tendency to dropsical effusions. According to Falk and Picot an injection into the jugular vein of a rabbit or dog of from $\frac{1}{30}$ to $\frac{1}{50}$ of its weight of water caused death. Picot found also, as had been previously stated by Richardson, that the injection of water altered the appearance of the coloured blood corpuscles, and he attributed the cause of death to interference with their respiratory functions.[1]

The water of the body is the general solvent, thus producing the

[1] Picot, *Comptes Rendus*, 1874, p. 62.

physical condition favourable to chemical operations, and the molecules of matter in solution are brought into such close proximity as to permit of those subtle interactions connected with the phenomena of life. As a general rule (and with the exception of such a tissue as exists in the vitreous humour of the eye) the greater the degree of activity of a tissue or organ the greater is the amount of water it contains.

2. **Mineral Matters.** These are chiefly chloride of sodium, chloride of potassium, sulphates of soda and potash, phosphates of soda, potash, lime, and magnesia, and carbonates of soda and lime. There are also small quantities of iron and silica. The following tables from Beaunis show the percentage of mineral matters in the ash of certain organs and of certain fluids—

TABLE I.—MINERAL MATTERS IN CERTAIN ANIMAL SOLIDS.

	Names of Authors of Analyses.					
	Heintz.	Staffel.	Breed.	Oidtmann.	C. Schmidt.	Oidtmann.
	Bone.	Muscles of Calf.	Brain.	Liver.	Lungs.	Spleen.
Chloride of Sodium,	...	10·59	4·74	...	13·0	...
Chloride of Potassium,	...	...	...	...	...	...
Soda,	...	2·35	10·69	14·51	19·5	44·33
Potash,	...	34·40	34·42	25·23	1·3	9·60
Lime,	37·58	1·99	0·72	3·61	1·9	7·48
Magnesia,	1·22	1·45	1·23	0·20	1·9	0·49
Oxide of Iron,	...	...	...	2·74	3·2	7·28
Chlorine,	...	...	...	2·58	...	0·54
Fluorine,	1·66	...	...	...	...	...
Free Phosphoric Acid,	...	...	9·15	...	...	...
Combined Phos. Acid,	53·31	48·13	39·02	50·18	48·5	27·10
Sulphuric Acid,	...	...	0·75	0·92	1·4	2·54
Carbonic Acid,	5·47	...	...	...	...	...
Silicic Acid,	...	0·81	0·12	0·27	...	0·17
Phosphate of Iron,	...	...	1·23	...	...	...

TABLE II.—MINERAL MATTERS IN CERTAIN ANIMAL FLUIDS.

	Names of Authors of Analyses.							
	Verdeil.	Weber.	Weber.	Dahnhardt.	Porter.	Wilderstein.	Rose.	Porter.
	Blood.	Serum.	Blood Clot.	Lymph.	Urine.	Milk.	Bile.	Excrements.
Chloride of Sodium,	58·81	72·88	17·36	74·48	67·26	10·73	27·70	4·33
Chloride of Potassium,	...	...	29·87	...	...	26·33	...	...
Soda,	4·15	12·93	3·55	10·35	1·33	...	36·73	5·07
Potash,	11·97	2·95	22·36	3·25	13·64	21·44	4·80	6·10
Lime,	1·76	2·28	2·58	0·97	1·15	18·78	1·43	26·40
Magnesia,	1·12	0·27	0·53	0·26	1·34	0·87	0·53	10·54
Oxide of Iron,	8·37	0·26	10·43	0·05	...	0·10	0·23	2·50
Phosphoric Acid,	10·23	1·73	10·64	1·09	11·21	19·00	10·45	36·03
Sulphuric Acid,	1·67	2·10	0·09	...	...	2·64	6·39	...
Carbonic Acid,	1·19	4·40	2·17	8·20	...	...	11·26	...
Silicic Acid,	...	0·20	0·42	1·27	4·06	...	0·36	3·13

On considering these tables, we may note the following points:—(1) That about $\frac{3}{4}$ of the total mineral matter in the solids consist of phosphoric acid and of lime; (2) the large amounts of potash and of phosphoric acid in muscle; (3) the large amounts of potash and of phosphoric acid in the brain; (4) the contrast as regards potash especially between the analyses of the liver and of the lungs; (5) the large amounts of soda and oxide of iron in the spleen; (6) the contrast of the analyses of blood, blood serum, and blood clot as regards chloride of sodium, potash, and phosphoric acid; (7) the general resemblance of lymph and urine to serum, except as regards potash and soda; (8) the large amount of chloride of potassium in blood clot and its absence from serum; (9) the large amounts of chloride of potassium, lime, and phosphoric acid in milk; and (10) the large amount of soda in bile. It will be observed that the analyses do not state precisely the amount of the various *salts* that are assumed to exist, but only the quantity of bases and of acids, and it would be impossible to allocate to the bases the requisite quantity of acids to form salts. Further, the phosphoric and sulphuric acids obtained in such analyses are derived partly from the decomposition of organic compounds, and they do not all exist in the tissues in combination with bases. It will therefore be seen that an analysis of the ash gives only an imperfect view of even the inorganic compounds. To get an approximate estimate of the amount of the more important salts another method is adopted in place of analyzing the ash, namely, that of estimating the quantity of the salt absorbed and eliminated daily.

1. *Chloride of sodium and potassium.*[1]—The chief salt of sodium is chloride of sodium (NaCl) or common salt, which exists in all the tissues and in all the fluids. Probably 200 grammes may be taken as the average amount in the body of an adult. From 15 to 20 grammes are eliminated daily chiefly in the urine, whilst a smaller amount is separated in sweat and in the excrements. To make up for this daily loss, common salt is taken in food, either as an ingredient of the food-stuff or as an adjunct. In carnivora, the amount in the food is sufficient for the wants of the organism; but herbivora and man appear to require an additional quantity. Bunge states that the food of herbivora is rich in carbonates, phosphates, and sulphates of potash, and that

[1] The tests for the mineral substances are no doubt familiar to the student, and the chemical processes, volumetric or gravimetric, by which the amount of each constituent may be ascertained, will be described in treating of the methods of analyzing the urine. The student of medicine, in particular, should make himself conversant with the more common reactions of the inorganic substances.

these salts, reacting on the chloride of sodium in the blood, are decomposed, chloride of potassium (KCl) and phosphates, carbonates and sulphates of soda being formed. These salts, then, in excess in the blood, are eliminated by the urine, whilst the deficiency of chloride of sodium must be met by additional supplies in the food. On the other hand the food of carnivora is deficient in salts of potash, and thus the amount of chloride of sodium in the food is quite sufficient as less of it is used in the manner indicated. If chloride of potassium be substituted for chloride of sodium, disturbances arise by-and-bye from a deficient amount of the latter salt. The tissues, however, retain common salt very tenaciously, so that during a dietary devoid of salt, the salt disappears slowly from the urine. (Beaunis, *op. cit.* vol. i. p. 79.)

A portion of the salt thus introduced may be supposed to pass through the body unchanged, but during its passage no doubt affecting nutritive processes. Thus it facilitates the absorption of albuminous matters, and it increases the metabolic changes in the blood or tissues, as shown by the increased amount of urea eliminated in the urine after the administration of salt. As already explained, a portion of the common salt taken in food is decomposed, giving its chlorine to produce chloride of potassium, a salt indispensable to muscular fibres and to the blood corpuscles. The relative amounts of chloride of sodium and of chloride of potassium in some of the principal fluids is shown, per 1000 parts, in the following table—

	NaCl.	KCl.
Blood,	2·70	2·05
Blood Corpuscles,	...	3·67
Plasma (*Liquor Sanguinis*),	5·54	0·35
Lymph,	5·67	...
Chyle,	5·84	...
Gastric Juice,	1.45	0·55
Pancreatic Juice (from permanent fistulæ),	2·50	0·93
Pancreatic Juice (from temporary fistulæ),	7·35	0·02
Bile,	5·53	0·28
Milk,	0·87	2·13
Urine,	11·00	4·50

2. *Salts of Soda and Potash.*—This table shows that the blood corpuscles are rich in KCl, and the plasma rich in NaCl, and it is a general fact that KCl exists largely in the solid parts such as blood corpuscles, muscular fibre, and nervous tissues, while NaCl exists in the fluids of the body. At one time it was supposed that the salts of potash had a high nutritive value, but the experiments of Panum indicate that this is not strictly the case. No doubt, as a rule, animals

thrive better on a diet containing salts of potash, but in small doses these have a stimulant rather than a nutritive action, increasing especially the action of the heart, both as regards force and frequency of beat. In larger doses the activity of the heart is weakened.

It would appear that salts of soda are more abundant in embryonic and early life than in adult life. Thus, Bunge found that for each kilogramme of weight there were the following proportions of soda and potash—

	Na_2O.	K_2O.
Rabbit's Embryo, - -	2·183	2·605
Rabbit, 14 days old, -	1·630	2·967
Kitten, 1 day old, - -	2·666	2·691
Cat, 19 days old, - -	2·285	2·790
Cat, 29 days old, - -	2·292	2·684
Dog, 4 days old, - -	2·589	2·667
Adult Mouse, - - -	1·700	3·280

3. *Salts of ammonia.*—Ammonia may be found in the urine, sweat, and gastric juice. About ·7 gramme is eliminated daily by the urine, and the urine of herbivora contains less than that of carnivora. Thus, Salkowsky shows that in normal acid urine the ratio of ammonia to the total nitrogen is from 1 to 17 to 1 to 20·5, while in the alkaline urine of the rabbit it is from 1 to 54 to 1 to 57. It is well known that under the action of an organic ferment (see URINE), ammonia arises from the decomposition of urea, and it has been suggested that possibly the small amount found in the urine, and in the sweat or expired air, may arise from the decomposition of urea in the intestine, the ammonia formed being absorbed by the blood-vessels and then eliminated by the kidney. (Beaunis.) In normal circumstances, there is no decomposition of urea in the blood leading to the formation of carbonate of ammonia, and Feltz and Ritter found that this did not occur even after injecting the ammoniacal ferment into the blood.

4. *Salts of Lime.*—Fluoride of calcium and phosphate, carbonate, sulphate, urate, and oxalate of lime exist in the body. The fluoride is found in the enamel of tooth and in bone. Phosphate of lime is found everywhere, but more especially in the bones and teeth, which may contain from 60 to 70 or 80 per cent. of this salt. In these structures it exists as the tribasic phosphate ($Ca_3 2PO_4$). The ash of all tissues, except elastic tissue, contains phosphate of lime, and urine contains phosphate of lime in solution, whilst alkaline urine, as in herbivora, shows it in a state of suspension. (Beaunis.) Carbonate of lime exists

in the otoliths (Fig. 17) or concretions in the internal ear, in the urine, in saliva and salivary concretions, and along with phosphate of lime in the bones, teeth, and hairs. Sulphate of lime has been found in bone, in the blood, and in pancreatic juice, whilst urate and oxalate of lime appear in urinary deposits. The salts of lime are derived from the food, more especially from vegetable products and from water, in which lime exists as bicarbonate. It would appear that even the small amount present in ordinary water may be sufficient for the wants of the organism. The carbonate in the food or in the water is changed during digestion into phosphate, and this passes into the blood and tissues. Valentin supposed that this change of carbonate into phosphate might go on even in the tissues, as he found that young bones were rich in carbonate of lime, and that the carbonate gave place to the phosphate as the bones grew older. Another source of carbonate of lime might be found in the formation of carbonic acid from the decomposition of such organic acids as tartrates, malates, citrates during their passage through the body. Salts of lime are eliminated by the bowels and kidneys, in herbivora, chiefly by the former channel, and in carnivora by the latter. The special function of the lime salts is to give firmness and solidity to the tissues, more especially to the bones. If supplied in deficient amount, or if they are removed from the solid tissues to too great an extent, the bones in particular may be imperfectly developed, or, yielding to the superincumbent weight, they may become deformed. Further, it appears that when food or drink contains too little of the salts of lime for the wants of the body, these salts are taken from the bones and muscles, indicating that they may have some other part to play in the metabolism of the body with which we are unacquainted.

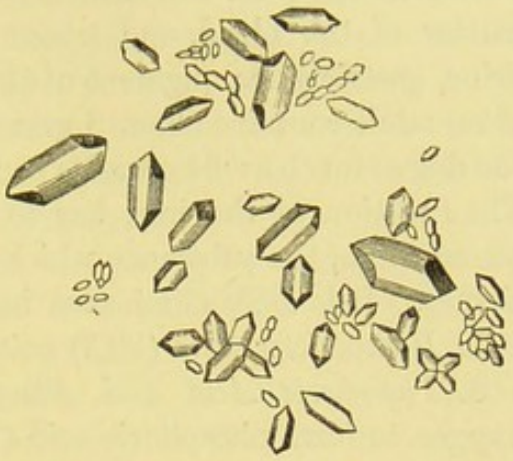


FIG. 17.—Otoliths of carbonate of lime, consisting of small thick columnar crystals, combinations of rhombohedrons and hexagonal prisms.

5. *Salts of Magnesia.*—Phosphate of magnesia ($Mg_3 2PO_4$) is usually found in small amount along with phosphate of lime in all the solids and fluids. Gorup Besanez states that in muscles and in the thymus gland the amount may be even greater than the amount of phosphate of lime. It is eliminated by the kidneys from carnivorous animals as phosphate held in solution in the acid fluid, whilst in the urine of herbivora it exists as the ammoniaco-magnesian, or triple phosphate,

or as the phosphate of magnesia. Excrements usually contain the same compound salt, along with palmitates and stearates of magnesia. The function of magnesia salts is unknown.

6. *Iron* forms an essential constituent of hæmoglobin, the colouring matter of the blood, and traces exist in the chyle, lymph, bile, milk, urine, gastric juice, pigment of the eye and hair. (Beaunis.) The blood of an adult contains about 3 grammes of pure iron, and in the spleen of the dog as much as ·24 gramme per 100 volumes has been found by Picard. The function of the iron has to do with the respiratory properties of hæmoglobin, the substance which conveys oxygen from the lungs to the tissues. It is daily eliminated in the fæces as the sulphide of iron.

7. *Hydrochloric Acid* (HCl) exists in the free state in gastric juice.

8. *Phosphoric Acid and Phosphates.*—Phosphorus, combined with oxygen to form phosphoric acid (PO_4), is derived from lecithin, nuclein, and glycero-phosphoric acids—three complex organic bodies, to be considered later—and it unites with soda, potash, lime, and magnesia to form the various phosphates already alluded to. There are three phosphates of soda: (*a*) Na_3PO_4, (*b*) Na_2HPO_4, and (*c*) NaH_2PO_4; three phosphates of potash, (*a*) K_3PO_4, (*b*) K_2HPO_4, and (*c*) KH_2PO_4; two phosphates of lime, (*a*) Ca_32PO_4, and (*b*) CaH_42PO_4; phosphate of magnesia, Mg_32PO_4; and the ammoniaco-magnesian, or triple phosphate, $NH_4Mg_2PO_4 + 6H_2O$. (Fig. 18.) An adult man eliminates by the kidneys from 2·5 to 3·5 grammes of phosphoric acid daily. Carnivora eliminate phosphates chiefly by the kidneys, only $\frac{1}{30}$ of the total being separated by the excrements as phosphate of lime and magnesia; whereas in herbivora the carbonates replace the phosphates in the urine, and the phosphates appear chiefly in the excrements. Compounds of phosphorus are evidently of great importance in the body, as indicated by their presence in the blood corpuscles, the muscles, and nervous tissues.

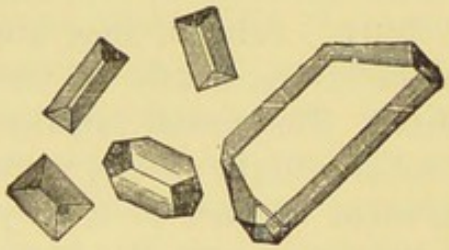


FIG. 18.—Phosphate of ammonia and magnesia—three-sided prisms bevelled at both ends and on one of the edges.

9. *Carbonates.*—These are always found in the ash of animal matters, but their presence may be due to the decomposition of organic acids. Still, the blood contains alkaline bicarbonates ($NaHCO_3$ and $KHCO_3$), and they may also be found in the urine, lymph, and saliva. Carbonates are derived from the food, directly or indirectly, by the decomposition of organic vegetable acids, such as malic, tartaric, or citric acids. Again, as will be seen hereafter, carbonic acid may be regarded, along with water, as the last of the series of bodies produced

by the decomposition of non-nitrogenous matters in the body, such as fats and carbo-hydrates.

10. *Sulphates.*—These are also always found in the ash of animal matter, but as sulphur is an essential constituent of albuminous matter, the sulphuric acid thus found arises from the oxidation of the sulphur during the process of incineration. The presence of sulphates in the ash is therefore no proof of the existence of such in the organic matter. Sulphates, however, are found in small quantity in the tissues, in the blood, and in the fluids, except milk, bile, and gastric juice. (Beaunis.) These are derived partly from the food and partly by oxidation of the sulphur in albuminous matter. That such oxidations do take place has been proved. Doses of sulphur are followed by an increase in the amount of sulphates eliminated. A diet rich in albuminous matter produces the same influence, whilst a vegetable diet has the reverse effect. Possibly albuminous matters may be decomposed, and sulphates be thus formed. At all events, the increased elimination of urea following a diet rich in albuminates is accompanied by an apparently correlative increase in the amount of sulphates. From 1·5 to 2·5 grammes of sulphuric acid are excreted by the kidneys of an adult man daily. The urine may also eliminate phenol-sulphuric acid and conjugated acids of a similar kind to the extent of ·2787 gramme daily. (*v. Velden.*) The urine of cats and dogs may contain alkaline hyposulphites. The excrements contain sulphide of iron, and, by the decomposition in the intestine of albuminous matters rich in sulphur, sulphuretted hydrogen may be produced.

Chap. II.—THE CHEMICAL CONSTITUTION OF THE ORGANIC CONSTITUENTS.

The organic compounds obtained from the body of a plant or of an animal were at one time supposed to be of a different constitution from those of inorganic nature. The chemist could decompose mineral matters into their elements and he could build them up again, but whilst he knew the amount of carbon, hydrogen, and oxygen in a given weight of starch, he could not build up this body from these elements. Hence it was assumed that the laws regulating the construction of the complex substances present in living bodies were different from those which rule inanimate matter. The synthesis of urea—a crystalline body found in the urine in 1828 by Wöhler—disproved this notion, and since then many organic bodies have been built up by the chemist. In the process, some insight has been obtained into their chemical constitution, the artificial distinction between inorganic and organic chemistry has been removed,

and Kekulé, one of the greatest authorities on such a matter, writes as follows—

"The chemical compounds of the vegetable and animal kingdom contain the same elements as those of inanimate nature. We know that in both cases the same laws of combination hold good, and hence no differences exist between organic and inorganic compounds, either in their component materials, or in the forces which hold these materials together, or in the number and the mode of grouping of their atoms. We notice continuous series of chemical compounds whose single members, when only those which lie close together are compared, exhibit strong analogy, so that between these no natural division is perceptible. If, however, for the sake of perspicuity a line of demarcation is to be drawn, we must remember that this boundary is purely arbitrary and is not a natural one, and may be drawn at any point which seems most desirable. If we wish to express by organic chemistry that which is usually considered under the name, we shall do best to include all carbon compounds. We, therefore, define organic chemistry as the chemistry of the carbon compounds, and we do not set up any opposition between inorganic and organic bodies. That to which the old name of organic chemistry has been given, and which we express by the more distinctive term of the chemistry of the carbon compounds, is merely a special portion of pure chemistry, considered apart from the other portion only because the large number and the peculiar importance of the carbon compounds render their special consideration necessary." [1]

Notwithstanding the weight of Kekulé's great authority, it must, however, be said that in the above passage he overshoots the mark, and that there is a real difference in character between organic and inorganic carbon compounds. Contrast, for example, sugar with prussiate of potash, and it will be seen that although both bodies are carbon compounds they are types of structure of an entirely different character. Organic carbon compounds have been happily defined by Schorlemmer as hydrocarbons and their more immediate derivatives, and organic chemistry is therefore that of the hydrocarbons. This definition is probably as good a one as can be given of organic substances.

It is clear then that we have to do now with the chemistry of carbon compounds. The student is at once brought face to face with a vast series of bodies the chemical constitution of which is represented by complex formulæ, and he soon finds also that the same substance may be represented by different formulæ according to the notions of the composition of the body held by different chemists. It is of interest therefore to understand what such formulæ mean and how the chemist is able to represent the constitution of an organic body by formulæ, because there is a great danger of attaching too much importance to formulæ and of imagining that they really represent the grouping of the atoms.

[1] Kekulé, *Lehrbuch d. Org. Chemie*, i. 11. The above, on comparison with the original, was modified from the translation of the same passage given in Roscoe and Schorlemmer's *Treatise on Chemistry*, vol. iii. part 1, p. 32.

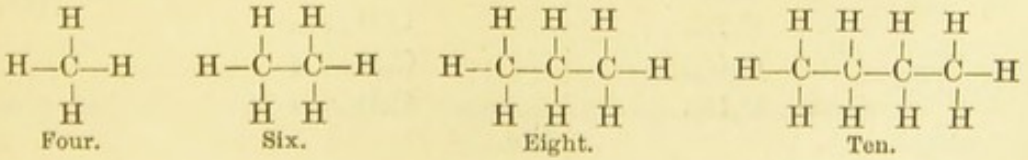
Without encroaching except to a very limited extent on the province of organic chemistry, it will be useful to give a brief account of the leading conceptions of the organic chemist in this most difficult department of the science.

Carbon is the one element essential to organic compounds, and the peculiarities of the chemical substances found in the body depend to a large extent on the chemical properties of this all-important element. It is a tetrad element, and its atomic weight (12) is capable of uniting with at most four atomic weights of hydrogen (1) or of any other monatomic element. The simplest hydrocarbon compound, marsh gas or methane, CH_4, is a *saturated* compound, that is, methane cannot combine with chlorine, bromine, or other monatomic element, but one or more of the atoms of hydrogen may be exchanged for an equivalent amount of another element. Thus—

Methane.	Methyl Chloride.	Methene Chloride.	Chloroform.
CH_4	CH_3Cl	CH_2Cl_2	$CHCl_3$
Carbon Tetra-chloride.	**Carbonic Acid.**	**Hydrocyanic Acid.**	**Cyanogen Chloride.**
CCl_4	CO_2	HCN	ClCN

The above may all be termed *substitution derivatives* of the first hydrocarbon, CH_4. But carbon may unite with itself, or, in other words, there may be many atoms of carbon in a carbon compound, and these may unite with each other and thus add greatly to the complexity of the substance. As shown by Kekulé, it is this fundamental property possessed by carbon, of its atoms uniting among themselves, which distinguishes it from all other elements, and this is also the cause of the large number of bodies that may be derived from the different groupings possible with three or four or more carbon atoms. Thus, if two atoms of carbon combine, two atomicities of the group are satisfied whilst six remain free; if three atoms of carbon combine, there are eight free atomicities; and if four atoms of carbon combine, there are ten free atomicities; and so on. Each of these free atomicities may be satisfied by combining with one of a monatomic element—say hydrogen—or two with a diatomic body—say oxygen—and in this way the compound may become a saturated compound, which cannot combine further with elementary bodies, but from which new bodies may be formed by substitution.

The addition of each atom of carbon to the end of the chain raises the combining power of the substance by two. Thus—



So that n-atoms of C will combine with $2n+2$ atoms of any monatomic substance. Each of these saturated compounds is the basis of a series of bodies, as shown in the following table—

Hydrocarbons.	Alcohols.	Aldehyde.	Acids.	Ketones.
C_nH_{2n+2}	$C_nH_{2n+2}O$	$C_nH_{2n}O$	$C_nH_{2n}O_2$	$C_nH_{2n}O$
CH_4	CH_4O	...	CH_2O_2	...
C_2H_6	C_2H_6O	C_2H_4O	$C_2H_4O_2$	...
C_3H_8	C_3H_8O	C_3H_6O	$C_3H_6O_2$	C_3H_6O
C_4H_{10}	$C_4H_{10}O$	C_4H_8O	$C_4H_8O_2$	C_4H_8O
C_5H_{12}	$C_5H_{12}O$	$C_5H_{10}O$	$C_5H_{10}O_2$	$C_5H_{10}O$
C_6H_{14}	$C_6H_{14}O$	$C_6H_{12}O$	$C_6H_{12}O_2$	..
C_7H_{16}	$C_7H_{16}O$	$C_7H_{14}O$	$C_7H_{14}O_2$	...
C_8H_{18}	$C_8H_{18}O$	$C_8H_{16}O$	$C_8H_{16}O_2$	...
C_9H_{20}	$C_9H_{20}O$	...	$C_9H_{18}O_2$	...
$C_{10}H_{22}$	$C_{10}H_{22}O$	...	$C_{10}H_{20}O_2$	...
$C_{11}H_{24}$	...	...	...	...
$C_{12}H_{26}$	...	...	$C_{12}H_{24}O_2$	...
$C_{13}H_{28}$	...	...	...	...
$C_{14}H_{30}$	...	...	$C_{14}H_{28}O_2$	...

Each of the individuals in this series of hydrocarbons differs from the one above it by CH_2. Such a series is termed homologous, and it is well known that the boiling points of the substances in a homologous series rise by nearly equal increments.

Since 1862, series of hydrocarbons represented by C_nH_{2n+2}, etc., have been grouped under the name of *paraffins*. It was found that American petroleum contained a mixture of homologous bodies represented by this formula, and their relation to the alcohols, aldehydes, acids, ethers, etc., already known to the organic chemist, was recognized. From each of these hydrocarbons numerous substitution products may be obtained. Thus, from any body having the general formula C_nH_{2n+2} substances may be derived by the substitution of OH, NH_2, CH_2. OH, CO . OH for one atom of H, or double each of these groups of OH, etc., for 2H, and so on. Further, from each hydrocarbon, C_nH_{2n+2} by theory at least, compounds poorer in hydrogen are formed by the withdrawal of one or more couples of atoms of hydrogen, and from each of these again substitution compounds may be produced. Thus, we have the following series of non-saturated hydrocarbons, each of which may be the basis of a group of chemical substances—

C_nH_{2n+2}	C_nH_{2n-14}
C_nH_{2n}	C_nH_{2n-16}
$C_{n2}H_{2n-2}$	C_nH_{2n-18}
C_nH_{2n-4}	C_nH_{2n-22}
C_nH_{2n-6}	C_nH_{2n-24}
C_nH_{2n-8}	C_nH_{2n-26}

Etc.

Thus, the series C_nH_{2n+2}, as shown in the above table, contains the well-known monatomic alcohols, such as methyl, ethyl, and propyl alcohols, and the corresponding acids—formic, acetic, propionic, etc. The second series (C_nH_{2n}), starting with olefiant gas, C_2H_4, contains the diatomic alcohols or glycols, $C_nH_{2n+2}O_2$, and the acids derived from these: (1) acids having the formula $C_nH_{2n}O_3$, such as glycollic acid, and (2) acids having the formula $C_nH_{2n-2}O_4$, of which oxalic acid is a representative. The third series (C_nH_{2n-2}) is illustrated by acetylene, C_2H_2; the fourth series (C_nH_{2n-4}) by terebinthene, $C_{10}H_{16}$; the fifth series (C_nH_{2n-6}) by benzol, C_6H_6; the sixth series (C_nH_{2n-8}) by cinnamene, C_8H_8, etc. A good example of bodies related to such a series as any of the above we have in the fifth series, C_nH_{2n-6}, which gives benzol, C_6H_6, and it in turn is related by substitution to numerous bodies, such as $C_6H_5.OH$, $C_6H_5.COH$, $C_6H_5.CO.OH$, $C_6H_5.NH_2$, etc., just as CH_4 yields the corresponding compounds, $CH_3.OH$, CH_3COH, $CH_3.CO.OH$, $CH_3.NH_2$, etc. There is, however, a far greater difference between $CH_3.NH_2$ and $C_6H_5.NH_2$ than between $CH_3.NH_2$ and any NH_2 substitution product of, for instance, C_2H_4, C_3H_6, etc. So marked are these differences that the benzol derivatives are classed as *aromatic compounds* to distinguish them from the more immediate derivatives of the paraffins which are termed *fatty compounds.*

Reference has already been made to the relation of the alcohols to the paraffins. Thus they may be represented by replacing the H of the hydrocarbon by OH. They are therefore substitution derivatives, and the analogues of metallic hydrates. For example, beginning with the hydrocarbon ethane, C_2H_6, we have $C_2H_5.OH$, ethylic hydrate or ethyl alcohol, analogous to $Na.OH$, sodium hydrate. In like manner, there is at least one alcohol for each hydrocarbon. But it is well known that there are alcohols having the same percentage composition and yet differing in their products of oxidation and decomposition. The same general formula therefore might represent different substances. For example, C_3H_8O represents propyl alcohol, but it is well known that there are two propyl alcohols—the one yielding by oxidation first an aldehyde and then an acid, and the second a ketone. To explain this, the chemist devises two formulæ. He supposes that in primary propyl alcohol there is a group of atoms, $CH_2.OH$, which is monatomic, and that in secondary propyl alcohol there is a diatomic group, $CH.OH$.

Primary propyl alcohol is then represented by the formula $\begin{matrix} CH_2.OH, \\ | \\ C_2H_5 \end{matrix}$

and secondary propyl alcohol by $\begin{matrix}CH_3\\|\\CH\\|\\CH_3\end{matrix}$.OH. In the oxidation of the first, the aldehyde is formed by the removal of the H_2 from the group $CH_2.OH$, leaving $\begin{matrix}C.OH\\|\\C_2H_5\end{matrix}$, or C_3H_6O, propyl aldehyde, and the acid by the substitution of O for H_2 in the same group: $\begin{matrix}CO.OH\\|\\C_2H_5\end{matrix}$, or $C_3H_6O_2$, propionic acid. Again, in the case of the oxidation of the secondary propyl alcohol, a ketone is formed by the removal of HH from the diatomic group, CH.OH—thus: $\begin{matrix}CH_3\\|\\CH\\|\\CH_3\end{matrix}$.OH, then $\begin{matrix}CH_3\\|\\CO\\|\\CH_3\end{matrix}$, or C_3H_6O, propyl ketone, or acetone. The ketone, when oxidized, cannot give any C_3 acid, because the group COH is wanting; it yields CO_2, H_2O and acetic acid. In like manner there are primary, secondary, and tertiary amyl alcohol, and four butyl alcohols are theoretically possible.

Compounds therefore having the same percentage elementary composition and molecular weight, and which show a similar behaviour under certain reactions, whilst they differ to a greater or less extent in other reactions, or at least in physical properties as boiling point, specific gravity, etc., are termed *isomeric*. Thus, as already pointed out, there are four isomeric butyl alcohols, all having the same formula, C_4H_9 (OH), and the products obtained by various reactions on these are also isomeric. Oxidation of the first butyl alcohol produces butyric acid ($C_4H_8O_2$); of the second, isobutyric acid ($C_4H_8O_2$); of the third, ethylmethyl ketone (C_4H_8O); and of the fourth, a mixture of acetic and formic acids.

Finally, bodies having the same percentage composition, but different molecular weights are *polymeric*. Thus, aldehyde C_2H_4O and $C_6H_{12}O_3$ paraldehyde, are polymeric; and the same is the case with the hydrocarbons of the general formula C_nH_{2n}, and with formal aldehyde, CH_2O, acetic acid, $C_2H_4O_2$, lactic acid, $C_3H_6O_3$, and grape sugar, $C_6H_{12}O_6$. It will be observed that the molecular weights of the polymeric bodies are integer multiples of the same empirical formula.

The alcohols hitherto considered are monatomic, but there are also diatomic and triatomic alcohols, and also bodies of a similar kind referable to the higher series of hydrocarbons. Thus the diatomic alcohols or glycols are represented by the formula $C_nH_{2n}(OH)_2$. It was pointed out that the primary monatomic propyl alcohol contains the group of atoms $CH_2.OH$, and its relation to the primary propyl

glycol may be represented by supposing two such groups existing in the latter. Thus $\begin{matrix}CH_2.OH\\|\\C_2H_5\end{matrix}$, or $\begin{matrix}CH_2.OH\\|\\CH_2\\|\\CH_3\end{matrix}$, is primary propyl alcohol, and $\begin{matrix}CH_2.OH\\|\\CH_2\\|\\CH_2OH\end{matrix}$ is primary propyl glycol. A diatomic alcohol is analogous to the oxide of a dibasic metal, such as $Ca(OH)_2$.

Again, we find the triatomic alcohol having the general formula $C_nH_{2n+2}O_3$, of which the best example is glycerine, of great importance in connection with the chemical constitution of the fats, as will be shown in another chapter. A triatomic alcohol is analogous to $Bi(OH)_3$.

Another group of bodies constitutes the *ethers* produced by treating the alcohols with dehydrating agents—

$$\underset{\text{Ethyl alcohol.}}{2C_2H_5(OH)} - H_2O = \underset{\text{Ethyl ether.}}{(C_2H_5)_2O}, \text{ or } \underset{\text{Ethyl ether.}}{C_2H_5 - O - C_2H_5}.$$

Observe also that ethylic ether $(C_2H_5)_2O$ is analogous to Na_2O, sodium oxide; and ethylene ether, C_2H_4O, to CaO, calcium oxide; and glycerine ether $(C_3H_5)_2O_3$, to Bi_2O_3, bismuthic oxide.

By the oxidation of the alcohols, another group named *aldehydes* is formed. They are bodies intermediate between alcohols and acids, and they may be described as hydrocarbons in which the monatomic radicle group COH replaces H (Hydrocarbon, CH_4)—

$$\underset{\text{Ethyl alcohol.}}{C_2H_5(OH)} + O = \underset{\text{Ethyl aldehyde.}}{CH_3.COH} + H_2O.$$

Further oxidation produces the corresponding *acids*, which may be regarded as compounds of the same radicles with CO . OH—

$$\underset{\text{Ethyl aldehyde.}}{CH_3.COH} + O = \underset{\text{Acetic acid.}}{CH_3.COOH}.$$

Thus each alcohol is related to a monobasic acid, and we have COH . OH, formic acid; $C_2OH_3.OH$, acetic acid; $C_3OH_5.OH$, propionic acid; $C_4OH_7.OH$, butyric acid; $C_5OH_9.OH$, valerianic acid; and $C_6OH_{11}.OH$, caproic acid. These acids form *metallic salts*, *compound ethers*, *amides*, and *haloid derivatives*. Thus, to form a salt—

$$2\underset{\text{Acetic acid.}}{(CH_3.CO.OH)} + K_2CO_3 = 2\underset{\text{Acetate of potash.}}{(CH_3.CO.OK)} + CO_2 + H_2O.$$

By acting on ethylic hydrate with acetic acid, *acetic ether* is formed, thus—

$$\underset{\text{Acetic acid.}}{CH_3.CO.OH} + \underset{\text{Ethyl alcohol.}}{C_2H_5.(OH)} = \underset{\text{Acetic ether.}}{CH_3CO.OC_2H_5} + H_2O.$$

The *compound ethers* in turn, when acted on by ammonia, yield *amides* and the corresponding alcohol—

$$\underset{\text{Acetic ether.}}{CH_3CO.OC_2H_5} + NH_3 = \underset{\text{Monacetamide.}}{CH_3CO.NH_2} + \underset{\text{Ethyl alcohol.}}{C_2H_5(OH)}.$$

Lastly, *amines* may be regarded as hydrocarbons in which $n(NH_2)$ are substituted for nH., and they are termed monamines, diamines, or triamines, according as they are related to one, two, or three molecules of ammonia, thus—

$N \left| \begin{matrix} C_6H_5 \\ H \\ H \end{matrix} \right.$ Phenylamine or Amidobenzine, a Monamine.

$N_2 \left| \begin{matrix} C_6H_4 \\ H_4 \end{matrix} \right.$ Phenylenediamine or Diamidobenzine, a Diamine.

$N_3 \left| \begin{matrix} C_6H_3 \\ H_6 \end{matrix} \right.$ Triamidobenzine, or a Triamine.

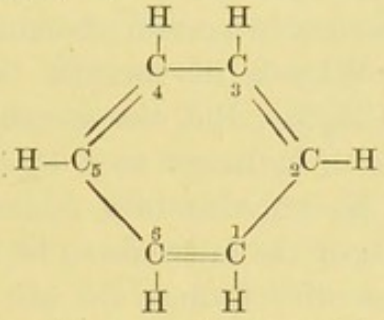
It will now be instructive to consider shortly how the chemist is enabled to give definite formulæ for complex organic bodies, so that we may understand what such formulæ imply.

At one time the structure of an organic compound was explained by the assumption of the existence of hypothetical groups of atoms or radicles which were made to take their place in various so-called types, such as the type of water, of ammonia, or of marsh gas. It is now, however, recognized that the nature of any compound is completely determined by the *atomic* constitution of its molecules. Suppose, for example, a substance is known to contain 3 atoms of carbon, 7 atoms of hydrogen, and 1 atom of chlorine in each molecule, that is C_3H_7Cl, there are at least two ways in which these might be arranged to represent two possible compounds. We might have the arrangement—

(1) $\begin{matrix} & H & H & H & \\ & | & | & | & \\ H- & C- & C- & C- & Cl, \\ & | & | & | & \\ & H & H & H & \end{matrix}$ or (2) $\begin{matrix} & H & H & H & \\ & | & | & | & \\ H- & C- & C- & C- & H. \\ & | & | & | & \\ & H & Cl & H & \end{matrix}$

A third body is impossible according to theory, and this is corroborated by experience, and the first body is known to be orthopropyl chloride and the second isopropyl chloride, bodies presenting certain chemical differences, but isomeric. Again, take C_2H_5Cl; this can be represented only under one possible form, namely $\begin{matrix} & H & H & \\ & | & | & \\ H- & C- & C- & Cl \\ & | & | & \\ & H & H & \end{matrix}$; there should be only one chloride of ethyl, as is the case. In like manner we find only one body, ethyl alcohol, C_2H_6O, having the arrangement of atoms $\begin{matrix} H & H \\ | & | \\ H-C-C-OH \\ | & | \\ H & H \end{matrix}$, but it does not follow that we have only one body with the formula C_2H_6O, and we accordingly find $\begin{matrix} H & & H \\ | & & | \\ H-C-O-C-H, \\ | & & | \\ H & & H \end{matrix}$

methyl ether. A formula is said to give the correct grouping of the atoms, if it indicates correctly how a body may split up into two or more portions and how it may be synthetically constructed. It usually accounts for all we know of the chemical nature of a substance, but it often expresses a great deal more than we know has been experimentally verified. Thus, take Benzol, C_6H_6; it is clear that the 6 atoms of C might be strung together in many ways so as to leave six surplus affinities for the 6 atoms of hydrogen. There are more than 30 different possible arrangements by which this might be accomplished, but only *one* benzol is known to exist. According to Kekulé the constitution of benzol is—



—that is, the 6 carbon atoms exist in a closed ring and every two neighbouring C's are united alternately by a single and a double band. According to this hypothesis there should be only one C_6H_5Cl, but there may be three $C_6H_4Cl_2$, according as to whether the chlorines are placed in the relative positions 1 and 2, or 1 and 3, or 1 and 4. That such bodies, and only such, exist has been proved experimentally.

Consideration will show that as the number of atoms increases the difficulty becomes greater and greater of determining what arrangements are possible and what are not possible, and as pointed out to me by Professor Dittmar, we now want a *law of limitation*, by which it may be possible to determine what arrangements exist naturally and what cannot exist.[1]

We are now in a position to understand what the requirements of the chemist are before he can construct a formula indicating the structure of an organic body. Such formulæ, when rightly understood, do not pretend to express the arrangement of the atoms in the body. Of that nothing is known. They only tell how the body will probably behave in various chemical reactions, how it may, as it were, fall to pieces, and how it may be possibly reconstructed. Chemical formulæ are a symbolic language expressing certain ideas in the mind of the chemist, and they should never be interpreted as dealing with the atomic constitution of the body being discussed.

[1] "What we call 'chemical constitution' is the constitution not of the body, but of its formula." (Dittmar.)

To determine a chemical formula the chemist must know (1) the percentage elementary composition of the body as ascertained by analysis;[1] and (2) the relative atomic weights of H, O, C, and N, namely 1, 16, 12, and 14; (3) then, from the percentage composition and the relative atomic weights the empirical formula is obtained, which expresses the simplest ratio of the weights of the elements entering into the composition of the substance; and (4) the specific gravity of the substance in a perfectly gaseous condition is, if possible, determined, and it is well known that the specific gravity is proportional to the molecular weight. From these data the formula is constructed, and then the possible arrangement of the atoms into groups is decided by the behaviour of the substance with reference to certain chemical reactions. Take, for example, acetic acid. Why is its formula $C_2H_4O_2$? Empirically it might be CH_2O, or $n(CH_2O)$. But the specific gravity of acetic acid vapour is 30, taking that of hydrogen as = 1; hence as the sp. gr. of a molecule of hydrogen, $H_2 = 2$, that of a molecule of acetic acid = 60, which shows the formula of the molecule to be, not CH_2O, but $C_2H_4O_2$. Again, acetic acid forms with sodium the salt acetate of soda, and in the reaction one fourth of the hydrogen is expelled for each equivalent of the sodium. As H_2 cannot be divided into fourths, it is clear that both the C and the H_2 must be doubled and that the formula of the molecule is $C_2H_4O_2$. In like manner, when chlorine is passed through acetic acid, chloracetic acid is formed, and for each atom of Cl one fourth of the hydrogen is displaced, again pointing to the molecular formula $C_2H_4O_2$. Apply the same mode of observation and reasoning as to the amount of O in acetic acid. When acted on by pentachloride of phosphorus half of the oxygen is removed to form chloride of acetyl, whilst the other half goes with the phosphorus, thus—

$$C_2H_3O.HO + PCl_5 = C_2H_3O.Cl + HCl + POCl_3.$$

Thus there must be more than 1 atom of O and not less than 2. Lastly, as to the number of atoms of carbon. When acetate of soda is heated with caustic potash, carbonate of potash is formed and marsh gas escapes. The C is thus parted, one half going to the carbonate and the other to the marsh gas. Consequently there must be at least 2 atoms of C. In the case of acids generally, as is well illustrated with reference to acetic acid, another method is to prepare the silver salt, and taking Ag = 108, to determine the relative quantities of C, H, and O, in combination with one atom of silver. In this case for 108 parts of silver, we find C = 24, that is 12×2; H = 3, that is 1×3; and O = 32, that is 16×2. Hence

[1] Details as to the methods of organic analysis may be found in any standard chemical work.

acetate of silver is $C_2H_3AgO_2$, or $n(C_2H_3AgO_2)$. But acetic acid is monobasic, and there is only one potassium, sodium, or silver salt, hence $C_2H_3AgO_2$ must be the formula of the salt, and $C_2H_4O_2$ that of the acid.

Lastly, and of the greatest importance, the chemist must study the decompositions of the body in question, and observe how the molecule divides and the number of atoms going into each portion. Suppose acetic acid to be burnt, it is resolved into H_2O and CO_2. This reaction might be explained on the supposition of the molecule being CH_2O as well as $C_2H_4O_2$ for $CH_2O + O = CO_2 + H_2O$. But as already pointed out, in the case of the formation of salts, one fourth of the hydrogen is replaced by K, or Na, or Ag; hence 4 and not 2 atoms of hydrogen must be present. As this is the case, we might represent the formula of acetic acid thus—$(C_2H_3O_2)H$, that is, placing the replaceable hydrogen outside. Again, in the formation of chloride of acetyl by the action of pentachloride of phosphorus, we found that 2 atoms of O existed, and that 1 of these went off in the phosphorous compound $POCl_3$. We might therefore place this O also outside, and the formula for acetic acid would become $(C_2H_3O)O.H$. This formula explains at once the constitution of anhydrous acetic acid $(C_2H_3O)—O—(C_2H_3O)$. Again, in the formation of marsh gas from acetic acid, we must split up the carbon group—thus $(CO.CH_3)OH$ becomes the formula. We have thus seen that there may be four ways of representing the composition of acetic acid according to the view necessary to account for a given reaction—(1) $C_2H_3O_2$; (2) $(C_2H_3O_2)H$; (3) $(C_2H_3O)OH$; and (4) $(CO.CH_3)OH$. This shows very clearly both how much and how little is indicated by the chemical formula of an organic substance. Sometimes an apparently anomalous case occurs that is puzzling to the chemist. For example, acetone is formed by the distillation of acetate of baryta, and it is found that three fourths of the carbon pass into acetone. Hence C_2 would not be sufficient and C_4 is the necessary number of atoms. This would make $C_4H_8O_4$ the formula of acetic acid, contrary to all the other observations and inferences. But it is more likely that in the process 2 molecules of acetic acid are involved, that is—

$$\left.\begin{matrix} C_2H_3O_2ba \\ C_2H_3O_2ba \end{matrix}\right\} = ba_2CO_3 + C_3H_6O.$$

The two ba = 1 Ba, and this suggests of course that two molecules of acetic acid are united to Ba.

These are the general considerations that guide the chemist in constructing formulæ. It can hardly be too strongly stated that when we speak of the chemical constitution of a substance we refer more

to the formula representing it than to the real molecular structure of the body itself.

The following quotation from Schorlemmer gives succinctly some of the leading properties of the carbon compounds (Schorlemmer, *Chemistry of Carbon Compounds*, p. 44).

"Most carbon compounds are colourless when in the pure state; but there exist also a great number having characteristic colours, and many of these are used as dye stuffs or for the preparation of pigments, such as indigo, the colours of madder-root, cochineal, aniline colours, etc. It appears that the colour of these bodies depends on their chemical constitution.

Odour and Taste. The odours of volatile carbon compounds vary very much, as the following examples show:—Spirits of wine, ether, acetic ether, acetic acid, chloroform, camphor, oil of cloves, etc. Compounds having a similar constitution often possess a similar smell. Thus the marsh gas hydrocarbons all possess a faint smell of flowers, which is more or less perceptible according to the volatility of the body. The compound ethers of the fatty acids smell like various kinds of fruits, and are on that account used by confectioners and perfumers. Most sulphur compounds, in which this element is not combined with oxygen, are characterized by their disagreeable odour, and many chlorides have a smell similar to that of chloroform. Relations between odour and chemical constitution certainly exist; but only a few such are known. Thus the amines or compound ammonias have an odour resembling that of ammonia, and many aldehydes, compounds which readily absorb oxygen from the air, have a peculiar suffocating smell.

The taste of carbon compounds is equally as varying as their odour; we find here also that analogous constitution produces a similar taste, and the alkaloids, as quinine, strychnine, etc., have an intensely bitter taste, whilst the taste of the alcohols of polygenic radicals, as glycerine, mannite, and sugar, is pleasantly sweet.

Solubility. A great number of carbon compounds are soluble in water, others only in alcohol, ether, acetic acid, benzine, etc. These different solvents are made use of in separating and purifying them. In homologous series the first members are generally more soluble in water than the higher ones. Thus in the series of the alcohols and fatty acids, the lower members are miscible with water in all proportions, whilst those following next dissolve only in certain proportions, and the highest are insoluble in water. All hydrocarbons are either very sparingly soluble or quite insoluble in water; by replacing in them a part of the hydrogen by hydroxyl or oxygen, compounds are formed which are more soluble, generally in proportion to the more oxygen they contain. Thus butyl, C_4H_{10}, is almost insoluble in water; butyl alcohol, $C_4H_9(OH)_2$, readily soluble; and butylene alcohol, $C_4H_8(OH)_2$, mixes with water in all proportions. Succinic acid, $C_4H_6O_4$, is more soluble than butyric acid, $C_4H_8O_2$; and malic acid, $C_4H_6O_5$, is very deliquescent."

It remains now to give in this chapter a classification of the organic compounds met with in the body. Two classifications might be given —the one based on chemical, the other on physiological considerations. Thus, one might classify the substances under the headings of the various series of hydrocarbons, but as not a few of the substances

of interest to physiologists cannot yet have their place accurately fixed, I prefer to give a physiological classification—that is, one in which they can be grouped according to the rôle they perform in the general economy.

I. *THE NITROGENOUS* include—

I. THE PROTEIDS, OR ALBUMINOIDS. No formula can be given.

A. TRUE ALBUMINS:

1. *Albumins*—(1) Serum albumin (blood), and (2) egg albumin (white of egg).
2. *Globulins*—(1) Vitellin (yolk of egg), (2) myosin (muscle), (3) paraglobulin (blood), and (4) fibrinogen (blood).
3. *Fibrin* (blood clot).
4. *Proteins*—(1) Casein (milk), (2) alkali albumin, and (3) syntonin (muscle).
5. *Peptones*—(1) Albumin peptones, (2) gelatin peptones (both digestive products).
6. *Crystallizable Albuminoids*—(1) Hæmoglobin (colouring matter of blood), (2) Vitellin plaques (?).
7. *Soluble Ferments*—(1) Ptyalin (saliva), (2) Pepsin (gastric juice), (3) Pancreatin (pancreatic juice), (4) Tripsin (pancreatic juice), (5) Inversive ferment (saliva ?), (6) Rennet (stomach of calf), (7) Lactic ferment (intestines), (8) Steatolytic ferment (pancreas), (9) Blood ferment (blood).

B. ALBUMINOUS DERIVATIVES:

1. Obtained by change of physical conditions or by chemical action from tissues—(1) Paralbumin (cysts), (2) Colloid matter (diseased liver, etc.), (3) Amyloid matter (diseased liver, kidney, etc.), (4) Mucin (mucus), (5) Nuclein (nuclei of cells), (6) Spermatin (semen).
2. Developed in tissues and obtained by boiling, etc.—(1) Collagen (yielding gelatin), (2) Chondrigen (yielding chondrin), (3) Elastin (from elastic tissue), (4) Keratin (from epidermis, etc.).

II. FATTY NITROGENOUS MATTERS—

1. Phosphoglyceric acid, $C_3H_9PO_6$, nervous matter.
2. Cholin, or neurin, $C_5H_{15}NO_2$, bile, etc.
3. Lecithin, $C_{44}H_{90}NPO_9$, in nervous tissues, blood corpuscles, yolk of egg, etc.
4. Cerebrin, $C_{19}H_{33}NO_3$, in nervous tissues.

III. AMINES—

1. Urea, CH_4N_2O, urine, etc.
2. Oxaluric acid, $C_3H_4N_2O_4$, urine.
3. Allantoin, $C_4H_6N_4O_3$, embryonic fluids.

IV. AMIDES—

1. Glycocolle, or glycin, $C_2H_5NO_2$, bile, etc.
2. Leucin, $C_6H_{13}NO_2$, pancreas, spleen, intestinal canal, etc.
3. Tyrosin, $C_9H_{11}NO_3$, pancreas, intestinal canal, etc.

4. Creatin, $C_4H_9N_3O_2$, muscles, etc.
5. Creatinin, $C_4H_7N_3O$, urine, etc.
6. Taurin, $C_2H_7NSO_3$, muscles, lungs, fæces.
7. Cystin, $C_3H_7NSO_2$, urine, etc.
8. Sarcosin, $C_3H_7NO_2$, muscle.

V. NITROGENOUS ACIDS—

1. Sulphocyanic acid, CNHS, saliva.
2. Uric acid, $C_5H_4N_4O_3$—and its derivatives, Parabanic acid, $C_3N_2H_2O_3$; Alloxan, $C_4H_2N_2O_4$; Guanin, $C_5H_5N_3O$; Sarcin, or hypoxanthin, $C_5H_4N_4O$; Xanthin, $C_3H_4N_4O_2$; Carnin, $C_7H_8N_4O_3$—urine, etc.
3. Hippuric acid, $C_9H_9NO_3$, urine.
4. Inosinic acid, $C_{10}H_{14}N_4O_{11}$, muscle.
5. Cryptophanic acid, $C_{10}H_{18}N_2O_{10}$, urine.
6. Bile Acids—(1) Glycocholic acid, $C_{26}H_{43}NO_6$.
   (2) Taurocholic acid, $C_{26}H_{45}NSO_7$.
   (3) Cholalic acid, $C_{24}H_{40}O_5$, and its derivative, dyslysin, $C_{24}H_{36}O_3$.
   (4) Choloidic acid, $C_{24}H_{38}O_4$.

VI. SALTS formed by union of organic acids and inorganic bases. These will be discussed under the heads of their respective acids.

1. Hippurate of soda, $C_9H_8NaNO_3$, urine.
2. Hippurate of lime, $C_9H_7CaNO_3$, urine.
3. Urate of soda, $C_5H_3NaN_4O_3$, urine.
4. Urate of potash, $C_5H_3KN_4O_3$, urine.
5. Oxalate of lime, C_2HCaO_4, urine.
6. Glycocholate of soda, $C_{26}H_{42}NaNO_6$, bile.
7. Taurocholate of soda, $C_{26}H_{44}NaNSO_7$, bile.
8. Sulphocyanide of potassium, CNKS, saliva.
9. Phenolsulphate of potash, $C_6H_5O.KSO_3$, urine.

VII. NITROGENOUS BODIES CONTAINING NO OXYGEN—

1. Trimethylamine, C_6H_9N, urine.
2. Naphthylamine, $C_{10}H_9N$, urine, fæces.
3. Indol, C_9H_7N, fæces, etc.
4. Skatol, C_9H_9N, fæces.
5. Pyrrol, CH_3N, fæces.

VIII. PIGMENTS—

1. Blood Pigments: (1) Hæmoglobin, no formula; (2) Hæmatin, $C_{34}H_{34}N_4FeO_5$; (3) Hæmatoidin.
2. Bile Pigments: (1) Bilirubin, $C_{16}H_{18}N_2O_3$; (2) Biliverdin, $C_{16}H_{18}N_2O_4$; (3) Choletelin, $C_{16}H_{18}N_2O_6$; (4) Bilifuscin, $C_{16}H_{20}N_2O_4$; (5) Biliprasin, $C_{16}H_{22}N_2O_6$; (6) Hydrobilirubin, $C_{32}H_{44}N_4O_7$.
3. Urine Pigments: (1) Urobilin, $C_{32}H_{40}N_4O_7$; (2) Indican, $C_{26}H_{31}NO_{17}$.
4. Other Pigments: (1) Lutein, yolk of egg, etc.; and (2) Melanin, eye, etc. (no formula).

II. *THE NON-NITROGENOUS* include—

I. ALCOHOLS: (1) Ethylic alcohol, $C_2H_5(OH)$, urine, etc.; (2) Cholesterin, $C_{26}H_{44}O.H_2O$, bile, nervous tissues, etc.; (3) Glycerine, $C_3H_5(OH)_3$, intestine, etc.; (4) Phenol, C_6H_6O, fæces, urine, etc.

II. FATS: (1) Tristearin, $C_3H_5(O.C_{18}H_{35}O)_3$; (2) Tripalmitin, $C_3H_5(O.C_{16}H_{31}O)_3$; (3) Triolein, $C_3H_5(O.C_{18}H_{33}O)_3$; (4) Soaps, such as tristearates, palmitates, and oleates of potash and soda.

III. CARBOHYDRATES—

1. *Glucoses*, $C_6H_{12}O_6$, : (1) Dextrose, (2) Chondroglucose, (3) Levulose, (4) Mannitose, (5) Galactose, and (6) Inosite (muscle sugar).
2. *Sucroses*, $C_{12}H_{22}O_{11}$: Sucrose (cane sugar), (2) Lactose (milk sugar), (3) Maltose.
3. *Amyloses*, $(C_6H_{10}O_5)_2$: (1) Starch, (2) Glycogen, (3) Dextrin, (4) Inulin, (5) Gums, and (6) Cellulose.

IV. THE NON-NITROGENOUS ACIDS—

1. *Acetic Acid Series*, $C_nH_{2n}O_2$: (1) Formic acid, CH_2O_2; (2) Acetic acid, $C_2H_4O_2$; (3) Propionic acid, $C_3H_6O_2$; (4) Butyric acid, $C_4H_8O_2$; (5) Valerianic acid, $C_5H_{10}O_2$; (6) Caproic acid, $C_6H_{12}O_2$; (7) Œnanthylic acid, $C_7H_{14}O_2$; (8) Caprylic acid, $C_8H_{16}O_2$; (9) Pelargonic acid, $C_9H_{18}O_2$; (10) Capric acid, $C_{10}H_{20}O_2$; (11) Laurostearic acid, $C_{12}H_{24}O_2$; (12) Myristic acid, $C_{14}H_{28}O_2$; (13) Palmitic acid, $C_{16}H_{32}O_2$; (14) Margaric acid, $C_{17}H_{34}O_2$; (15) Stearic acid, $C_{18}H_{36}O_2$.
2. *Glycollic Acid Series*, $C_nH_{2n}O_3$: (1) Carbonic acid, CH_2O_3; (2) Glycollic acid, $C_2H_4O_3$; (3) Lactic acid, $C_3H_6O_3$; (4) Oxybutyric acid, $C_4H_8O_3$; (5) Oxyvalerianic acid, $C_5H_{10}O_3$; (6) Oxycaproic acid, $C_6H_{12}O_3$.
3. *Oxalic-Acid Series*, $C_nH_{2n-2}O_4$: (1) Oxalic acid, $C_2H_2O_4$; (2) Malonic acid, $C_3H_4O_4$; (3) Succinic acid, $C_4H_6O_4$; (4) Adipic acid, $C_6H_{10}O_4$; (5) Pimelic acid, $C_7H_{12}O_4$; (6) Suberic acid, $C_8H_{14}O_4$; (7) Sebacic acid, $C_{10}H_{18}O_4$.
4. *Oleic Acid Series*, $C_nH_{2n-2}O_2$: (1) Acrylic acid, $C_3H_4O_2$; (2) Crotonic acid, $C_4H_6O_2$; (3) Angelic acid, $C_5H_8O_2$; (4) Oleic acid, $C_{18}H_{34}O_2$.

CHAP. III.—THE PROTEIDS OR ALBUMINOIDS.

A. CHEMICAL CHARACTERS.

These organic compounds of complex chemical constitution are the most important of all proximate principles, inasmuch as none of the phenomena characteristic of life can occur without their presence. They form the substratum of all tissues, and especially are they found in protoplasm, forming the chief mass of the nitrogenous matter of plants and animals. As a type of these substances we may take egg albumin, a viscous, non-crystalline, colourless, inodorous, tasteless substance, which analysis shows to consist of carbon, hydrogen,

oxygen, nitrogen, and sulphur. The name proteid (πρωτεῖον, pre-eminence) was first given by Mulder to a substance he obtained by the action of potash on albuminous matter, and he considered it to be the basis or radicle of all albuminous substances, with the hypothetical formula $C_{36}H_{26}N_4O_{10}$. It has been shown, however, that no such definite chemical compound exists, and that what Mulder obtained was merely albuminous matter more or less modified. The composition of the proteids, according to Hoppe-Seyler, varies between the following numbers—

	C	H	N	S	O
From	51·5	6·9	15·2	0·3	20·9
To	54·5	7·3	17·0	2·0	23·5

In addition, they always contain a small quantity of the chlorides and phosphates of the alkalies, however carefully they may have been purified. Considerable difference of opinion still exists among chemists as to the constitution of these bodies. The view of Mulder, above alluded to, that all the proteids contain the same radicle, *protein*, is now abandoned. Nasse has attempted to get an insight into their molecular construction by studying experimentally the mode in which the nitrogen exists in them. He treated various proteids in the dried and powdered state with caustic baryta for 40 or 50 hours and examined the products of decomposition. The conclusion arrived at is that part of the nitrogen is held firmly, whilst part can easily be displaced, showing that it exists in different states of combination. Comparing the action of caustic baryta on albumin with its action on compounds, the molecular structure of which is fairly well known, such as amines, amides, or leucin, Nasse thinks that "a definite quantity of the loosely-combined nitrogen of proteids is combined as in amides; another part as the nitrogen in creatin or the more loosely-held portion of the nitrogen of uric acid; and most of the remainder as in the acid amides and the difficultly expulsible nitrogen of creatin or sarcosin." [1]

More extended researches in a similar direction have been made by Schützenberger who heated coagulated albumin or other proteids with caustic baryta for several hours. Ammonia and acetic acid were thus obtained, and a friable light yellow residue remained, to the extent of 96 per cent. of the albumin used. It would appear in the first instance that the action of the baryta caused a fixation of water and a decomposition into (1) the fixed residue in which a portion of the nitrogen is firmly combined; (2) volatile products, such as pyrrol, indol, etc.; (3) ammonia, the nitrogen of which is about $\frac{1}{4}$ part of the total

[1] Watt's *Dict. of Chemistry*, vol. vii. p. 1023. Nasse's Experiments—*Studien über die Eiweisskörper*. Pflüger's *Archives*, vi. p. 589.

nitrogen in the albumin; (4) carbonic acid; (5) fatty acids, more especially acetic acid; and (6) oxalic acid. The following equation illustrates this decomposition—

$$\underset{\text{Albumin.}}{C_{240}H_{392}N_{65}O_{75}S_3} + 57H_2O = \underset{\text{Fixed residue.}}{C_{219}H_{431}N_{48}O_{106}} + \underset{\text{Ammonia.}}{16NH_3} + \underset{\text{Oxalic acid.}}{3C_2H_2O_4} + \underset{\text{Carbonic acid.}}{3CO_2} + 4C_2H_4O_2 + C_4H_5N.$$

Acetic acid. Pyrrol.

The fixed residue, by the prolonged action of the baryta and a high temperature, yields (1) tyrosin, $C_9H_{11}NO_3$; (2) amido-acids of the fatty series, such as leucin (amido-caproic acid), butalanin (amido-valerianic acid), alanin (amido-propionic acid); (3) glutamic and aspartic acids; (4) glutimic acid; (5) tyroleucin; (6) leuceïns, which are combinations of leucins and tyroleucins; (7) acids of the general formula $C_{2n}H_{2n-1}NO_6$; and (8) gluco-proteins, bodies of a sweetish taste, having the formula $C_n^1H_{2n}N_2O_4$ ($n = 10$ or 12). The bodies most easily obtained were tyrosin and leucin, the former to the amount of from 2·3 to 3·5 per cent. The leucins, having the general formula $C_nH_{2n+1}NO_2$, and the leuceïns, having the general formula $C_nH_{2n-1}NO_2$, are derived from the splitting up of the gluco-proteins. The longer the action of the baryta is continued the greater is the proportion of leucins and leuceïns formed, but at the beginning of the process, and whilst the solution is diluted, gluco-proteids form the chief part of the fixed residue. These in turn are split up into leucins and leuceïns, and into intermediate compounds of gluco-proteins with leucins and leuceïns. Thus, according to Schützenberger, albumin is an amido-derivative, a ureide, which by hydration splits up into a large number of amido-derivatives.[1]

The researches of the chemists have been conducted of course on dead albumin. But, as already pointed out, the dead state is very different from the living, and we must beware of drawing conclusions as to the chemical condition of living matter from an analysis of the substance after death. This was emphasized so long ago as 1837 by John Fletcher, a very suggestive writer, whose speculative opinions are still well worthy of study;[2] but it was not until 1875 that the chemical difference between living and dead protoplasm was again pressed on the attention of physiologists by E. Pflüger.[3] Dead albumin, more especially in the dry state, can be kept without change for a great length of time;

[1] Schützenberger, *Recherches sur l'Albumine et les Matières Albuminoïdes, Bulletin de la Soc. Chemique*, v. 23 and 24. Also Watt's *Dict. of Chem.* v. viii. p. 1682.

[2] Fletcher, *Rudiments of Physiology*. Edinburgh, 1837.

[3] Pflüger. See his *Archives*, vol. x. p. 251.

it is indifferent to oxygen, and it has the character of chemical stability. But when it becomes living all those characters are reversed. It is now unstable, readily decomposing into simpler substances, and, as has been well said, "the molecule of albumin begins to live by breathing oxygen."[1] Pflüger supposes that when assimilated the nitrogen of the proteid matter passes from the state of more or less stable amides in the dead condition to the more unstable condition in which it exists in cyanogen and its compounds. Further, Pflüger showed that no better proof could be given of the difference between the constitution of the dead and of the living molecule of albumin than the affinity of the living state for oxygen. He introduced living frogs into chambers containing no oxygen and kept at a low temperature, and observed that all the processes of life continued for many hours, that is to say there was constant self-oxidation. By a rearrangement of the atoms or groups of atoms of the molecule of dead proteid matter it becomes alive. It has a strong affinity for oxygen, and it may then split up into simpler bodies, some of which do not exist among the decomposition products of dead proteid matter. Whether Pflüger's conjecture, that the constitution of living proteid matter is that it contains cyanogen radicles in which the nitrogen is loosely combined, be true or not, there can be no doubt his mode of viewing the subject has been of great value to science.

Following in the same direction are the able researches of Loew and Bokorny.[2] Approaching the subject from the point of view of chemists interested in the chemical changes in the plant, they made the remarkable discovery that living protoplasm (or proteid matter) has the property of reducing silver from a weak alkaline solution of silver nitrate, whilst dead proteid matter has no such effect. The observation was made with the protoplasm of various algæ, but the results were not so satisfactory with the protoplasm of the higher plants, and still less so with that of animal tissues. This is accounted for by Loew and Bokorny by the statement that animal protoplasm is extremely sensitive to even weak silver solutions, quickly dying, and that thus the usual reaction does not occur. By careful experiment and reasoning, they show that the effect is not due to anything in the vegetable cell except protoplasm, and they then inquire what substance of a chemical nature in the protoplasm would have such a reducing effect. They come to the conclusion that in the protoplasm there is something of the nature of an aldehyde. They then direct attention to the formation of proteid matter in plants, showing that these absorb

[1] Gamgee, *Physiological Chemistry*, vol. i. p. 22.

[2] Oscar Loew and Thomas Bokorny, *Die Chemische Kraftquelle im Lebenden Protoplasma*. Munich, 1882.

inorganic nitrogen-compounds, such as nitrates and sulphates of ammonia. These are probably decomposed in the plant by the action of the salts of organic acids, setting free nitric and sulphuric acids, and these in turn may be further split up, so as to supply the nitrogen and sulphur required in the formation of proteids. Further, Loew and Bokorny start with formic aldehyde, and by uniting it with ammonia they produce a body termed the aldehyde of aspartic acid.[1] Aspartic acid, $C_4H_7NO_4$, is obtained by the decomposition, under the action of acids or alkalies, of asparagin, $C_4H_8N_2O_3$, a substance of the nature of an amide (the amide of succinamic acid, $C_2H_3 . NH_2 . CONH_2 . COOH$), frequently found in leaves and in young shoots kept in the dark. The accumulation of asparagin in young shoots kept in the dark is explained by supposing that in these circumstances some of the proteid already in the shoot decomposes, yielding among other substances asparagin, and that this asparagin does not meet with the appropriate non-nitrogenous matter with which it could combine to form proteid. Loew and Bokorny show the synthesis of a proteid in the following manner—

(1) $$4CHOH + NH_3 = \begin{matrix} NH_2 . CH . COH \\ | \\ CH_2 . COH \end{matrix} + 2H_2O.$$

Formic aldehyde. Ammonia. Aspartic aldehyde.

Then by polymerization of the aspartic aldehyde, we have—

(2) $$3\left\{\begin{matrix} NH_2 . CH . COH \\ | \\ CH_2 . COH \end{matrix}\right\} = C_{12}H_{17}N_3O_4 + 2H_2O.$$

By further polymerization, in the presence of a sulphur compound, we have—

(3) $$6(C_{12}H_{17}N_3O_4) + H_2S + 6H_2 = C_{72}H_{112}N_{18}SO_{22} + 2H_2O,$$

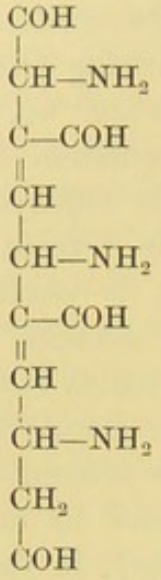
a formula representing the composition of ordinary albumin. The synthesis may also be illustrated in another manner.

Representing the aldehyde of aspartic acid thus—

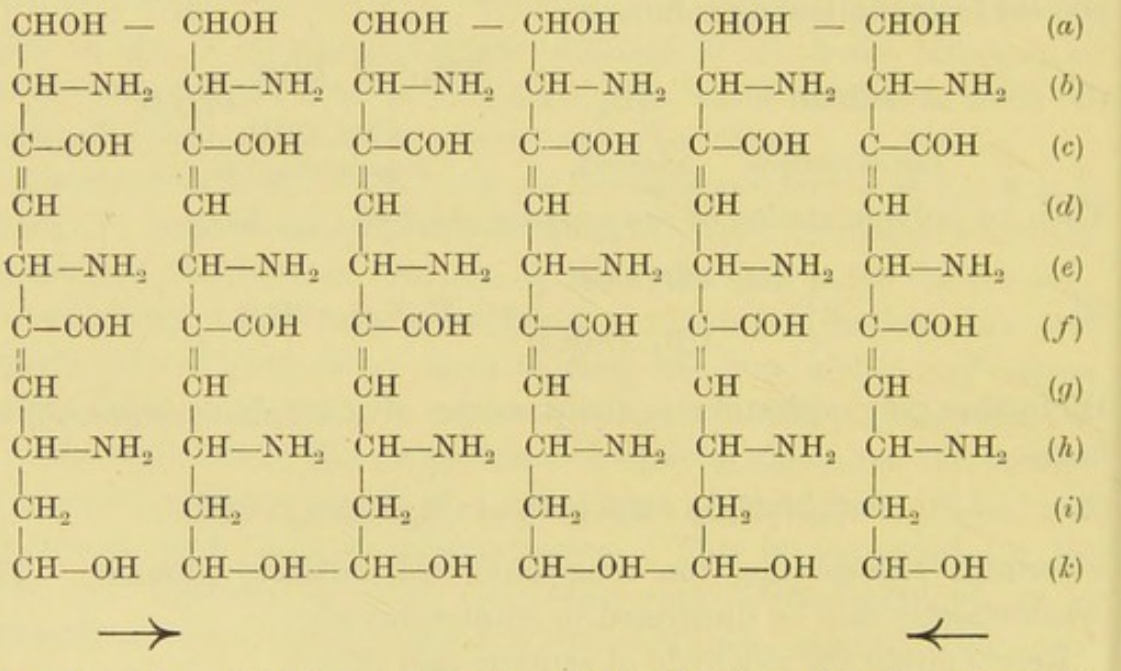
$$\begin{matrix} COH \\ | \\ CH—NH_2 \\ | \\ CH_2—COH, \end{matrix}$$

[1] The weak point in this theory is that the aldehyde of aspartic acid is unknown to chemists, but Loew and Bokorny say that it has not been diligently looked for, and that it is no doubt unstable and evanescent.

we obtain by condensation, as above, the product—



Then in a manner similar to the building up of complex bodies by successive duplications, along with condensation, we have six of the above grouped as follows to form proteid matter—

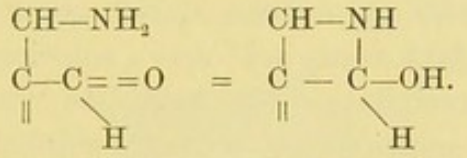


The formula of this product is $C_{72}H_{114}N_{18}O_{24}$, differing from the formula Lieberkühn gave for albumin, $C_{72}H_{112}N_{18}O_{22}$, S, by 2 atoms of H, 2 of O, and 1 of S. This ingenious formula also shows how, by cleavage of the molecule of albumin, various bodies may be produced. Thus, a carbohydrate might arise from *a* and *k*; from the rows *b*, *e*, *h*, rich in N, by taking up O, we obtain guanin, uric acid, kreatin; lines *g* and *d* constitute a benzol nucleus; from *i* a fatty acid group, which by three-fold condensation leads to stearic acid. All these substances have been obtained from proteid matter, either by the chemist in the laboratory, or as the result of physiological experiment.

Similarly, by cleavage in the vertical direction, and the absorption of water, leucin, tyrosin, and other bodies may be produced. Further, Loew and Bokorny attribute the remarkable energy of living proteid matter to the fact of the existence in it of an aldehyde group of atoms, in which molecular movements are constantly taking place. Thus, representing any monatomic radical by R, we have an aldehyde expressed by the formula, $R-\!-\!-C\overset{O}{\underset{H}{<}}$. Now, in such a compound two affinities are opposed—(1) the O to the H, and (2) the C to each of O and H. A struggle occurs between the antagonistic actions, causing much atomic movement, and one may conceive the following changes to occur in rapid succession—

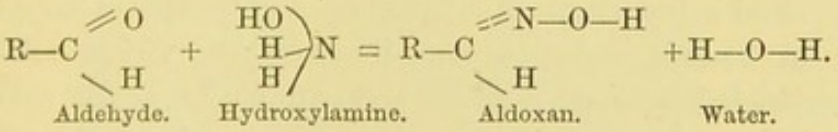
(1) $R-\!-\!-C\overset{O}{\underset{H}{<}}$, (2) $R-\!-\!-C\overset{OH}{<}$, (3) $R-\!-\!-C\overset{O}{\underset{H}{<}}$, (4) $R-\!-\!-C\overset{}{\underset{OH}{<}}$.

If the affinities in 2 and 4 be satisfied by combining with another substance, there will then be a state of comparative rest; but a neighbouring aldehyde group would probably impair the stability, and again set up molecular action. Thus—

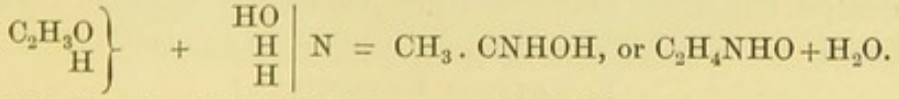


Group in active albumin. Group in passive albumin.

A striking fact in support of Loew and Bokorny's theory that active proteid matter contains a chemical substance of the nature of an aldehyde is that a weak solution of hydroxylamine quickly kills protoplasm, a result that may be explained by assuming that a compound is at once formed by the action of hydroxylamine on the aldehyde. Thus—



Aldehyde. Hydroxylamine. Aldoxan. Water.

Or, 

Acetic aldehyde. Hydroxylamine. Acetic aldoxan.

I have entered somewhat fully into these theoretical views regarding the composition of proteids because it is only by a consideration of such that we can understand how it is that when proteids are oxidized there is the simultaneous production of fatty acids, of aromatic compounds,

and of bodies analogous to urea. Further, the consideration of the complexity and the instability of the molecule leads the mind to the conception of certain forms of vital action being due to molecular movements of a chemical character.

Let us next examine some of the more obvious chemical characters of the proteids. These may be demonstrated by making a filtered solution of egg albumin (white of egg). "Only certain of the proteids are soluble in water, all are soluble with the aid of heat, in concentrated acetic acid, and in solutions of caustic alkalies; they are insoluble in cold absolute alcohol and in ether." (Gamgee, *op. cit.*) It can be readily shown that the albumin is precipitated (1) by the strong mineral acids; (2) by acetic acid and ferrocyanide of potassium; (3) by acetic acid and a large amount of a concentrated solution of any neutral salt of the alkalies or alkaline earths; (4) by basic acetate of lead; (5) by mercuric chloride; (6) by tannic acid; and (7) by alcohol. In the presence of free alkali they are slightly soluble in hot alcohol. The following special tests may then be applied—(1) Boil and then add a few drops of strong nitric acid; the precipitate formed is insoluble in nitric acid. Serum and egg albumin solutions show opalescence at a temperature of from 60° to 65° C., and coagulation occurs from 72° to 73° C. (Hoppe-Seyler.) (2) Add acetic acid and a solution of ferrocyanide of potassium; a white precipitate. (3) Acidulate with acetic acid, and add a concentrated solution of sodium sulphate; a precipitate. (4) *Millon's reaction.* Dissolve 1 part by weight of mercury in 2 parts of nitric acid of a specific gravity of 1·42, and dilute each volume of liquid with two volumes of water. Add a few drops of this solution to solution of proteid, and on heating, the fluid becomes of a purple-red colour. (5) *Xantho-protein reaction.* Add a few drops of strong nitric acid; boil; cool; and then add a few drops of solution of ammonia; a yellow colour is produced if proteid matter is present. (6) *Piotrowski's reaction.* Add a few drops of a solution of sulphate of copper and of a solution of caustic soda or potash; heat; a violet colour results. (7) *Adamkiewicz's reaction.* Add excess of glacial acetic acid and then concentrated sulphuric acid; a violet colour with feeble fluorescence is produced.

B. Physical Characters.

If a solution of albumin, such as serum-albumin, in serum of blood, which also contains saline matters and crystalline organic bodies, be placed in a dialyzer (see Figs. 19 and 20) in distilled water, it will be found that the crystalline matters pass through the parchment membrane into the water, whilst the proteid matter remains in the dialyzer.

Solutions of other proteids behave in a similar way, unless they are combined with saline matters, when a portion may pass through.

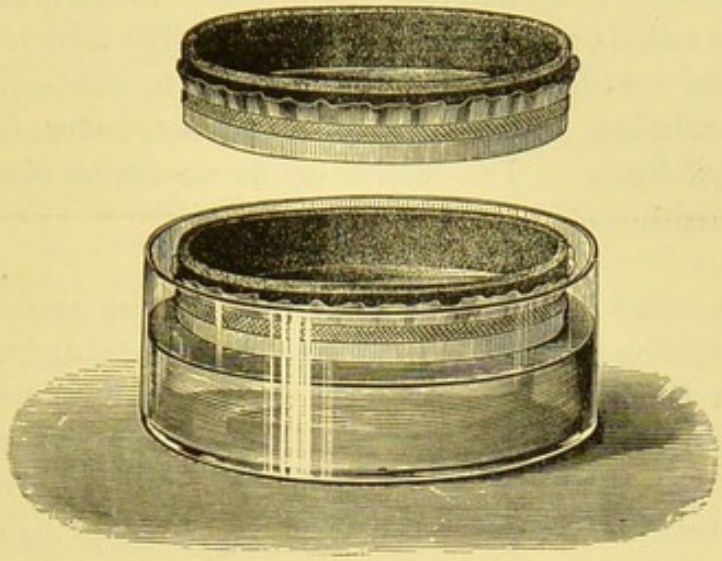


FIG. 19.—Hoop Dialyzer, consisting of a sheet of moist parchment paper stretched and tied over a ring of gutta-percha.

Proteids belong to the group of colloids, thus described in the classical researches of Thomas Graham—"Although often largely soluble in water, they are held in solution by a most feeble force. They

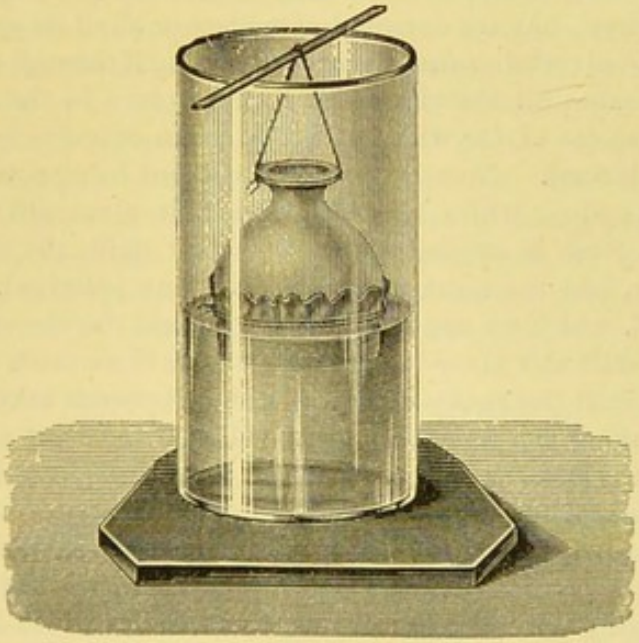


FIG. 20.—Another form of Dialyzer, in which the parchment is stretched and tied across the wide end of a bulb.

appear singularly inert in the capacity of acids and bases, and in all the ordinary chemical relations. But, on the other hand, their peculiar physical aggregation with the chemical indifference referred to, appears

to be required in substances that can interfere in the organic processes of life. The plastic elements of the animal body are found in this class. As gelatin appears to be its type, it is proposed to designate substances of the class as *colloids*, and to speak of their peculiar form of aggregation as the *colloidal condition of matter*. Opposed to the colloidal is the crystalline condition. Substances affecting the latter form will be classed as *crystalloids*. The distinction is no doubt one of intimate molecular constitution."[1]

Along with other organic bodies, solutions of the proteids have the property of rotating the plane of the rays of polarized light. A general knowledge of the principles on which the optical properties of various organic substances are examined is required of the physiological student, and as the use of the polariscope will be frequently referred to, I shall here give a brief account of the nature of polarized light, and of the structure of a Polarizing Apparatus.

The nature of polarized light is thus happily illustrated by a distinguished writer on *Physics*—

"Ordinary light consists of vibrations taking place always in planes at right angles to the direction of the ray, but in all directions in those planes. That is, if the ray travels along the axle of a wheel, the vibrations composing it are all in the plane of the wheel, but are executed along any or all of its spokes. The effect of reflecting light from certain substances, or of passing it through certain crystalline substances, is to cause all the vibrations to take place in the same direction—that is, along one spoke of the wheel and the spoke opposite to it. The light is then said to be polarized. Now, if the wheel, without being rotated, be slid along the axle, the spoke along which the vibrations take place will trace out a plane. When no rotative force is applied to the polarized light, the vibrations all take place in this plane, and the light is said to be plane polarized. If we twist the reflector or crystal, which we use as a polarizer, round the direction of the ray as an axis, we shall shift this plane in the same way as if we cause the wheel to turn on its axis and so shift the spoke along which the vibrations take place; but when the wheel is slid along the axis, this spoke will still trace out a plane—only that plane will not be the same as before. That is, if we turn the polarizing mirror or crystal, we turn the plane of polarization, but the light still remains plane polarized. We cannot detect by the eye in what plane light is polarized, or, indeed, whether or not it is polarized at all. In order to do so, we have to take advantage of the following natural law: Transparent bodies which have the power of polarizing light in any given plane are opaque to light already polarized in a plane at right angles to that plane." . . . "Thus to determine in what plane light is polarized, we have only to take a crystal which has the power of polarizing light in a certain plane fixed with regard to its axis, and to turn it round till the light

[1] See Young's edition of Graham's *Physical and Chemical Researches*, p. 553. The paper from which quotation is made appeared in *Phil. Trans.* 1861.

is extinguished. We then know that the light is polarized in a plane at right angles to that plane in the crystal." [1]

Light is readily polarized by passing it through an optical apparatus, called a *Nicol's Prism*. This consists (see Fig. 21) of two prisms of transparent calc-spar or Iceland spar *ABCD*, *CDEF*, cemented together with Canada balsam at the faces *CD*, and the faces *AB*, *EF*, cut so as to make an angle of 68° with the edges *AE*, *BF*. The incident ray *mn* is divided into two rays, both of which are polarized; the one, *no*, termed the ordinary ray, follows the ordinary laws of refraction, remaining always in the plane of incidence and having a constant index of refraction; the other, *ne*, called the extraordinary ray, being polarized perpendicularly to the principal section of the crystal, has its direction not confined to the plane of incidence, "unless that plane coincides with or is perpendicular to the principal section, and its index of refraction, excepting in the last-mentioned case, varying continually with the angle of incidence." [2] By the Nicol's prism, the ordinary ray suffers total reflection in the direction *oP*, whilst the extraordinary ray passes in the direction *ef*, and thence issues as *fg* parallel to *mn*. An eye placed at *g* would see one image formed by the extraordinary ray *nefg*. Hence only one ray passes through a Nicol's prism, that is, a ray polarized in a plane perpendicular to the principal section, or the short diagonal of the rhomb *ab*. One Nicol's prism, suitably mounted, is termed a *polarizer*, because by it light is brought into a polarized state; if now another Nicol's prism be held between the first one and the eye, it will be found that no light passes through when this second prism is so rotated as to have its principal section at right angles to that of the first, that both are perfectly transparent when their principal sections are parallel, and that light of diminished intensity is transmitted in intermediate positions. This second Nicol's prism is termed the *analyzer*, because by it the state of the light polarized by the first Nicol is determined.

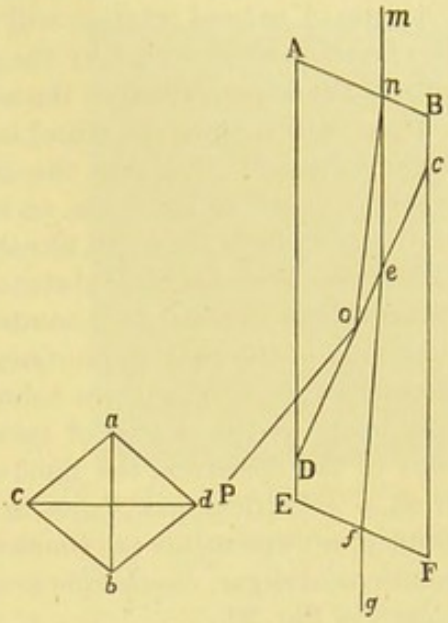


FIG. 21.—Diagram showing action of Nicol's Prism.

If now a beam of light, polarized by a Nicol's prism in a horizontal plane, be passed through a tube containing a solution of albumin or of sugar, its plane of polarization on emergence will be rotated to one side or the other of the horizontal. To understand this, we must conceive the case of two rays of light of the same amplitude travelling along the same path, polarized so as to be at right angles to each other, and differing in phase by a quarter of a wave length, then the resultant wave would have its molecules rotating in circles transversely to the direction of the ray. This is termed *circular polarization*, and the effect is the same as "if the ray were polarized in one plane, and that plane were made to rotate round the direction of the ray as an axis, making a complete revolution during the time of one vibration." [3] Mr. Gordon gives an ingenious mechanical

[1] Gordon, *Physical Treatise on Electricity and Magnetism*, vol. ii. p. 206.
[2] Watt's *Dict. of Chem.* vol. iii. p. 654. [3] *Ibid.* vol. iii. p. 674.

illustration of this state of matters : " Let us, as before, represent the plane of polarization by the direction of the spokes of a wheel, and let us slide the wheel backward and forward on the axis, and cause it to rotate at the same time so that the spoke is always in the plane of polarization at the point where the wheel is on the axis. The case of natural rotation will then be illustrated by considering the turning of the wheel to be produced by the guiding action of a spiral or long screw thread cut on the axis. Thus, as the wheel moves along, the spoke traces out a twisted surface, while, when the wheel is slid back again, the spoke comes back along the same surface."[1] Further, the direction of the movement of the ether molecules from the centre of the circle, or in other words, the direction of the plane of polarization, may be to the right, like the movement of the hands of a watch, or to the left, the reverse direction, and substances rotating to the right are dextro-rotatory, whilst those to the left are lævo-rotatory. This effect on polarized light is due to the molecules of the body in solution, and it follows that the amount of rotation will depend on the length of the column of the solution and upon the strength of the solution. As the degree of rotation varies according to the wave lengths of the rays of the spectrum, the light chosen should be yellow rays, such as are emitted from a colourless gas flame in which common salt is volatilized. On these principles instruments are constructed termed *Polarimeters*, or when employed for solutions of sugar, *Saccharimeters*, of which a convenient form, made by Soleil, is shown in Fig. 22.

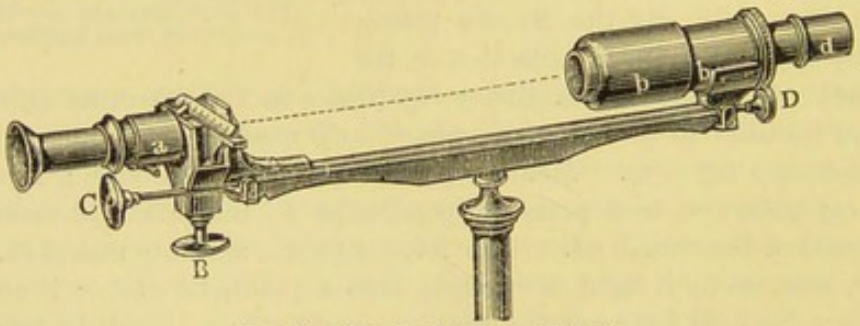


Fig. 22.—Saccharimeter of Soleil.

Suppose then, to put the matter in a simple form, that the fluid to be examined is placed in a glass tube surrounded by brass, and closed at both ends with plate glass discs, to fit watertight, screw-caps pressing firmly on the discs. The length of the column of fluid is usually 1 decimetre. The tube may be placed on the support in the position of the dotted line (Fig. 22), between two Nicol's prisms at *a* and *b*. The one Nicol is the polarizer, the other the analyzer. Set the two Nicol's prisms so that their principal sections are at right angles and then, as above explained, on looking through the apparatus at a yellow light the field will be dark so long as the tube is not interposed. When the tube is put in position the field is at once lit up, and then the analyzing prism must be rotated until the light disappears. The angle through which the analyzer has been turned measures the rotation produced by the solution in the tube. With this arrangement the difficulty is in determining when the light disappears, and to obviate this an appeal is made to the sensitiveness of the eye to colour. It is well known that when a plate of the uniaxial crystal of quartz, cut at right angles to the optic axis, is placed between two Nicol's prisms with their axes crossed (that is with the field dark) a series of brilliant colours appears—red, yellow, green, blue, according to the thickness of the quartz plate. This is because quartz exhibits

[1] Gordon, *op. cit.* p. 208.

circular polarization in a striking way. It will also be found by experiment with different colours that to cause the colour to disappear the analyzer must be rotated through a different angle for each colour, an angle constant for a given thickness of plate for each colour. Thus, with a quartz plate 3·75 mm. thick the angles of rotation are as follows :—Medium red, 56°·25 ; orange, 71°·25 ; yellow, 90° ; green, 101°·25 ; blue, 120°; indigo, 142°·5 ; and violet, 165°, as shown in Fig. 23. Observe, the angle is least for red and greatest for violet. Some varieties of quartz are dextro- whilst others are lævo-rotatory, but for a given thickness of plate in both cases the angle of rotation is the same. Experiment has shown that with a quartz plate 3·75 mm. thick, when the principal sections of the polarizer and analyzer are parallel, the red and violet rays are transmitted with only slightly diminished intensity, the resultant colour being purple, which passes quickly to red or violet on rotating the analyzer. This special tint (sensitive-tint, transition-tint, couleur sensible, teinte de passage) is used in optical arrangements as a standard tint because it is easy for the eye to determine when this tint has been obtained and when it disappears.

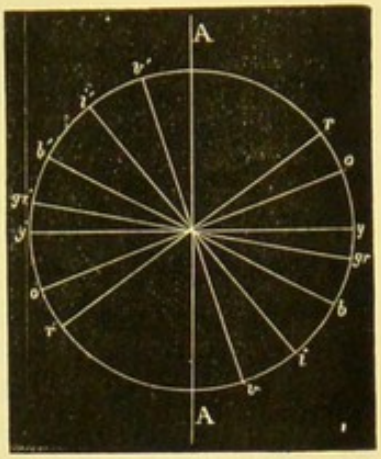


FIG. 23.—Diagram showing the angular rotation of a beam of white light (polarized by a Nicol's prism whose principal axis is parallel to A A) caused by its passage through a quartz plate 3·75 mm. in thickness. *r*-*r*′ plane of vibration of red, *o*-*o*′ of orange, *y*-*y*′ of yellow, *gr*-*gr*′ of green, *b*-*b*′ of blue, *i*-*i*′ of indigo, and *v*-*v*′ of violet.

Soleil has taken advantage of these facts in the construction of the Saccharimeter, a diagram of which is shown in Fig. 24. The light from a gas flame is

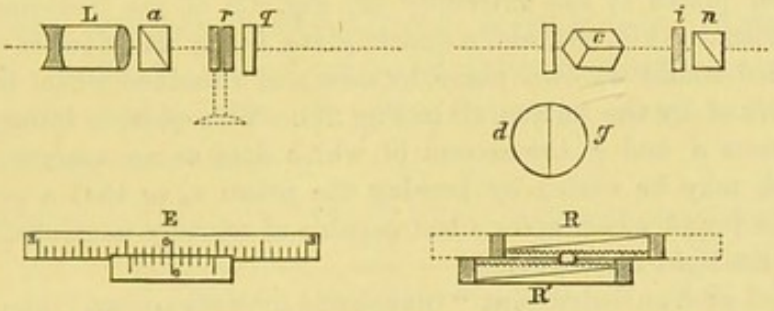


FIG. 24.—Diagram showing the optical arrangements in Soleil's Saccharimeter. For further description, see text.

polarized by a Nicol's prism, *c* (*d* in Fig. 22); and it then passes through a double plate of quartz 3·75 mm. thick (at *b* in Fig. 22). One half of this plate is dextro- (*d*), whilst the other is lævo-rotatory (*g*), as seen in diagram, *gd*. The light passes through the tube containing the solution, placed in the position of the dotted line in Fig. 22, then traverses a quartz plate *q*, cut perpendicularly to its axis, then through an arrangement called the compensator at *r*, then through the analyzer, a Nicol's prism, *a*, and lastly through a telescope L. The following description of the compensator and of the general action of the instrument is given by the late Mr. Henry Watts in the article "Light" in Watt's *Dictionary of Chemistry*, vol. iii. p. 674. (See Fig. 24.)

"RR is a horizontal section of the compensator, consisting of two quartz-prisms perpendicular to the axis, and of contrary rotation to the plate *q*. These prisms can slide one over the other horizontally and in contrary directions, so as to vary the thickness which the modified light has to traverse. They are set in motion by

a toothed pinion fixed to the button (B in Fig. 22), and acting on two racks adapted to the lower part of the mounting of the prisms. One of these mountings carries an ivory scale (seen a little to the right of a in Fig. 22), represented separately at E; the other, a vernier, which slides along the scale, and serves to measure the opposite displacement of the two prisms. When the zeros of the scale and vernier coincide the two prisms are opposite to one another, and their thicknesses are together equal to that of the plate q, the rotatory power of which is therefore neutralized by them. The analyzer may then be turned by means of an endless screw into such a position that the two halves of the double quartz plate may exhibit the sensitive tint. If the tube containing the liquid be then put in its place, the two halves gd will then exhibit very different colours; and to restore them to equality the compensator r must be so adjusted as to produce, together with the plate q, an inversion opposite to that of the liquid, either by increasing the thickness of the double prism, r, or by diminishing it, so as to allow the action of the plate q to predominate. The direction in which the vernier is moved along the rule, which is marked with two series of divisions on opposite sides of the zero-point, indicates the direction of the rotation exerted by the liquid, and the displacement of the vernier gives the angle of deviation when the thickness of quartz corresponding to one division of the scale is known. These divisions are usually made to correspond with the tenth of a millimetre; the vernier indicates a tenth of these so that the estimation may be made to the hundredth of a millimetre. The half of this quantity suffices to produce an appreciable difference of tint in the two halves of the double quartz plate. When either the light or the liquid in the tube is coloured, this colour, added to that produced by the polarization, modifies the sensitive tint and diminishes the accuracy of the observation. To neutralize this colour, Soleil places at the extremity (d, Fig. 22) of the instrument a doubly-refracting prism, n (Fig. 24), and a quartz plate, i (Fig. 24), fixed in a socket which can be turned round its own plane by means of a toothed wheel or pinion (D in Fig. 22), worked by the button (C in Fig. 22). This plate is interposed between the two prisms n and c, the second of which acts as an analyzer, and yields a colour which may be varied by turning the prism n, so that a position of this prism may be found which gives a tint capable of sensibly neutralizing that of the liquid or of the light employed."[1]

By means of such an instrument "the specific rotatory power," [a], of a solution of any substance may be determined in degrees, and the term designates "the rotation of the plane of polarized light produced by 1 gramme of substance dissolved in 1 cubic centimetre of liquid when examined in a column 1 decimetre thick." Let ϵ=the quantity of substance in a unit weight of solution; l the length of column; and a the angle of rotation, the angle of rotation for unit of length will be $\frac{a}{\epsilon l}$. But as solution may cause condensation of volume, divide the quantity $\frac{a}{\epsilon l}$ by the density, δ, of the solution. The specific rotatory power then $=\frac{a}{\epsilon l \delta}$. Suppose a solution containing 155 milligrammes of cane sugar in a gramme of water has a specific gravity of 1·06 and that the transition tint is obtained by a rotation of 24° in a column of fluid 20 centimetres long, then the specific rotatory power is

$$[a] = \frac{24}{\cdot 155 \times 20 \times 1\cdot 06} = 7°\cdot 3.$$

[1] A description of Laurent's Polarimeter, a very sensitive instrument, is given

The specific rotatory power [a] of serum albumin is $-56°$, of egg albumin $-33°{\cdot}5$ (Hoppe-Seyler) or $-38°{\cdot}08$ (Haas), of paraglobulin $-59°{\cdot}75$ (Haas), of sodium albuminate $-55°$, of syntonin, prepared by the action of dilute hydrochloric acid on myosin of muscle, $-72°$ (Hoppe-Seyler), and of casein dissolved in sulphate of magnesia $-80°$. These measurements are for yellow light near D line of spectrum, and hence represented by $[a]_D$.

C. Physiological Characters.

Proteid matters, such as albuminates, or substances related to them, are found in all the tissues and fluids. The following table from Gorup-Besanez, gives the proportion in 1000 parts—

FLUIDS.		TISSUES.	
Cerebro-spinal fluid,	0·9	Spinal cord,	74·9
Aqueous humour,	1·4	Brain,	86·3
Liquor amnii,	7·0	Liver,	117·4
Pericardial fluid,	23·6	Thymus of calf,	122·9
Lymph,	24·6	Egg,	134·3
Pancreatic juice,	33·3	Muscles,	161·8
Synovial fluid,	39·1	Middle coat of arteries,	273·3
Milk,	39·4	Cartilage,	301·0
Chyle,	40·9	Bone,	345·0
Blood,	195·6	Crystalline lens,	383·0

All are derived from the vegetable or animal substances constituting food. Ultimate analysis shows a great resemblance between animal and vegetable proteids, and it may be inferred that the proteids of animals are only modifications of those found in plants. A. Brittner[1] gives the following analysis—

	C	H	N	O	S
Egg albumin,	54·00	16·55	6·99	22·82	1·63
Blood albumin,	53·50	15·60	7·14	22·46	1·30
Plant albumin,	54·98	15·84	7·31	20·65	1·22
Blood fibrin,	52·40	18·07	7·03	21·29	1·22
Plant fibrin,	53·82	16·04	7·30	21·80	1·04
Animal casein,	54·67	15·78	7·46	22·97	1·12
Plant casein,	53·94	16·47	7·16	21·93	0·50

As will be afterwards studied, proteids are converted by the digestive process into peptones and in this condition pass into the blood. From the blood they are partly absorbed by the tissues, becoming for a time part of the living matter, and they may partly exist in the blood in a

in Gamgee's *Physiological Chemistry*, p. 8 *et seq.* For full details regarding instruments of this description see Landolt's *Handbook of the Polariscope*, 1882.

[1] A. Brittner, N. Rep. Pharm. xxi. 66-108, 129-150. Quoted in Watt's *Dict. of Chem.* vol. viii. p. 1684.

state of combination with saline matters. The processes occurring in the living matter lead again to a splitting up of the complex molecule, and simpler bodies are formed; these again decompose, and after a series of descending steps, urea, carbonic acid, and sulphates become the ultimate products eliminated from the body by various channels. It is important to observe that the product carrying off nearly the whole of the nitrogen is urea, and it is instructive now to compare the percentage composition of proteid and urea—

	C	H	O	N	S
Proteid, - - - -	53	7·30	23·04	15·53	1·13
Urea, - - - -	20	6·6	26·67	46·67	...

This table shows that urea contains about three times as much nitrogen as proteid matter, or 100 grms. of urea contain as much nitrogen as 300 grms. of proteid. (Foster.)

Chap. IV.—CHARACTERS OF THE SPECIAL PROTEIDS.

A. True Albumins.

1. **The Albumins.**—Their general characters are thus summed up:—They all contain nitrogen and sulphur; their chemical constitution oscillates round the following: $C_{54}H_7N_{16}O_{22}S$. Amorphous, soluble in water and various acids, usually soluble in alkalies; almost insoluble in alcohol and ether. Aqueous solutions are neutral. They yield in flame an odour of burned horn, giving off ammoniacal products, and leaving a residue of ash which consists mostly of phosphate of lime. Left to themselves they decompose very easily. Calcined with potash or boiled with sulphuric acid, they furnish leucin and tyrosin. Concentrated nitric acid, when hot, transforms them into a yellow body, *xanthoproteic acid*. Treated by acids, alkalies, or after putrid decomposition, they yield the following products: oily volatile acids, oxalic, acetic, formic, valerianic, fumaric, and asparagic acids; leucin, tyrosin, ammonia, etc. By oxidation of albumin, formic, acetic, propionic, butyric, valeric, capric, and benzoic acids, and the aldehydes of these acids, various organic volatile bases, acetonitrile, valeronitrile, and propionitrile may be obtained.

(1) *Serum Albumin.*—When dried, it is a clear yellow, transparent, vitreous substance, soluble in water; the solution is somewhat opalescent, and lightly fluorescent. At 70° C. heat coagulates it if the solution is not very alkaline. In this coagulation there always remains a small quantity of alkaline albuminate, and the liquid may even become alkaline. According to Mathieu and Urbain, the carbonic acid dissolved in

the albumin combines with it under the influence of the heat and is the cause of the coagulation. Solutions of albumin, deprived of carbonic acid by being placed in a vacuum, become incoagulable. Alcohol precipitates it from its solutions; carbonic, acetic, tartaric, phosphoric acids do not precipitate it; the concentrated acids precipitate it, especially nitric, metaphosphoric, picric, carbolic, and tannic acids. The alkalies transform it into basic albumin. It dissolves in concentrated nitric acid; most of the metallic salts precipitate it. After being deprived of all its salts by the dialyzer, it is not precipitated by heat or alcohol, but is precipitated by ether. Deprived of its volatile salts, and especially of the carbonate of ammonia by the absolute vacuum, it is transformed into a matter said to be identical with fibrinogen and fibrinoplastic substances. Kept several days in the vacuum, at a temperature of 40° to 60° C., it abandons considerable quantities of gas, consisting mostly of carbonic acid, hydrogen, and a small quantity of nitrogen. It deviates polarized light to the left.

(2) *Egg Albumin.*—When dried in air it forms a pale, yellowish, translucent mass, easily triturated to a white powder. It does not dissolve readily in water, but the presence of any alkaline salt causes it to dissolve easily. An aqueous solution deviates the plane of polarized light to the left. A solution on being heated becomes opalescent at 60° C., begins to deposit a coagulum at 60° to 63° C., and at a temperature a little higher the whole mass coagulates. It is insoluble in alcohol or ether. Ether only coagulates a portion of albumin in solution. The reactions with acids are the same as those of serum albumin. The chief distinction between serum albumin and egg albumin is that ether does not precipitate the former whilst it precipitates the latter.

(3) *Vegetable Albumin.*—In the juices of many plants a substance exists having similar properties to the albumins above described. It coagulates when heated from 65° to 70° C., and it gives the same reactions as animal albumin with the acids, etc. Existing in neutral or acid fluids in the plant, it is found in the juice of carrots, turnips, cabbages, the green stems of peas, potatoes, and in wheat flour; it is also found in oleaginous seeds. It differs from legumin (vegetable casein) by being precipitated by heat, but not by acetic acid.

2. **The Globulins.**—Albuminoid matters insoluble in water, soluble in a strong solution of chloride of sodium; their solution coagulated by heat; changed into syntonin by very dilute hydrochloric acid, and into alkali-albumins by the action of alkalies. They include—

(1) *Vitellin*, a substance existing in yolk of egg; it is not precipitated from its solutions by common salt. Its solutions coagulate at 70° to

75° C. It is changed into syntonin by the action of dilute hydrochloric acid.

(2) *Myosin.*—This substance exists in a fluid condition in living muscle, and, by its spontaneous coagulation after death, the condition called cadaveric rigidity is produced. It may be obtained by pressure from the muscles of a frog recently killed. It is transformed by weak acids into syntonin; its saline solution is coagulated by heat like albumin at 55° to 60° C.; alcohol precipitates it. It is readily soluble in a weak solution of common salt, and it is precipitated from such a solution on saturation with common salt.

(3) *Paraglobulin.*—Serum-globulin or fibrinoplastic substance: a substance existing in the blood, soluble in weak solutions of common salt; from weak alkaline solutions it is precipitated by a small quantity of common salt; "a further addition of this body leads to re-solution of the precipitate, which is thrown down again when the amount of sodium chloride in solution exceeds 20 per cent." (Gamgee, *op. cit.*) It is precipitated completely by magnesium sulphate. "The temperature of coagulation varies (according to amount of salts present and mode of heating) between 68° and 80°, on an average, 75° C." (Gamgee.)

(4) *Fibrinogen*, a substance also existing in the blood. It is soluble in water and in weak solution of sodium chloride. It is "precipitated from them completely by the addition of sodium chloride when this amounts to 12 to 16 per cent." (Gamgee.) Carbonic acid gives a precipitate which forms with difficulty; it is also precipitated by a mixture of 3 parts of alcohol to 1 part of ether, and by sulphate of copper; the precipitate is insoluble in an excess of the reagent. The temperature of coagulation is 56° C.

3. **Fibrin.**—This is an albuminous body formed during the coagulation of blood, chyle, or lymph. It is produced, according to one theory, by the combination of the two soluble albuminoids above described, *fibrinogen* and *fibrinoplastic substance.* Fibrinogen is found in all fluids which may transude from the blood into the tissues, in the fluid part of the blood itself, called the *liquor sanguinis*, in lymph and in chyle: whilst fibrinoplastic substance exists in the blood corpuscles and in small quantity in the liquor sanguinis. The conditions favouring the union of these two substances to form fibrin will be described in treating of the coagulation of the blood.

When obtained by washing blood clot, it consists of white amorphous filaments; insoluble in water, alcohol, and mineral acids, it swells in weak acids and in solutions of alkaline salts; it is soluble in weak acids (acetic, lactic, and phosphoric acids), potash, solutions of alkaline salts, $\frac{1}{10}$ % solution of chloride of sodium; ferrocyanide of potassium

precipitates it from its acid, and acetic acids from its alkaline, solutions. "When heated for many hours at 40° C. in dilute hydrochloric acid it dissolves, and the solution contains acid albumin." (Gamgee.) Fibrin decomposes ozone, evolving oxygen without seeming to suffer change. With ozone and a few drops of tincture of guaiacum, it gives a blue colour.

4. **The Proteins or Derived Albumins.**—These are proteid bodies, insoluble in pure water and in solutions of common salt, but are readily soluble in dilute hydrochloric acid and in dilute alkalies. They are not coagulated by heat. The proteins include—

(1) *Casein.*—This substance is coagulated from milk by heat with the addition of an acid, and by rennet. If any albuminous substance be treated with an alkali, and if the solution be neutralized by acetic acid, a substance chemically identical with casein is obtained. *Legumin*, found in the grains of leguminous plants, is identical with casein. It is insoluble in water, and soluble in waters lightly alkalinized. Its solution is not coagulated by heat; it is soluble in hydrochloric acid, less so in acetic acid. Its solutions are precipitated by alcohol, sulphate of magnesia, chloride of calcium, and metallic salts. When heated with a strong solution of caustic potash, potassium sulphide is formed. (Gamgee.) By lengthened boiling with water, it gives lactic acid and creatinin.

(2) *Alkali Albumin.*—This is a substance obtained by the action of dilute alkalies upon the proteids. In the presence of alkaline phosphates its solutions are not precipitated by neutralization; thus it is distinguished from acid albumin or syntonin. When heated with a strong solution of caustic potash, potassium sulphide is *not* formed, thus differing from casein, to which it is very closely related. (Gamgee.)

(3) *Acid Albumin or Syntonin.*—This substance is obtained by the action of dilute acids, especially hydrochloric acid, on proteids in solution, and by the action of strong hydrochloric acid on solid proteids. It is also the first product of digestion in the stomach. It is distinguished from basic albumin, because its solution in weak acids and alkaline carbonates is precipitated by neutralization (even in presence of alkaline phosphates). It has two other principal tests: (1) its solution in lime water is partly coagulated by heat; (2) the same solution gives a precipitate, while hot, with chloride of calcium, sulphate of magnesia, and chloride of sodium.

5. **The Peptones.**—These are products of the digestive process, and they are distinguished by the following characters:—They are very soluble in water, insoluble in absolute alcohol and ether, but alcohol precipitates them with difficulty from an aqueous solution; heat does not coagulate them; they are neither precipitated by acids nor by

alkalies, but they are precipitated by bichloride of mercury, or acetate of lead, or ammonia; ferrocyanide of potassium, and acetic acid throw down a precipitate. They are precipitated by a large excess of absolute alcohol and by tannic acid. "In the presence of much caustic potash or soda, a trace of a solution of copper sulphate produces a beautiful rose colour." (Gamgee.) They are very diffusible. They rotate to the left the plane of a ray of polarized light.

6. **Crystallizable Albuminoids.**—These are (1) hæmoglobin, the colouring matter of the blood, which will be described in treating of blood, and (2) vitellin plaques, a peculiar albuminoid substance found in the yolk of the eggs of fishes, and said to resemble aleuron grains in plants.

7. **Soluble Ferments.**—As will be seen in treating of fermentation, soluble ferments are albuminoid or proteid matter in a peculiar molecular condition.

B. Albuminous Derivatives.

The albuminous derivatives consist of two groups: (1) those arising from proteids in consequence of chemical transformations; and (2) those arising from physical changes or from the changes happening in the development of tissue.

1. **Albuminous Derivatives Chemically produced.**—These are substances closely related to those just described. Among them, in addition to certain substances found in diseased tissues we have the intercellular substance found in bone and connective tissue, known as *collagen*, which yields *gelatin* on boiling. Cartilage supplies an analogous body by prolonged boiling, called *chondrin*. In like manner, elastic tissues yield *elastin*; and epithelium, hair, nail, and epidermis yield *keratin*, which is remarkable for its richness in sulphur. Analyses of the albuminous derivatives show that gelatin and chondrin are a little richer in oxygen and a little poorer in carbon than albuminous bodies, whilst elastin contains, on the contrary, more carbon and less oxygen. Gelatin is not precipitated by any acid except tannic acid, while chondrin is precipitated by nearly all acids. Both are soluble in water. Keratin is not dissolved by boiling water or by weak acids, but it is dissolved by alkalies and concentrated acids. Elastin is dissolved only in concentrated alkalies.

(1) *Paralbumin.*—This is a form of albumin often found in ovarian cysts and in dropsical fluids, and is distinguished from the albumin of serum by two characters: the precipitate obtained by alcohol is soluble in water; and it coagulates incompletely by heat.

(2) *Colloid matter.*—This is a peculiar gelatinous, colourless or faint-

yellow, dull, or translucent substance resulting from a physiological metamorphoses of cells. It is often found in the thyroid gland, causing a tumour called *goître*. Acetic acid causes it sometimes to swell; it is readily stained by carmine; it is soluble in cold and hot water, for the most part not in alcohol or ether, but in caustic alkalies. "It has not a single positive character; it is distinguished from albumin by its insolubility in acetic acid, from mucus by the extent of coagulation with acetic acid, and from lardaceous substance by the want of colour with iodine and sulphuric acid." (Wagner's *Manual of General Pathology*, p. 329.) Colloid matter is held by some to be modified mucin, whilst others regard it as albumin which has become insoluble in acetic acid on account of the presence of chloride of sodium. It is undoubtedly a modified proteid substance, having most of the chemical characteristics of proteids.

(3) *Amyloid matter, or Lardacein.*—This substance exists in a form of degeneration of tissue (waxy degeneration), specially affecting the small arteries and capillaries, in which the tissue acquires a dull lustre and translucent appearance. The parts become brownish-red on the addition of diluted tincture of iodine, whilst parts containing no amyloid matter are coloured yellow. After the addition of dilute or concentrated sulphuric acid, the tissues assume a violet, rarely a blue hue, which may remain unchanged for hours or weeks, or after a short time may disappear. Water does not dissolve amyloid matter; concentrated alkalies dissolve it; alcohol and ether have no effect. Dilute acetic acid has no effect; but the strong acid may cause it to swell up. It is insoluble also in alkaline carbonates, and it is not acted on by the gastric juice at the temperature of the body. Kekulé has shown that amyloid matter contains cholesterin, that it contains no body similar to starch or cellulose as the reaction with iodine and sulphuric acid would lead one to infer, and that it is modified proteid matter, possibly an intermediate step between albuminous matter and fat and cholesterin. In the nervous centres (brain and cord), in the prostate gland, and in the lungs, small, round, oval, homogeneous, or concentrically laminated bodies are occasionally met with, called *corpora amylacea*, which become blue or bluish-grey by iodine alone, or quite blue after the addition of sulphuric acid. Some of these bodies, containing more albuminous substance than others, become green, brown, or yellow. Hot water and caustic alkalies dissolve them; alcohol and ether have no effect. Pathologists are of opinion that the matter forming these bodies is not the same as ordinary amyloid matter.

(4) *Mucin.*—This is a substance existing in the mucus secreted by mucous membranes; it exists in the connective, and more especially in

embryonic tissues; it is said to form the chief constituent of the bodies of some invertebrates (*Helix*), and it is found in large amount in the bile. It is a glutinous substance, soluble in water, in weak solutions of the alkalies and alkaline earths; it is precipitated by alcohol, by weak acids, and acetic acid; it is not coagulated by heat. Alkaline or neutral solutions of mucin are not precipitated by sulphate of copper, bichloride of mercury, nitrate of silver, perchloride of iron, etc. It is not precipitated by acetic acid and ferrocyanide of potassium, nor by tannic acid, but it is precipitated by acetate of lead from neutral or weak alkaline solutions. It contains C, H, N, O, but no S. Dr. Gamgee gives the following elementary analyses of mucin by various observers--

	I. From mucous contents of a cyst. (Scherer.)	II. From sub-maxillary gland. (Obelensky.)	III. From *Helix pomatia*. (Eichwald.)
C. - -	52·17	52·31	48·94
H. - -	7·01	7·22	6·81
N. - -	12·64	11·84	8·50
O. - -	28·18	28·63	35·38

These analyses show that it is a substance related to the proteids. It is probably a product of the differentiation of the protoplasm of certain animal cells. (Gamgee, *op. cit.*)

(5) *Nuclein.*—The nuclei of cells are believed to contain an organic body containing phosphorus. It has been isolated from pus cells in sufficient quantity for purposes of analysis, and appears as a greyish mass, insoluble in dilute hydrochloric acid but soluble in a weak solution of caustic soda. The analysis given by Miescher of the nuclein from salmon-melt and by Hoppe-Seyler of the nuclein of pus cells agree in finding from 2·28 (pus) to 9·59 (spermatozoa) per cent. of phosphorus present. Further, in the nuclein of yolk of egg Worm-Müller found 2·2, 2·68, and 7·9 per cent. of phosphorus. Gamgee considers the results of analyses so unsatisfactory as to lead him to deny the existence of nuclein as "a definite chemical individual possessed of constant composition." (Gamgee, *op. cit.* p. 243.)

(6) *Spermatin* is the name given to a substance found by Vauquelin in the semen of a man and by Kölliker in that of a bull and stallion. It was not coagulated by heat, became slightly turbid on the addition of acetic acid, whilst excess of the acid dissolved it. It was also precipitated by ferrocyanide of potassium. Probably it is not a distinct substance but modified mucin or an albuminate of an alkali.

2. Albuminous Derivatives physically and histogenetically produced.

(1) *Collagen.*—The organic basis of all the connective tissues, such as ordinary white fibrous tissue, tendon, fascia, and the organic matter of bone, consist of a substance termed collagen (κόλλα, glue) which yields *gelatin* on boiling. This well known substance swells in cold water; is soluble in boiling water, and gelatinizes on cooling; it is soluble in acids and alkalies, but is insoluble in alcohol, ether, and chloroform. Dissolved with the aid of heat in glycerine, on cooling, a jelly—glycerine-jelly—is formed. Solutions of gelatin are precipitated by tannin and bichloride of mercury; they are not precipitated by solutions of acetate of lead, thus differing from chondrin, nor by acetic acid and ferrocyanide of potassium. The aqueous solutions are lævo-rotatory of polarized light to the extent at 30° C. of [*a*] 130°. (Hoppe-Seyler.) Collagen is regarded as the anhydride of gelatin, as Hofmeister found that by heating gelatin for some time at 130° C. it lost ·755 per cent. of water and became a body identical with gelatin. (Gamgee.) Thus—

$$\underset{\text{Gelatin.}}{C_{102}H_{151}N_{31}O_{39}} + H_2O = \underset{\text{Collagen.}}{C_{102}H_{149}N_{31}O_{38}}.$$

Prolonged boiling causes gelatin to lose its property of coagulating, and Hofmeister states that the mixture then contains two bodies related to peptones, to which he gives the names *semiglutin* ($C_{55}H_{85}N_{17}O_{22}$) and *hemicollin* ($C_{47}H_{70}N_{14}O_{19}$). Further, he found that in thus decomposing, collagen combines with water. Thus—

$$\underset{\text{Collagen.}}{C_{102}H_{149}N_{31}O_{38}} + 3H_2O = \underset{\text{Semiglutin.}}{C_{55}H_{85}N_{17}O_{22}} + \underset{\text{Hemicollin.}}{C_{47}H_{70}N_{14}O_{19}}.$$

Semiglutin is precipitated by platinum tetrachloride whilst hemicollin is not. Further, by boiling in sulphuric acid gelatin yields, like other proteids, ammonia, leucin, glycin, and perhaps aspartic acid; the action of caustic baryta in sealed tubes is to give "ammonia, carbon dioxide, acetic and oxalic acids, and a mixture of amido-acids (containing glycin and alanin, amido-butyric acid, traces of glutamine, etc.)." (Gamgee, *op. cit.*)

(2) *Chondrigen.*—The basis of the matrix of cartilage is a substance, chondrigen, which yields chondrin on boiling. If chondrigen is digested in water at a temperature of 120° C. for several hours in a Papin's digester it dissolves and the solution contains *chondrin.* This substance is soluble in hot water, and gelatinizes in the cold. It is insoluble in alcohol and ether and in cold water. Its solutions are precipitated by alcohol as well as by mineral acids, acetic acids, alum, perchloride of

iron, acetate of lead, and nitrate of silver; the precipitate is soluble in an excess of the reagent. The precipitate by acetic acid is redissolved by alkaline salts, which distinguishes chondrin from albuminoid matters. Prolonged boiling in dilute hydrochloric or sulphuric acids produces a substance termed *chondroglucose*, which is lævo-rotatory to polarized light and reduces cupric salts. A similar substance is obtained from mucin by boiling in dilute acids. With prolonged boiling in dilute sulphuric acid, chondrin yields leucin but no tyrosin or glycocin. Morochowitz denies the existence of chondrin as a distinct substance, and regards it as a mixture of gelatin, mucin, and salts. This view is supported by Gamgee in the following suggestive sentence—"If these views be, as the author believes, correct, all the tissues belonging to the connective tissue group possess common chemical character in that their ground substance is in all cases a body transformed into gelatin by the prolonged action of boiling water; this being mixed in greater or less proportion with mucin which, as we have seen, undoubtedly plays the part in many forms of connective tissue of a connecting or cementing substance." (Gamgee, *op. cit.* p. 271.)

(3) *Elastin.*—From the *ligamentum nuchæ* and wherever elastic tissue exists, a substance called elastin may be extracted by boiling first in ether and alcohol, then for a long time in water, then in acetic acid, and lastly in a concentrated solution of caustic soda. When dried, it is a hard, brittle, yellowish substance, insoluble in water, ammonia, acetic acid, or alcohol. In solution it is not precipitated by the mineral acids, but tannic acid precipitates it from a neutralized solution. "It is soluble in boiling solution of caustic potash, in cold concentrated sulphuric acid, and in concentrated nitric acid." (Gamgee.)

(4) *Keratin.*—This substance is obtained by boiling horn, hair, feathers, or cuticle, in ether, alcohol, water, and dilute acids successively. It is insoluble in alcohol and ether; it swells in water, and still more so in acetic acid. It is soluble in solutions of caustic soda and potash. When boiled with dilute sulphuric acid, keratin yields leucin, tyrosin, volatile fatty acids, and aspartic acids. "Nitric acid dissolves it, and oxalic acid is formed as an ultimate product." (Gamgee.) Keratin is remarkable for the large proportion of sulphur existing in it in a loose state, and hence it yields sulphuretted hydrogen when heated in sealed glass tubes up to 150° to 200° C., and the residue is first dissolved by boiling in alkalies and then treated with acetic acid. The amount of sulphur varies in specimens of keratin prepared from different epidermic tissues. Keratin from human hair contains sulphur varying from 3 to 8·23 per cent. (Gamgee.)

The chemical composition of the four albuminous derivatives—gelatin,

chondrin, elastin, and keratin—is contrasted with albumin in the following table[1]—

	Serum Albumin.	Mucin. (Obolensky.)	Gelatin. (Hofmeister.)	Chondrin. (Schützenberger.)	Elastin. (Mulder.)	Keratin. (Mulder.)
C. - -	52·7	52·31	50·75	50·16	55·47	51·00
H. - -	6·9	7·32	6·47	6·58	7·54	6·94
N. - -	15·4	11·84	17·86	14·18	16·09	17·51
O. - -	20·9	28·63	24·92	29·18	20·90	21·75
S. - -	0·8	—	doubtful.	doubtful.	—	2·80

The following table contrasts the chief reactions of gelatin, chondrin, and mucin[2]—

	Gelatin.	Chondrin.	Mucin.
Acetic acid.	No precipitate but dissolves in acid. Is precipitated when heated with alkaline salts.	Precipitate, soluble in solutions of alkaline salts.	Precipitate, insoluble in solutions of alkaline salts.
Mineral acids.	No precipitate.	Precipitate, soluble in excess.	Precipitate, soluble in excess.
Tannic acid.	Yellow precipitate.	Opalescence.	No effect.
Acetate of lead.	No precipitate.	Precipitate, partly soluble in excess.	Precipitate by basic acetate of lead.
Alum.	No precipitate.	Precipitate, soluble in excess.	Precipitate.
Mercuric chloride.	White precipitate.	Opalescence only.	Slight turbidity.
When decomposed by boiling in dilute acids.	Leucin and glycocin formed; no sugar stuff.	Leucin; no glycocin; also chondroglucose.	Leucin and tyrosin and body similar to chondroglucose.

Chap. V.—NITROGENOUS PROXIMATE PRINCIPLES OTHER THAN THE PROTEIDS.

A. Fatty Nitrogenous Matters.

There are at least four bodies that may be classed under this head—lecithin, cholin or neurin, phospho-glyceric acid, and cerebrin—all of

[1] Compiled from Gamgee's *Physiological Chemistry*, vol. i.

[2] See Charles' *Physiological and Pathological Chemistry*, p. 134.

which are related chemically and physiologically to a substance called protagon, already referred to.

1. *Protagon*, $C_{160}H_{308}N_5PO_{35}$, is a substance existing in nervous matter, and specially in the brain and spinal cord; it is also found in the coloured and colourless blood corpuscles, in most animal fluids, and in yolk of egg. It is a neutral substance, insoluble in water, soluble in boiling alcohol and in boiling fats, but is insoluble in cold alcohol. When boiled with baryta water it yields glucose, phospho-glyceric acid ($C_3H_9PO_6$), and a body similar to neurin, but differing from it by having one molecule of water less than neurin—thus, $C_5H_{13}NO$. According to Hoppe-Seyler, protagon is a mixture of lecithin and cerebrin, whilst Bäyer regards it as a glucoside. It has, however, been conclusively established by Gamgee and Blankenhorn that it is a definite chemical body, thus confirming the original opinion of its discoverer, Liebreich.

2. *Lecithin*, $C_{44}H_{90}NPO_9 + H_2O$, is also found in nervous matter, in the blood corpuscles, and in yolk of egg. When obtained from eggs, it is a white, waxy, hygroscopic mass, colourless, soluble in alcohol, ether, chloroform, sulphide of carbon, benzol, and in the fats. When heated with baryta water, it is split up into phospho-glyceric acid, cholin, neurin, and stearate of baryta. When ignited it burns away, leaving as a residue only metaphosphoric acid. (Gamgee.) Dr. Gamgee doubts its existence *in quantity* in the brain tissue, and he says that among the numerous phosphorized matters obtained from an alcoholic solution of brain matter, lecithin is to be reckoned. No sufficient proof of its identity has, however, yet been furnished. Such matters from an alcoholic extract of brain are protagon, cholesterin, and a group of phosphorized bodies, to which the general name *cerebrin* may be applied. To understand the constitution of lecithin, consider, in the first place, its relations to phospho-glyceric acid and neurin.

a. Phospho-glyceric acid, $C_3H_9PO_6$, or $C_3H_5 . (OH)_2 . O . PO . (OH)_2$, may be obtained pure as a syrupy liquid, easily decomposed by heat into glycerine and phosphoric acid, of which substances it is really a combination. Thus—

$$\underset{\text{Glycerine.}}{C_3H_5\begin{cases}OH\\OH\\OH\end{cases}} + H_3PO_4 = \underset{\text{Phospho-glyceric acid.}}{C_3H_5\begin{cases}OH\\OH\\PO_4H_2\end{cases}} \text{ or } C_3H_5\begin{cases}OH\\OH\\O . PO(OH)_2\end{cases} + H_2O.$$

One molecule of water is formed, and it may be said that phospho-glyceric acid is produced by the union of glycerine and phosphoric acid with the loss of one molecule of water.

b. Neurin or Cholin, $C_5H_{15}NO_2$, is also formed by the decomposition of lecithin, and it is identical with one of the constituents of the bile

acids. It is a syrupy liquid, soluble in alcohol and ether, and alkaline in reaction. It may be thus represented: $C_2H_4 . N(CH_3)_3 . (OH)_2$. When heated it decomposes into ethylene glycol and trimethylamine, and it will be observed that the hydroxyls are united to the ethylene glycol. Thus—

$$\underset{\text{Neurin.}}{C_2H_4 . N(CH_3)_3 . (OH)_2} = \underset{\text{Ethylene glycol.}}{C_2H_4 . (OH)_2} + \underset{\text{Trimethylamine.}}{N(CH_3)_3}.$$

Neurin has been formed synthetically. (Gamgee.)

Now, *lecithin* may be regarded as built on the type of phospho-glyceric acid, in which two of the hydrogens are replaced by the radicle of a fatty acid (oleic, stearic, palmitic), while one atom of hydrogen of the phosphoric acid is replaced by one molecule of cholin, less one molecule of hydroxyl. Thus—

$$\underset{\text{Phospho-glyceric acid.}}{C_3H_5\begin{cases} OH \\ OH \\ O . PO(OH)_2 \end{cases}};$$

then $C_{18}H_{37} - H_2O + O = C_{18}H_{35}O$, radicle of stearic acid; then

$$\underset{\text{Lecithin.}}{C_3H_5\begin{cases} O . C_{18}H_{35}O \\ O . C_{18}H_{35}O \\ O . PO\begin{cases} OH \\ O . C_2H_4 . N(CH_3)_3 . OH \end{cases} \end{cases}};$$

or, $C_{44}H_{90}NPO_9$, supposing the radicle of stearic acid exists in the compound, which may be called distearyl-lecithin.

Thus, by its decomposition, lecithin may yield phospho-glyceric acid, glycerine, trimethylamine, and ethylene glycol, and this may account for the occasional appearance of trimethylamine in some of the secretions. A consideration of its constitution also suggests its relationship to cholin—one of the products of the decomposition of the bile acids.

3. *Cerebrin* is a substance of varying formulæ extracted from nervous matter, and differing from lecithin in not containing phosphorus. There is no trustworthy evidence that it is a definite chemical compound. Gamgee has extracted from the brain by alcohol at 45° C. a body which he has provisionally named *pseudo-cerebrin*, and to which he attaches the formula $C_{44}H_{92}NO_8$. Dr. Thudichum states, as the result of an elaborate research, that he has separated from brain matter phosphorized bodies belonging to three groups—the *kephalins* ($C_{42}H_{79}NPO_{13}$), the *myelins*, of varying formulæ ($C_{40}H_{83}NPO_8$, $C_{40}H_{75}NPO_{10}$, $C_{40}H_{82}N_2PO_{10}$, $C_{52}H_{103}N_2PO_9$, $C_{39}H_{81}NPO_8$, and $C_{39}H_{82}N_2PO_8$), and the *lecithins*—bodies of great instability, and hence of uncertain composition.[1]

[1] For a full critical account of this difficult subject, see Gamgee's *Physiological Chemistry*, vol. i. pp. 425-447; also Dr. Thudichum's Research, *Reports of Medical Officer of the Privy Council and Local Government Board*, 1874, p. 113 *et seq.*

B. The Amides.

1. **Urea.**—The typical substance belonging to this group is *urea*, CH_4N_2O. It may be regarded as constructed on the type of two molecules of ammonia, in which two hydrogens are replaced by the dyad radical CO, thus—

$$\begin{matrix} N\left|\begin{matrix}H\\H\\H\end{matrix}\right. \\ \\ N\left|\begin{matrix}H\\H\\H\end{matrix}\right. \end{matrix} \!\!\!\

matter. When obtained pure, it consists of elongated, prismatic, four-sided crystals, terminated by one or two oblique surfaces. (See Fig. 25.) Nitric acid forms *nitrate of urea*, a salt which takes the form of octahedra, or lozenge-shaped tablets, or hexagons (Fig. 26). Oxalic acid forms flat or prismatic crystals of *oxalate of urea* (Fig. 26). Urea is

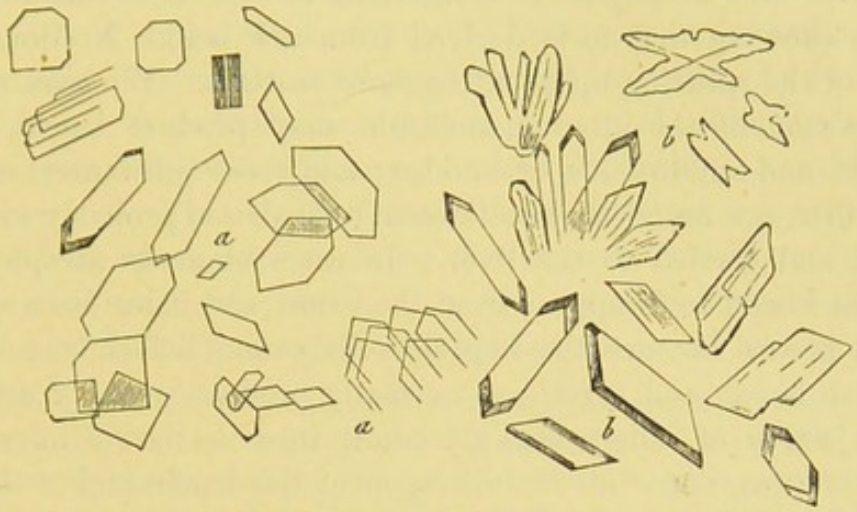


FIG. 26.—Nitrate and Oxalate of Urea. *a*, *a*. Nitrate of urea; *b*, *b*. Oxalate of urea.

inodorous, and has a bitterish-saline taste. It is soluble in water and alcohol, but not in ether. It is not precipitated by acetate, nor by subacetate of lead, but it is thrown down by mercuric nitrate, forming a whitish precipitate, having the formula $(CON_2H_4)_2Hg(NO_3)_2 + 3HgO$, and containing urea and mercuric oxide in the proportion of 10 to 72. Nitrous acid, chlorine, hypochlorite and hypobromite of soda decompose it into nitrogen and carbonic acid—

$$CON_2H_4 + N_2O_3 = CO_2 + 2H_2O + 2N_2.$$
$$CON_2H_4 + H_2O + 3Cl_2 = CO_2 + N_2 + 6HCl.$$
$$CON_2H_4 + 3NaBrO = CO_2 + N_2 + 2H_2O + 3NaBr.$$

On the reaction of mercuric nitrate with urea depends Liebig's well-known volumetric process for the estimation of the amount of urea in a fluid, and on its reaction with hypobromite of soda is founded the method of determining the amount of urea from the amount of nitrogen evolved, devised first by Knop and since modified and improved by many chemists. These methods will be described in treating of the analysis of the urine.

By fermentation, under the action of a specific organism (*Micrococcus Ureæ*), it is transformed into carbonate of ammonia—

$$\underset{\text{Urea.}}{CON_2H_4} + 2H_2O = \underset{\text{Carbonate of ammonia.}}{(NH_4)_2CO_3}.$$

When dry chlorine is passed over urea, the following reaction occurs—

$$\underset{\text{Urea.}}{3(CON_2H_4)} + 3Cl_2 = \underset{\text{Cyanuric acid.}}{C_3H_3O_3N_3} + N_2 + 5HCl + NH_4Cl.$$

When heated with a mixture of caustic potash and permanganate of potash, it is decomposed into carbonic acid and ammonia. There is still considerable obscurity as to the origin of urea, but the evidence will be better appreciated after a study of the metabolic changes occurring in the tissues, and more especially after the study of the functions of the liver and kidneys. It is sufficient to state here that there is no evidence showing that it is derived from uric acid. No doubt it is a product of the splitting up of nitrogenous matters. We have seen that decomposition of the albumin-molecule may produce leucin, tyrosin, glycocolle, and amido-acids or amides; and these substances, especially the two first, are found in the alimentary canal, and probably are thence absorbed and carried to the liver. In cases of acute atrophy of the liver, urea is said to disappear from the urine, and it has been supposed that this occurs because the hepatic cells cannot effect such chemical changes on leucin and tyrosin as normally produce urea. Further, the direct injection of leucin and glycocolle into the bowel increases the amount of urea, and "all the nitrogen of the leucin and of the glycocolle appears in the urea." (Beaunis.) It will be seen that this is strong presumptive evidence in favour of the view that urea may be a decomposition product of such bodies; but one must admit that the effect may be due not to a direct transformation, but to the action of leucin and its allies on the general metabolism of proteid matter in the liver. It has also been shown that amides, such as glycocolle, sarcosin, and taurin, when introduced into the body so as to be carried to the liver, increase the urea (Salkowski), but they may appear in the urine as uramides, that is, bodies formed by a combination of NHCO (cyanic acid) with the amide introduced. Thus glycocin and cyanic acid form hydantoïnic acid, sarcosin (methyl-glycocolle) with cyanic acid forms methyl-hydantoïnic acid (Schultzen), and taurin with cyanic acid forms tauro-carbamic acid (uramo-isthionic acid) (Salkowski)—

$$\underset{\text{Glycocolle.}}{NH_2 . CH_2 . CO . OH} + \underset{\text{Cyanic acid.}}{CO . NH} = \underset{\text{Hydantoïnic acid.}}{NH . CONH_2 . CH_2 . CO . OH.}$$

$$\underset{\text{Taurin.}}{NH_2 . CH_2 . CH_2 . SO_2 . OH} + \underset{\text{Cyanic acid.}}{CO . NH} = \underset{\text{Tauro-carbamic acid.}}{NH . CONH_2 . CH_2 . CH_2 . SO_2 . OH.}$$

There is evidently then some relation between glycocolle, sarcosin, leucin, tyrosin, and urea, although the chemist has not succeeded in effecting a direct transformation of any of these bodies into urea. But if not directly derived, may not urea originate from certain bodies in their turn springing from the amides, glycocolle, sarcosin, etc., or at all events having a common origin with these? For example, suppose by decomposition of albuminoids that cyanic acid were produced, then one can conceive urea originating thus—

$$\underset{\text{Cyanic acid.}}{NHCO} + \underset{\text{Cyanic acid.}}{NHCO} + H_2O = \underset{\text{Urea.}}{CON_2H_4} + \underset{\text{Carbonic acid.}}{CO_2}.$$

Chemists have not yet found cyanic acid as a product of the decomposition of the amides, so that this origin of urea is doubtful. Another theory that has been put to the test of experiment with unsatisfactory results is, that urea may be produced by the formation from albuminoids of ammonia, and that the carbonate of ammonia produced by the union of ammonia with carbonic acid may then, by losing water, become urea. To test this view, salts of ammonia have been administered to animals, and the effect, if any, on the elimination of urea has been scrutinized. Kniriem and Salkowski state that the urea eliminated by the rabbit is increased by giving the animal chloride of ammonium or nitrate of ammonia; in the dog, however, Salkowski did not observe this effect. The point in dispute is thus stated by Beaunis—

"This fact, which led Voit and Feder to deny the results obtained by Kniriem and Salkowski, has been explained by Schmiedeberg and his pupils, by showing how differently the dog and the rabbit are influenced by acids. Schmiedeberg and Walter have shown that in the dog, the ingestion of hydrochloric acid increases notably the elimination of ammonia by the urine; this ammonia, necessary for the elimination of hydrochloric acid, the latter takes from the organism; but if, instead of the hydrochloric acid being introduced in a free condition, it is introduced in chloride of ammonium, there is no formation of urea, because the ammonia is retained by the hydrochloric acid, which, in place of borrowing the ammonia from the organism itself, employs that directly supplied. This is shown by the fact that if a dog receives carbonate of ammonia instead of the chloride of ammonium, a part of the carbonate appears in the urine as urea. If, however, following the example of Munk, the organism of the dog is placed in the same conditions as that of a rabbit by rendering the urine alkaline by a vegetable diet, then the chloride of ammonium only appears partially in the urine, a half, or less, of the salt introduced contributing to the formation of urea."[1]

If then ammoniacal salts increase the amount of urea eliminated, we may suppose with Salkowski that cyanic acid arising from the decomposition of proteids unites with the ammonia to form cyanate of ammonia, which, by molecular transformation, then becomes urea. Normally then the reaction would be thus—

$$\underset{\text{Cyanic acid.}}{CO.NH} + \underset{\text{Cyanic acid.}}{CO.NH} + H_2O = \underset{\text{Urea.}}{CON_2H_4} + CO_2.$$

whilst the reaction after the administration of ammonia would be—

$$\underset{\text{Cyanic acid.}}{2CONH} + \underset{\text{Ammonia.}}{2NH_3} = \underset{\text{Urea.}}{2CON_2H_4}.$$

Thus, whilst it is clear that urea results from decomposition of proteid matters, the exact steps by which this is accomplished are unknown,

[1] Beaunis, *op. cit.* vol. i. p. 147.

and the question whether urea may arise from decomposition only, or from the synthesis of products of decomposition, is still unsettled.

2. *Oxaluric acid*, $C_3H_4N_2O_4$.—This substance is urea in which one atom of hydrogen is replaced by the residue of oxalic acid, that is oxalic acid $C_2H_2O_4$, minus hydroxyl, HO or C_2O_3H—

$$CO\begin{matrix}NH_2\\NH_2\end{matrix} \qquad CO\begin{matrix}NH_2\\NH.C_2O_3H.\end{matrix}$$

Urea. Oxaluric acid.

Heated in the presence of water, it is decomposed into oxalic acid and oxalate of urea—

$$\underset{\text{Oxaluric acid.}}{2(C_3H_4N_2O_4)} + \underset{\text{Water.}}{2(H_2O)} = \underset{\text{Oxalate of urea.}}{(CH_4N_2O)_2C_2H_2O_4} + \underset{\text{Oxalic acid.}}{C_2H_2O_4}.$$

The nature of this acid will be further referred to in discussing uric acid and its derivatives, of which oxaluric acid is one.

3. *Allantoin*, $C_4H_6N_4O_3$, is also a derivative of uric acid, found in the amniotic and allantoic fluids of the embryo, in the urine of newly-born children, and in the urine of certain animals, such as the dog and cat. It may be formed artificially by treating uric acid with water and peroxide of lead, or by acting on uric acid with potassium ferrocyanide and caustic potash. It forms shining colourless prisms (Fig. 27), is tasteless, and is soluble in 160 parts of water at 20° C. and in 30 parts of boiling water. Heated with nitric or hydrochloric acids, it is changed into uric and allanturic acids; sulphuric acid splits it up into carbonic acid, carbonic oxide, and ammonia, whilst, when boiled with baryta water, it gives off ammonia and precipitates oxalate of barium. When 1 part of glyoxalic acid is heated with 2 parts of urea to 100° C. for eight or ten hours, allantoin is formed, and from this reaction it is regarded as the diureide of glyoxalic acid, thus—

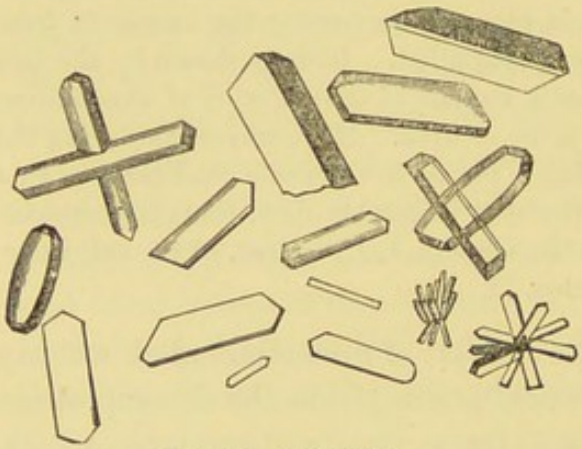


Fig. 27.—Allantoin.

$$CO\begin{matrix}NH-CH-NH-CO-NH_2\\|\\NH-CO\end{matrix}$$

Pelouze discovered an acid, *allanturic acid*, $C_7H_{10}N_6O_6$(?), produced from allantoin by the action of dilute nitric acid, thus—

$$\underset{\text{Allantoin.}}{2(C_4H_6N_4O_3)} + H_2O = \underset{\text{Allanturic acid.}}{C_7H_{10}N_6O_6} + \underset{\text{Urea.}}{CON_2H_4}.$$

CHAP. VI.—NITROGENOUS PROXIMATE PRINCIPLES.—CONTINUED.

THE AMIDES, OR AMIDO-ACIDS.

When we examine the chemical constitution of plants, we find they contain non-nitrogenous and nitrogenous bodies. As examples of the former, we have (1) carbohydrates, such as cellulose, starch, dextrin, gum, and cane and grape sugar; (2) the varieties of the essential oils and resins; and (3) vegetable acids. The nitrogenous compounds comprise in addition to proteids, the colouring matters of plants, the principal of which is chlorophyll, and organic bases termed *alkaloids*. These bases contain nitrogen, in addition to carbon, oxygen, and hydrogen. The following are examples—

Aconitine,	$C_{30}H_{47}NO_7$	Aconitum napellus.
Asparagine,	$C_4H_8N_2O_3$	Asparagus officinalis.
Atropine,	$C_{17}H_{23}NO_3$	Atropa belladonna.
Biberine,	$C_{19}H_{21}NO_3$	Biberis vulgaris.
Brucine,	$C_{28}H_{26}N_2O_4$	Strychnos nux vomica.
Caffein,	$C_8H_{10}N_4O_2$	Tea, coffee, etc.
Cinchonine,	$C_{20}H_{24}N_2O$	Cinchona bark.
Codeine,	$C_{18}H_{21}N_2O_3$	Opium.
Conine,	$C_8H_{15}N$	Conium maculatum.
Morphine,	$C_{17}H_{19}NO_3$	Opium.
Narceine,	$C_{24}H_{29}NO_9$	Opium.
Narcotine,	$C_{22}H_{23}NO_7$	Opium.
Nicotine,	$C_{10}H_{14}N_2$	Tobacco.
Papaverine,	$C_{20}H_{21}NO_4$	Opium.
Quinine,	$C_{20}H_{24}N_2O_2$	Cinchona bark.
Kinoline,	C_9H_7N	Cinchona bark.
Sinapine,	$C_{16}H_{23}NO_5$	White mustard.
Strychnine,	$C_{21}H_{22}N_2O_2$	Strychnos nux vomica.
Thebaine,	$C_{19}H_{21}N_2O_3$	Opium.
Theine,	$C_8H_{10}N_4O_2$	Tea, coffee.
Theobromine,	$C_7H_8N_4O_2$	Cacao beans.

Chemically, these bodies are regarded as compound ammonias—that is, bodies in which one, two, or three equivalents of hydrogen are replaced by radicles. Thus conine may be represented as ammonia in which two atoms of hydrogen are replaced by the monatomic radicle, C_4H_7, and nicotine as a condensed ammonia in which all the atoms of hydrogen are replaced by the triatomic radicle C_5H_7. Example—

$$\left.\begin{matrix}(C_4H_7)_2\\H\end{matrix}\right\}N = C_8H_{15}N. \qquad \left.\begin{matrix}C_5H_7\\C_5H_7\end{matrix}\right\}N_2 = C_{10}H_{14}N_2.$$

Conine. Nicotine.

In the animal economy certain bodies are found which apparently

have a somewhat similar chemical structure. They differ in chemical composition from the vegetable alkaloids, however, by containing less nitrogen and oxygen in proportion to the amount of carbon. These substances are—

Leucin,	$C_6H_{13}NO_2$	Cystin,	$C_3H_7NSO_2$
Tyrosin,	$C_9H_{11}NO_3$	Sarcin,	$C_5H_4N_4O$
Glycocin or glycocolle,	$C_2H_5NO_2$	Xanthin,	$C_5H_4N_4O_2$
Taurin,	$C_2H_7NO_3S$	Guanin,	$C_5H_5N_5O$
Creatin,	$C_4H_9N_3O_2$	Alloxan,	$C_4H_2N_2O_4$
Creatinin,	$C_4H_7N_3O$	Urea,	CH_4N_2O

It is probable that certain of these bodies are *amides*—ammonias in which one or more atoms of hydrogen are replaced by radicles of an acid, or *amido-acids*—that is, acids in which one or more hydrogen atoms of the radicle of the acid are replaced by NH_2. Thus urea, as already explained, may be regarded as carbamide, or ammonia containing the diatomic radicle of carbonic acid. Example—

$$\left.\begin{matrix}H_3\\H_3\end{matrix}\right\}N_2 \qquad CO \qquad \left.\begin{matrix}CO\\H_2\\H_2\end{matrix}\right\}N_2 = CH_4N_2O.$$

Ammonia. Radicle of CO_2. Carbamide. Urea.

In like manner, on the type of one molecule of water, glycocin may be regarded as amido-acetic acid, leucin as amido-caproic acid, and sarcosin, one of the derivatives of creatin, as amido-methyl-acetic acid. In the amido-acid group of the latter substance one atom of hydrogen is replaced by CH_3 (methyl), thus—

$$\left.\begin{matrix}C_2H_2ONH_2\\H\end{matrix}\right\}O \qquad \left.\begin{matrix}C_6H_{10}ONH_2\\H\end{matrix}\right\}O \qquad \left.\begin{matrix}C_2HO(CH_3)NH_2\\H\end{matrix}\right\}O.$$

or $C_2H_5NO_2$ — Glycocin or amido-acetic acid.

or $C_6H_{13}NO_2$ — Leucin or amido-caproic acid.

or $C_3H_7NO_2$ — Sarcosin or methyl-glycocin, or amido-methyl-acetic acid.

The amides are often conjugated bodies, and they may behave either as bases forming unstable compounds with acids, or they may play the part of feeble acids, which, with the loss of water, unite with alkaline bases. The chief point of physiological importance is their instability, especially under the conditions prevailing in the living body.

1. *Glycocolle*, or *Glycin*, or *Glycocin*, $C_2H_5NO_2$, as above shown, is amido-acetic acid. It forms a constituent of glycocholic acid, one of the acids of the bile. Combining with benzoic acid, it forms hippuric acid. When uric acid is acted on with warm hydriodic acid, it splits up into glycocolle, iodide of ammonium, and carbonic acid, a reaction which indicates that glycocolle is related to uric acid—

$$\underset{\text{Uric acid.}}{C_5H_4N_4O_3} + \underset{\text{Hydriodic acid.}}{3HI} + 5H_2O = \underset{\text{Glycocolle.}}{C_2H_5NO_2} + \underset{\text{Iodide of ammonium.}}{3NH_4I} + \underset{\text{Carbonic acid.}}{3CO_2}.$$

The decomposition of gelatin yields glycocolle among other substances, and it is possible that glycocolle may be produced in the liver from gelatin absorbed from the alimentary canal. Glycocolle, when thus formed, unites with cholalic acid to produce glycocholic acid, and it may also unite with any benzoic acid present to form hippuric acid. In the intestine, no doubt, glycocholic acid is decomposed, and the glycocolle liberated passes into the blood. It has been suggested that it may then be decomposed into methylamine and carbonic acid, thus—

$$\begin{array}{l} CH_2 \cdot NH_2 \\ | \\ CO \cdot OH \cdot \end{array} = N \left| \begin{array}{l} H \\ H \\ CH_3 \end{array} \right. + CO_2$$

Glycocolle. Methylamine.

This methylamine (not found free in the body), may, in certain circumstances, form trimethylamine, which sometimes is present. Again, the oxidation of glycocolle produces carbonic acid, oxamic acid, oxalic acid, and water; and oxamic acid, in turn, is readily decomposed into ammonia and oxalic acid. In a pure state, glycocolle appears in the form of rhombohedric or prismatic crystals (Fig. 28), having a sweetish taste. These are soluble in cold water, and they are insoluble in cold alcohol and ether. The solution is acid. When a solution of glycocolle is boiled with a solution of hydrated cupric oxide, a blue solution is formed, from which bluish needle-shaped crystals separate on cooling.

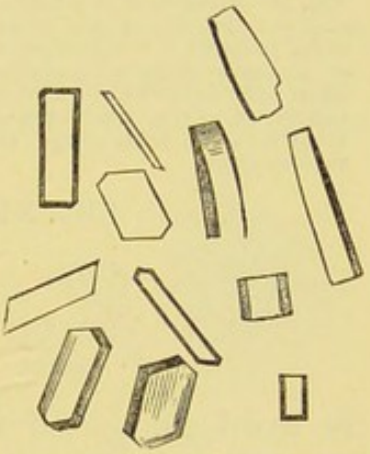


FIG. 28.—Glycocin, or glycocolle, or glycin.

2. *Leucin*, $C_6H_{13}NO_2$, is amido-caproic acid. It is related to an acid, leucic acid, or oxy-caproic acid, that is to say, the hydroxyl of leucic acid is replaced in leucin by the radicle NH_2. Thus—

CH_3	$CH_2 \cdot OH$	$CH_2 \cdot NH_2$
$(CH_2)_4$	$(CH_2)_4$	$(CH_2)_4$
$CO \cdot OH$	$CO \cdot HO$	$CO \cdot HO$
Caproic acid.	Leucic acid or oxy-caproic acid.	Leucin.

It has already been pointed out (p. 59) that leucin exists in considerable amount among the decomposition products of proteids when the proteids are split up by the action of strong caustic alkalies or sulphuric acid. Leucin is also formed when proteids are decomposed by putrefaction and by pancreatic digestion. It is found in many of the tissues and organs, more especially in the pa creas, and in smaller quantities

in the liver, kidneys, spleen, thymus, lymphatic glands, and the brain. In some pathological conditions, such as leukæmia and acute atrophy of the liver, it is found in the urine in the form of yellowish-brown balls (Fig. 29) or spheres, highly refractive, and consisting of masses of fine needle-shaped crystals. Leucin is inodorous, it is soluble in water, slightly in alcohol, but not in ether. Its solutions are neutral in reaction and almost tasteless. Leucin originates in the body by the decomposition of proteids. In turn, it splits up into fatty acids and ammonia. Thus, by heat it is decomposed into carbonic acid and amylamine—

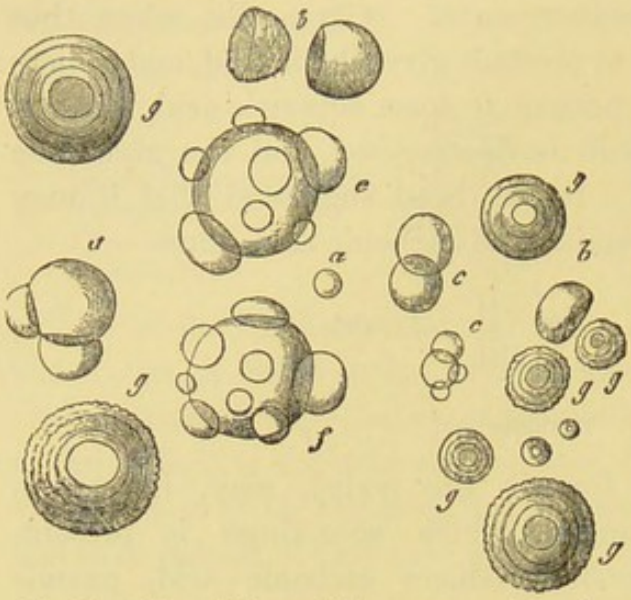


FIG. 29.—Spheroidal crystalline masses of leucin. *a*, A very minute simple spherule; *b*, hemispheroidal masses; *c*, *c*, aggregates of small globules; *d*, a larger globule supporting two halves; *e*, *f*, large spheroids richly studded with minute segments; *g*, *g*, *g*, *g*, laminated globules, some with smooth, some with rough surface, and of very various sizes.

$$\underset{\text{Leucin.}}{CH_2.NH_2\,(CH_2)_4\,CO.OH.} = \underset{\text{Amylamine.}}{CH_2.NH_2\,(CH_2)_3\,CH_3} + CO_2$$

By hydriodic acid it is decomposed into caproic acid and ammonia, thus—

$$\underset{\text{Leucin.}}{CH_2.NH_2\,(CH_2)_4\,CO.OH.} + 2H = \underset{\text{Caproic acid.}}{CH_3\,(CH_2)_4\,CO.OH.} + NH_3.$$

With concentrated sulphuric acid, leucin yields ammonia and valeric acid, and with permanganate of potash, oxalic acid, carbonic acid, valeric acid, and ammonia. Possibly, in the body, similar decompositions may occur, which accounts for the traces of these substances found in some organs. Thus, valeric acid has been found in the sweat and in epidermic matters containing leucin. It has also been conjectured that leucin may be one of the steps towards the formation of urea, and this is supported experimentally by the fact that the administration of leucin to dogs increases the amount of urea eliminated by the kidneys.

3. *Tyrosin*, $C_9H_{11}NO_3$, is the amide of oxyphenylpropionic acid, and is related on the one hand to a fatty acid, propionic acid, and on the other, to phenic acid, an aromatic substance, thus—

CH_3	CH_3	CH_3	CH_3
|	|	|	|
CH_2	$CH . NH_2$	$CH . (C_6H_4 . OH)$	$C . (C_6H_4 . OH)NH_2$
|	|	|	|
$CO . OH$	$CO . OH$	$CO . OH$	$CO . OH .$
Propionic acid.	Alanin.	Oxyphenylpropionic acid.	Tyrosin.

Here observe that one atom of hydrogen in propionic acid is replaced by the radicle oxyphenyl ($C_6H_4 . OH$), and when the remaining hydrogen in oxyphenylpropionic acid is replaced by NH_2, tyrosin is produced; or, as shown above, tyrosin may be looked on as alanin (the amide of propionic acid), in which one atom of hydrogen is replaced by oxyphenyl. Tyrosin is usually found along with leucin, and like it, results from the decomposition of proteids. Occasionally found in the urine, it is probably entirely decomposed in the body, and it has been shown that the administration of tyrosin to a living animal is not followed by its appearance in the urine, but by the appearance of phenol and phenol-sulphates, indicating its relation to the aromatic compounds. Tyrosin crystallizes in slender, colourless, microscopic needles (Fig. 30). It is slightly soluble in cold water, but is insoluble in alcohol and ether. Whilst burning, it smells like burnt horn. Oxidation by bichromate of potash yields oil of bitter almonds, hydrocyanic, benzoic, formic, acetic, and carbonic acids. *Piria's test:* Heat the substance with a few drops of concentrated sulphuric acid in a watch-glass; when the solution is cold, add a little water and a few morsels of chalk, when there will be effervescence; filter; evaporate to small bulk, and add two drops of a neutral solution of perchloride of iron; a violet colour indicates tyrosin. *Hoffmann's test:* Dissolve substance in water; add several drops of a neutral solution of mercuric nitrate; boil, and a rose-coloured fluid with a reddish precipitate indicates tyrosin.

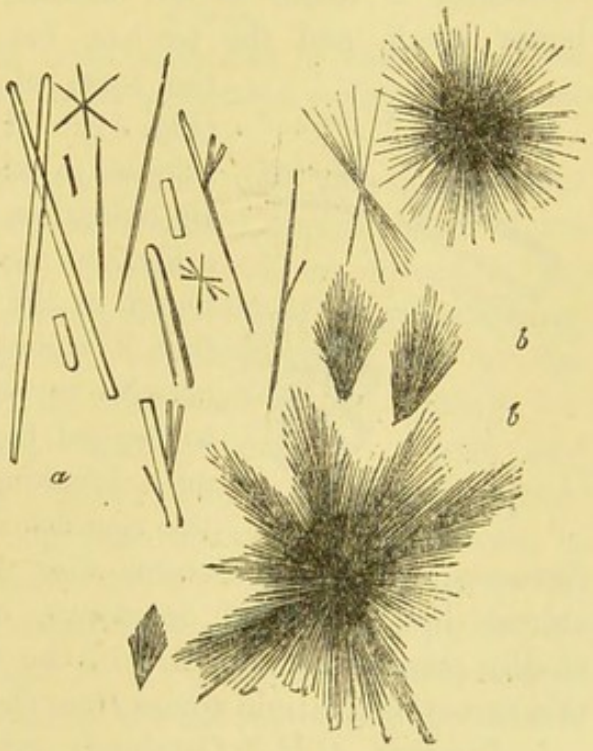
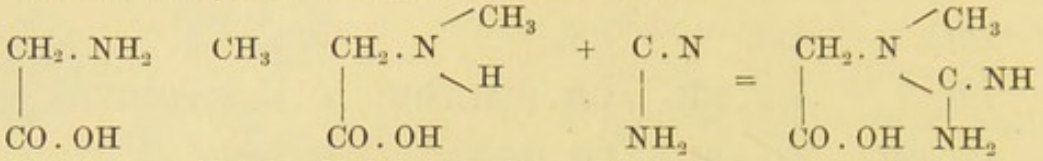
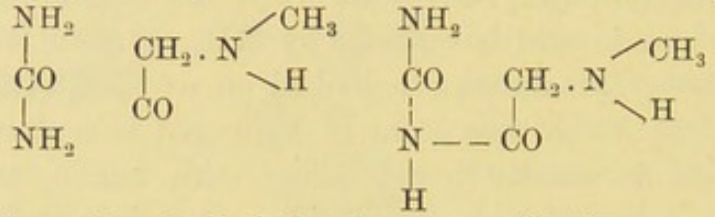


FIG. 30.—Acicular crystals of tyrosin. *a*, Single crystals; *b*, *b*, smaller and larger groups of the same.

4. *Creatin*, $C_4H_9N_3O_2 + H_2O$, is related to another amide, sarcosin, (methyl-glycocin, or amido-methyl-acetic acid). This substance, sarcosin, united to cyanamide, gives creatin, thus—



Another view is that it is related to urea, in which one atom of hydrogen is replaced by sarcosin, less one molecule of hydroxyl—



This view is supported by the fact that creatin boiled with baryta water yields sarcosin and urea—

$$\underset{\text{Creatin.}}{C_4H_9N_3O_2} + H_2O = \underset{\text{Sarcosin.}}{C_3H_7NO_2} + \underset{\text{Urea.}}{CON_2H_4}$$

Creatin is found in the muscles, nervous tissues, the blood, the liquor amnii, and the testicle, but not in glands. It appears in the form of colourless rhombohedric prisms (Fig. 31). It is soluble in water, almost insoluble in alcohol; its solutions are neutral. By oxidation, creatin gives oxalic and carbonic acids and methyluramine, $C_2H_7N_3$. Although found in the muscles, it has not been proved that it is increased in amount by causing the muscles to contract, and its presence appears to depend more on the nature of the diet than on a breaking down of the proteid matter of the muscles during activity. An animal diet increases, whilst a vegetable diet decreases its amount. With an animal diet, an allied substance, creatinin, into which creatin is readily transformed, appears in the urine, but it has been held that this excess of creatinin comes from the creatin in the animal food.

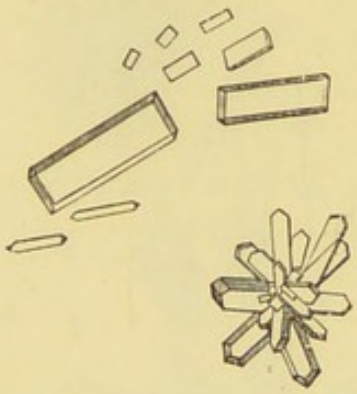
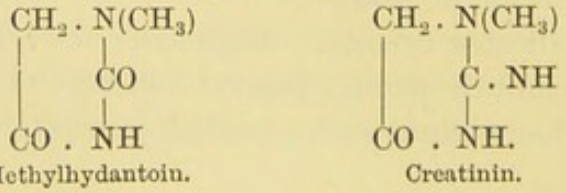


Fig. 31.—Creatin.

5. *Creatinin*, $C_4H_7N_3O$, closely related to creatin, is also related to a body named methylhydantoin, being transformed into it and ammonia when heated with baryta water to 100° C.—



It is readily derived from creatin. Thus, when creatin is heated with hydrochloric acid, or even when creatin is subjected to prolonged boiling, it parts with water, and creatinin is formed, thus—

$$\underset{\text{Creatin.}}{C_4H_9N_3O_2} = \underset{\text{Creatinin.}}{C_4H_7N_3O} + H_2O$$

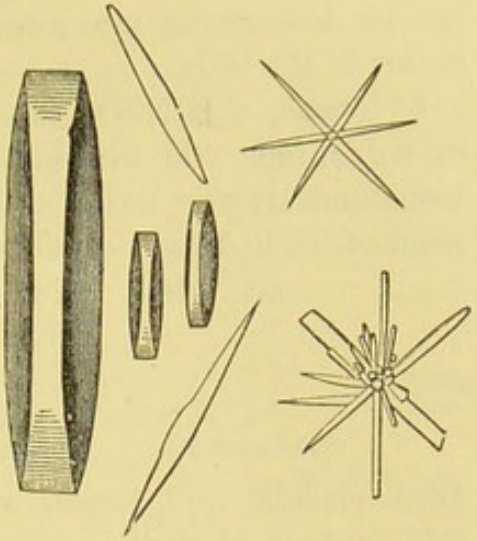


Fig. 32.—Creatinin.

Creatinin forms large, brilliant, colourless prisms, having an alkaline taste (Fig. 32). It is soluble in water and alcohol, scarcely soluble in ether. It has a strongly alkaline reaction. Oxidized, it yields methyluramine, $C_2H_7N_3$. A concentrated solution of chloride of zinc (not acid) produces a finely granular precipitate, or groups of fine needles or prisms, of a double chloride of zinc and creatinin; this chloride, treated with sulphide of ammonium, reproduces creatin by taking an equivalent of water—

$$\underset{\text{Creatinin.}}{C_4H_7N_3O} + H_2O = \underset{\text{Creatin.}}{C_4H_9N_3O_2}$$

Test.—A solution of creatinin, as in urine, acidulated by nitric acid, gives with phospho-molybdic acid a yellow crystalline precipitate, soluble in hot nitric acid.

The transformation of creatin into creatinin does not occur in the blood (which contains no creatinin), and it would appear that it occurs in the kidneys, as there is always creatinin and not creatin in the urine. Ligature of the ureters, however, is not followed by the appearance in the kidney of creatinin, but of creatin, pointing to the transformation happening, not in the substance of the kidney, but in the urine. As to the possible transformation of creatin into urea, there is conflicting evidence. Munk found that the administration of creatin to man and the dog was followed by an increase in the amount of creatinin in the urine, and also by an increase in the amount of urea. The change of creatinin into urea was supposed by Oppler, Zaleski, and others to occur in the kidney, as extirpation of the kidney was found by them to cause not an increase of the urea in the blood, but of creatin in the muscles. On the other hand, if the ureters were ligatured, urea accumulated in the blood and the amount of creatin in the muscles was normal. Similar experiments by Voit and Meissner, and by Voit and Gscheidlen have, however, given negative results. When one considers the severe nature of the operations, and the impossibility of the animals surviving for more than a few hours, it

is not surprising tha tresults should be contradictory. The matter then stands thus—that whilst in the laboratory by various methods creatin can be decomposed into sarcosin and urea, there is no proof that this occurs in the body.

6. *Taurin*, $C_2H_7NSO_3$, is an organic body related to isthionic acid, or sulphurous acid in which an atom of hydrogen is replaced by a monatomic radicle oxyethylene ($(CH_2)_2 . OH$), and in turn the replacement of the hydroxyl in isthionic acid by NH_2 produces taurin, thus —

$SO_2 . OH_2$	$CH_2 . OH$	$CH_2 . OH$	$CH_2 . NH_2$
	CH_2	CH_2	CH_2
		$SO_2 . OH$	$SO_2 . OH.$
Sulphurous acid.	Oxyethylene.	Isthionic acid.	Taurin.

With cholalic acid, taurin forms one of the bile acids, taurocholic acid, just as glycocin, unites with the same acid to produce glycocholic acid. Another point of resemblance between glycocin and taurin is that cyanic acid combines with glycocin to form hydantoïnic acid, whilst cyanic acid with taurin leads to tauro-carbamic acid, thus—

$CH_2 . NH_2$	$CH_2 . NH . CO$
$CO . OH$	$CO . OH \quad NH_2$
Glycocin.	Hydantoïnic acid.
$CH_2 . NH_2$	$CH_2 . HN . CO$
$CH_2 . SO_2 . OH$	$CH_2 . SO_2 . OH . NH_2$
Taurin.	Tauro-carbamic acid.

Tauro-carbamic acid exists in the urine and may originate by the union of taurin with cyanic acid. Taurin, arising from the decomposition of taurocholic acid, is found in small amount in the intestinal canal and fæces, and also in muscle, in the lungs, in the urine of the ox, and in the liver and spleen of certain fishes. Its origin is unknown. From the small amount in the fæces, and the relatively large amount found in the liver, it is probable that part of the taurin is decomposed into simpler substances. As potash splits it up into carbonate of ammonia and the sulphite and acetate of potash, it has been conjectured that the alkalies of the bile may so transform it, and that the sulphates of the urine may

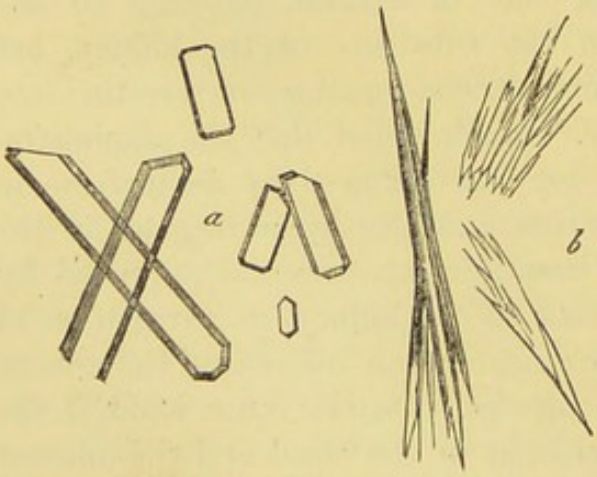
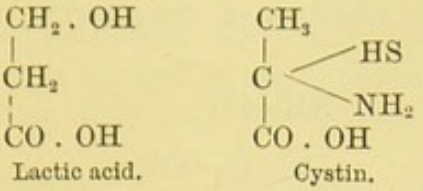


FIG. 33.—Taurin. *a*. Six-sided prisms; *b*. Irregular sheaf-like masses from an impure solution.

be partly derived from this source. Taurin crystallizes in the form of colourless six-sided prisms (Fig. 33). It is soluble in water, and is insoluble in alcohol and in ether.

7. *Cystin*, $C_3H_7NSO_2$, is amido-lactic acid, thus—



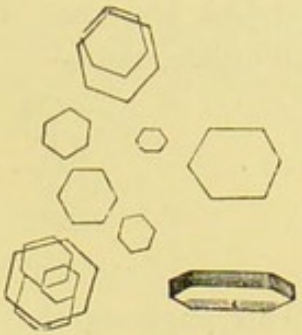


Fig. 34.—Cystin.

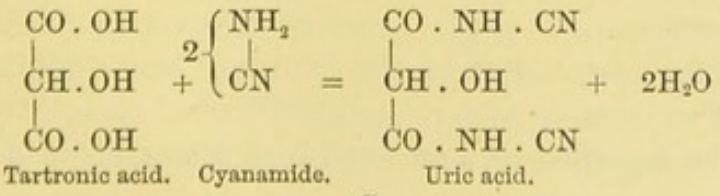
It is found as a constituent of a rare form of urinary calculus, and occasionally in the urine, more especially of the dog. Its origin is unknown. It forms rhombohedric or hexagonal colourless plates (Fig. 34). It is insoluble in water, alcohol, and ether, but is soluble, like uric acid, in ammonia, in the mineral acids, and in a solution of oxalic acid. If heated on a silver surface with a little caustic soda, it gives a brownish-black spot of sulphide of silver.

8. *Sarcosin*, $C_3H_7NO_2$, has been already referred to in treating of creatin, and we have seen that it is methyl-glycolle, $C_2H_4(CH_3)NO_2$. It has not been found in the body, but it may be obtained by heating creatin with baryta water. It crystallizes in rhombohedric colourless columns. It is very soluble in water, less so in alcohol, and not at all in ether.

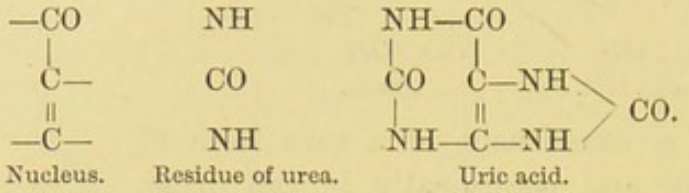
Chap. VII.—THE NITROGENOUS ACIDS.

1. *Sulphocyanic Acid*, CNHS, united to potassium or sodium to form a sulphocyanide, is almost invariably found in the saliva, and it has occasionally been found in the urine, in milk, and in the blood. It may be detected by giving a red colour (sulphocyanide of iron) with a solution of perchloride of iron. Some have supposed that it may originate from the decomposition of carious teeth, but it has been found where this condition did not exist.

2. *Uric Acid*, $C_5H_4N_4O_3$, is one of the most important bodies of this group, inasmuch as it is related to a number of nitrogenized substances, often met with in the solids and fluids. Much diversity of view still exists as to its chemical nature. Baeyer regards it as formed by the union of a radicle cyanamide with tartronic or oxymalonic acid—

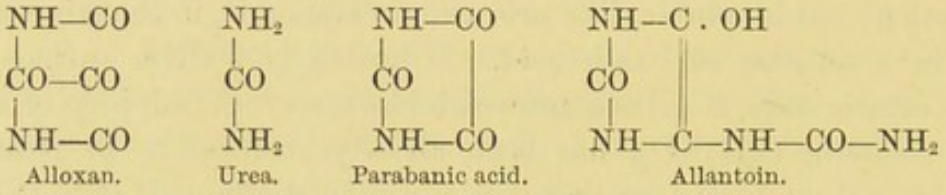


or, two molecules of hydroxyl in tartronic acid are replaced by two cyanamides, which each lose an atom of hydrogen, and these, with the molecules of hydroxyl, form water. Another view put forward by Medicus is to consider uric acid as containing a nucleus of 3 atoms of carbon, and that this with two residues of urea forms uric acid or a di-ureide, thus—

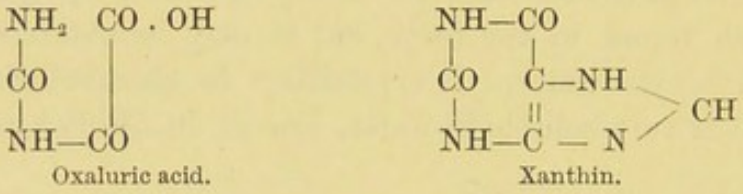


Nucleus. Residue of urea. Uric acid.

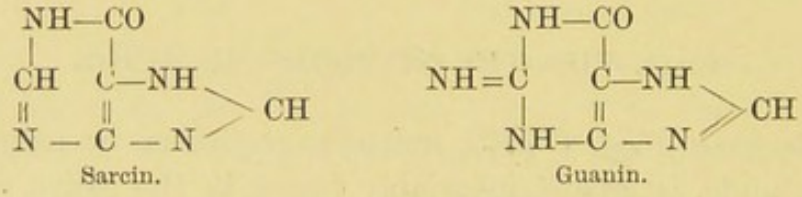
This suggests the relationship of a number of the bodies allied to uric acid, such as alloxan, urea, parabanic acid, allantoin, oxaluric acid, xanthin, sarcin, and guanin, thus—



Alloxan. Urea. Parabanic acid. Allantoin.



Oxaluric acid. Xanthin.



Sarcin. Guanin.

Most oxidizing substances convert uric acid into alloxan or parabanic acid; and when boiled with water and peroxide of lead, it yields allantoin. When oxidized in the presence of water it gives up two of its hydrogen atoms to the oxidizing agent, while the dehydrogenized residue (dehyduric acid) reacts with water to form mesoxalic acid and urea—

$$\underset{\text{Dehyduric acid.}}{C_5H_2N_4O_5} + 4H_2O = \underset{\text{Mesoxalic acid.}}{C_3H_2O_5} + \underset{\text{Urea.}}{2CN_2H_4O}$$

Dilute nitric acid acts upon uric acid and produces alloxan, and this when heated with baryta water is resolved into mesoxalic acid and urea—

$$\underset{\text{Dehyduric acid.}}{C_5H_2N_4O_5} + 2H_2O = \underset{\text{Alloxan.}}{C_4N_2H_2O_4} + \underset{\text{Urea.}}{CN_2H_4O}$$

$$\underset{\text{Alloxan.}}{C_4N_2H_2O_4} + H_2O = \underset{\text{Mesoxalic acid.}}{C_3H_2O_5} + \underset{\text{Urea.}}{CN_2H_4O}$$

Alloxan may be regarded as a *mon-ureide* of mesoxalic acid formed by

the union of one molecule of the acid with one molecule of urea, less two of water, and formed thus—

$$\underset{\text{Mesoxalic acid.}}{C_3H_2O_5} + \underset{\text{Urea.}}{CN_2H_4O} - 2H_2O = \underset{\text{Alloxan.}}{C_4N_2H_2O_4}$$

Again, the hypothetical dehyduric acid is a *di-ureide* of mesoxalic acid, that is, it is formed by the union of one molecule of mesoxalic acid with two molecules of urea, less four molecules of water, thus—

$$\underset{\text{Mesoxalic acid.}}{C_3H_2O_5} + \underset{\text{Urea.}}{2(CH_4N_2O)} - 4H_2O = \underset{\text{Dehyduric acid.}}{C_5H_2N_4O_3}$$

By hydrogenizing mesoxalic acid, tartronic acid is produced; and by hydrogenizing alloxan, we obtain another compound known as dialuric acid. These bodies, tartronic acid and dialuric acid, bear the same relation to uric acid as mesoxalic acid and urea bear to dehyduric acid, thus—

$C_3H_2O_3$	$C_4N_2H_2O_4$	$C_5N_4H_2O_3$
Mesoxalic acid.	Alloxan.	Dehyduric acid.
$C_3H_4O_5$	$C_4N_2H_4O_4$	$C_5N_4H_4O_3$
Tartronic acid.	Dialuric acid.	Uric acid.

Thus we have three groups of bodies related to uric acid: (*a*) *an-ureides*, non-nitrogenous acids, such as tartronic and mesoxalic acids; (*b*) *mon-ureides*, consisting of a residue of the acid added to one residue of urea, such as dialuric acid and alloxan; and (*c*) *di-ureides*, consisting of a residue of the acid added to *two* residues of urea, such as uric acid itself.

It is important also to observe the origin of other bodies belonging to these groups. Thus mesoxalic acid, the most complex non-nitrogenous product that can be obtained by the oxidation of uric acid, is one of a series of *an-ureides*, each of which contains one CO group more than the preceding, thus—

CH_2O_3 Carbonic acid.
$C_2H_2O_4$ Oxalic acid.
$C_3H_2O_5$ Mesoxalic acid.

Acted on by nascent oxygen, mesoxalic acid loses its excess of CO and becomes oxalic acid, thus: $C_3H_2O_5 + O = CO_2 + C_2H_2O_4$. Hence when uric acid is oxidized beyond the point of producing mesoxalic acid, oxalic acid may be formed, and this may conjugate with one mol. of urea to form a mon-ureide, parabanic acid, or with two mol. of urea to form a di-ureide, mycomelic acid. If the oxidation of uric acid proceeds further than the oxalic acid stage, carbonic acid is formed, and this is also theoretically capable of forming ureides. Thus allophanic acid is the mon-ureide of carbonic acid, but no di-ureide exists. Alloxan, the mon-ureide of mesoxalic acid, is formed from mesoxalate of urea by the elimination of two mol. of water, but if only one mol. of water is removed, a

related body, alloxanic acid, is produced. Similarly, oxalic acid yields two mon-ureides, parabanic acid and oxaluric acid. The relation of these bodies is seen in the following table, modified from that given by Odling. (*Lectures on Animal Chemistry*, p. 133.)

An-ureides.	Mon-ureides.	Di-ureides.
CH_2O_2 Carbonic.	$C_2N_2H_4O_3$ Allophanic.	—
$C_2H_2O_3$ Glyoxalic.	$C_3N_2H_4O_3$ Lantanuric.	$C_4N_4H_6O_3$ Allantoin.
$C_2H_2O_4$ Oxalic.	$C_3N_2H_4O_4$ Oxaluric.	—
—	$C_3N_2H_2O_3$ Parabanic.	—
—	—	$C_5N_4H_4O$ Hypoxanthin.
$C_3H_4O_4$ Malonic.	$C_4N_2H_4O_3$ Barbituric.	$C_5N_4H_4O_2$ Xanthin.
$C_3H_4O_5$ Tartronic.	$C_4N_2H_4O_4$ Dialuric.	$C_5N_4H_4O_3$ Uric acid.
$C_3H_2O_5$ Mesoxalic.	$C_4N_2H_2O_4$ Alloxan.	—

The substance termed alloxantin is formed by the union of two consecutive mon-ureides, dialuric acid and alloxan, with the elimination of water, thus—

$$\underset{\text{Dialuric acid.}}{C_4N_2H_4O_4} + \underset{\text{Alloxan.}}{C_4N_2H_2O_4} - H_2O = \underset{\text{Alloxantin.}}{C_8N_4H_4O_7}.$$

It will also be seen that xanthin is the di-ureide of barbituric acid, which again is the mon-ureide of malonic acid, and this in turn can be obtained from tartronic acid by deoxidation. Closely related to xanthin is hypoxanthin. Uric acid deoxidized by sodium amalgam yields a mixture of xanthin and hypoxanthin, and hypoxanthin may be converted into xanthin by oxidation with nitric acid.

Uric acid is found in the urine, either in the free condition to a very small extent or in the form of the *urates of potash and soda*, perhaps the most common forms of urinary deposit. The daily amount eliminated by an adult man with a mixed diet is from ·5 to 1 gram.; with a vegetable diet it may fall to ·3 gram., and with a rich animal diet it may amount to as much as 1·5 gram. It also is met with in urinary calculi and in the chalky deposits in the joints of gouty persons. Traces also are found in the blood, kidneys, spleen, lungs, brain, and muscular tissue. It forms a large part of the excrement of birds and of reptiles.

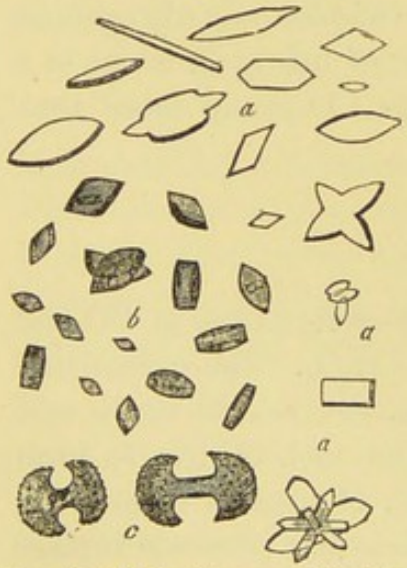


FIG. 35.—Uric acid. *a*, *a*, Rhombs and rectangular prisms; *b*, forms from human urine; *c*, dumb-bell crystals. In right hand corner, rosette.

Pure uric acid crystallizes in colourless rhombic rectangular or hexagonal plates, or in rectangular prisms. When obtained from urine, it is coloured more or less by the urinary pigment, and forms rhombs, lozenges, or rectangular prisms, and these are grouped so as to

form rosettes, barrels, or dumb-bell like forms. (See Fig. 35.) Uric acid is tasteless and inodorous. Its solubility is small as it requires for its solution 1,900 parts of cold water and 15,000 parts of hot water; it is insoluble in alcohol and ether. Its solutions are feebly acid. It is dibasic.

There are several well known salts of uric acid.

(*a*) *Urates of soda.* These form the chief part of the deposit of urates commonly found in urine and are readily recognized by their disappearance on heating. There are two urates of soda—the neutral salt, $C_5H_2N_4O_3Na_2$, which forms nodular masses, and the acid urate, $C_5H_3N_4O_3Na$, which is usually amorphous and rarely crystallizes in urine. The latter is soluble in 1,200 parts of cold water and in 25 parts of boiling water. The chalky deposits in the joints of gouty persons are almost entirely composed of this salt, and from these it may be obtained in forms represented in Fig. 36.

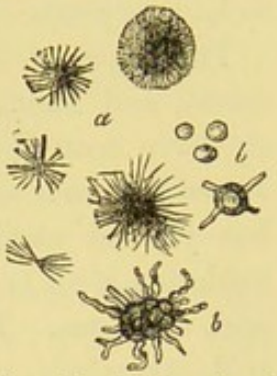


FIG. 36.—Acid urate of soda. *a*, Needles, usually aggregated; *b*, *b*, spheroidal masses.

(*b*) *Urates of potash* corresponding to those of soda may be made by saturating a solution of potassium hydrate with uric acid, when they may be crystallized as fine needles, but they do not often occur in urinary sediments.

(*c*) *Acid urate of ammonia*, $C_5H_3N_4O_3(NH_4)$, is found in alkaline urine, either as a pinkish amorphous powder or in the form of globular masses having little spicules proceeding from them (Fig. 37). The neutral salt is unknown.

FIG. 37.—Urate of ammonia.

(*d*) *Acid urate of lime*, $(C_5H_3N_4O_3)_2Ca$, occurs in urinary sediments and calculi, and appears in the form of fine needles.

(*e*) *Acid urate of lithia*, $C_5H_3N_4O_3Li$, is the most soluble salt of uric acid. "It may be artificially prepared by dissolving uric acid in a warm solution of lithium carbonate. It crystallizes in needles, which dissolve in 60 parts of water at 50° C., and do not separate when the solution is cooled." (Witthaus, *Manual of Chemistry.*)

Tests for Uric Acid.—(1) *Murexide test:* Put a little of the substance to be examined on a white porcelain plate or lid, add two drops of nitric acid, heat and evaporate till dry; if the substance is uric acid, it dissolves in nitric acid, and gives on evaporation a yellow or reddish-yellow residue, which becomes reddish-purple by adding a drop of caustic ammonia, and bluish-violet with soda or potash. The purple colour is readily brought out by bringing a glass rod dipped in ammonia solution near the residue after evaporation. (2) Dissolve the substance to be examined in a few drops of solution of caustic soda, and filter; add

to the liquid chloride of ammonium in excess, and there will be a precipitate of urate of ammonia which, by the addition of hydrochloric acid, yields crystals of uric acid. (3) The microscopic examination of the crystals so as to determine their form. (4) *Garrod's test:* To detect uric acid in serum, place about 2 c.c. of the serum in a flat watch-glass and acidulate with acetic acid; a fine fibril of linen thread is placed in the liquid, which is set aside and allowed to evaporate for two or three hours; the fibril is then examined microscopically when it will be found to have crystals of uric acid adherent to it.

The *origin* of uric acid is still doubtful. Its relationship to guanin, sarcin, and xanthin has already been pointed out. Guanin and sarcin have been changed into xanthin, but the latter has not been converted into uric acid. Uric acid, however, reduced by sodium amalgam, has yielded sarcin and xanthin, and the ultimate products of the oxidation of all four bodies are the same, namely, parabanic acid, oxaluric acid, and urea. These four bodies—guanin, sarcin, xanthin, and uric acid—therefore, probably represent four stages of oxidation, of which uric acid is the last.

Uric acid forms the principal product of the decomposition of nitrogenous matters in animals so different as birds and reptiles. In the bird there is rapid oxidation and a high temperature, while the reverse conditions exist in the reptile, and still the chief nitrogenous substance in the excrements of both classes is uric acid, a fact for which no satisfactory explanation can be offered. Even urea in the bird appears to be changed into uric acid, as it has been found that the ingestion of urea by fowls is followed by an increased elimination of uric acid. On the other hand, the ingestion of sarcosin by fowls diminishes the amount of uric acid, and in other animals, inhalations of oxygen or of protoxide of nitrogen, the administration of sulphate of quinine, and a vegetable diet, also diminish it. Quinic acid and benzoic acid and an animal diet increase the amount of uric acid eliminated.

Further, it is supposed that a portion of the uric acid formed is eliminated as such, while another portion is transformed by oxidation into other substances. Thus it may readily be changed, first into alloxan, then into parabanic acid, then into oxaluric acid, then into oxalic acid and urea, and ultimately into carbonic acid and water, thus—

(1) $$\underset{\text{Uric acid.}}{C_5H_4N_4O_3} + H_2O + O = \underset{\text{Alloxan.}}{C_4H_2N_2O_4} + \underset{\text{Urea.}}{N_2H_4CO}.$$

(2) $$\underset{\text{Alloxan.}}{C_4H_2N_2O_4} + O = \underset{\text{Parabanic acid.}}{C_3H_2N_2O_3} + CO_2.$$

(3) $$\underset{\text{Parabanic acid.}}{C_3H_2N_2O_3} + H_2O = \underset{\text{Oxaluric acid.}}{C_3H_4N_2O_4}.$$

(4) $$\underset{\text{Oxaluric acid.}}{C_3H_4N_2O_4} + H_2O = \underset{\text{Urea.}}{CN_2H_4O} + \underset{\text{Oxalic acid.}}{C_2H_2O_4}.$$

(5) $$\underset{\text{Oxalic acid.}}{C_2H_2O_4} + O = 2CO_2 + H_2O.$$

Another view is that uric acid by oxidation is changed into allantoin and that this substance is resolved by successive stages, in which new compounds appear, into urea and parabanic acid, the latter being then split up into urea, carbonic acid, and water, as has already been shown, thus—

(1) $$\underset{\text{Uric acid.}}{C_5H_4N_4O_3} + H_2O + O = \underset{\text{Allantoin.}}{C_4H_6N_4O_3} + CO_2.$$

(2) $$\underset{\text{Allantoin.}}{C_4H_6N_4O_3} + H_2O = \underset{\text{Urea.}}{CN_2H_4O} + \underset{\text{Allanturic acid.}}{C_3H_4N_2O_3}.$$

(3) $$2(\underset{\text{Allanturic acid.}}{C_3H_4N_2O_3}) = \underset{\text{Parabanic acid.}}{C_3H_2N_2O_3} + \underset{\text{Hydantoïnic acid.}}{C_3H_6N_2O_3}.$$

Allanturic, parabanic, and hydantoïnic acids have not been found in the body, but allantoin is met with in the urine of the newly born. Further, Salkowski has found that the ingestion of uric acid by dogs is followed by the appearance of allantoin in the urine. The evidence both from chemical considerations and the observation of some phenomena of disease is in favour of the view that a portion of uric acid may be resolved into urea, and that this transformation is aided by active oxidations.

The substances specially related to uric acid are the following—

(1) *Guanin*, $C_5H_5N_5O$, has been found in the liver and pancreas. It is a white or yellowish, amorphous, odourless, tasteless substance, almost insoluble in water, alcohol, and ether, but easily soluble in acids and alkalies. Evaporated on a bit of platinum foil with a drop of fuming nitric acid, it gives a yellowish residue which becomes red on the addition of a drop of caustic soda, and gives a purple colour on heating. Derived from the splitting up of albuminous matters, it probably gives origin to xanthin and ultimately to urea.

(2) *Sarcin or Hypoxanthin*, $C_5H_4N_4O$, has been found in the spleen, pancreas, supra-renal capsules, muscles, liver, marrow of bone, the urine, the liver in acute yellow atrophy, and the blood and urine of leucocythæmia. It occurs in the state of nodular masses formed of fine colourless needles, soluble in 300 parts of cold and 78 parts of boiling water. (Witthaus.) Oxidized by nitric acid it gives xanthin, thus—

$$\underset{\text{Hypoxanthin.}}{C_5H_4N_4O} + O = \underset{\text{Xanthin.}}{C_5H_4N_4O_2}.$$

(3) *Xanthin*, $C_5H_4N_4O_2$, sometimes termed *xanthic oxide*, is found in a rare form of urinary calculus, in human urine after the use of sulphur

baths and inunctions, and in the pancreas, spleen, liver, thymus, and brain. It is a pale yellow amorphous powder, slightly soluble in cold water, insoluble in alcohol and ether, and soluble in ammonia. Dissolved in a drop or two of nitric acid and evaporated, a yellowish residue is left which turns reddish-yellow on the addition of a drop of solution of caustic potash, and violet-red on being heated. Calculi of xanthin vary in size from that of a pea to that of a pigeon's egg. They are hard, brownish-yellow, smooth, shining, and show well-defined concentric layers. Their broken surface assumes a waxy polish when rubbed.

(4) *Allantoin* and *oxaluric acid* have been described. (See p. 88.)

(5) *Carnin*, $C_7H_8N_4O_3 + H_2O$, occurs in the form of chalky crystals, readily soluble in warm water but insoluble in alcohol and ether. It is intimately related to sarcin and it may be considered as a compound of that body and acetic acid, thus—

$$\underset{\text{Sarcin.}}{C_5H_4N_4O} + \underset{\text{Acetic acid.}}{C_2H_4O_2} = \underset{\text{Carnin.}}{C_7H_8N_4O_3}$$

It may be obtained from extract of meat. Doses of from ·05 to ·2 gramme exert a slightly depressing influence on the heart.

3. *Hippuric acid*, $C_9H_9NO_3$, or benzyl-glycocol, is glycocolle in which one atom of hydrogen has been replaced by the radicle benzyl, $C_6H_5.CO$, thus—

$$\underset{\text{Glycocolle.}}{\begin{matrix} CH_2-NH_2 \\ | \\ CO.OH \end{matrix}} \qquad \underset{\text{Hippuric acid.}}{\begin{matrix} CH_2.N(C_6H_5.CO)H \\ | \\ CO.OH \end{matrix}}$$

It is isomeric with acetamidobenzoic acid, $C_7H_6(C_2H_3O)NO_2$. When heated with the mineral acids, or the alkalies, or when influenced by certain ferments, such as that of decomposing urine, hippuric acid is converted into glycocolle and benzoic acid—

$$\underset{\text{Hippuric acid.}}{C_9H_9NO_3} + H_2O = \underset{\text{Glycocolle.}}{C_2H_5NO_2} + \underset{\text{Benzoic acid.}}{C_7H_6O_2}$$

Hippuric acid is a constant constituent of the urine of herbivora and of human urine in the form of hippurate of soda ($C_9H_8NaNO_3.H_2O$) or of lime $Ca(C_9H_8NO_3)_2.3H_2O$. Only a very small amount exists in urine in the free state. It crystallizes in transparent, colourless prisms (Fig. 38). By slow evaporation from dilute solutions, crystals are obtained similar to those of the triple phosphate of ammonia and magnesia. (Frey.) It is soluble sparingly in water, and more readily in hot alcohol or ether. It dissolves unchanged in hydrochloric acid, and on boiling the solution it is converted into benzoic acid and glycocolle. The following are the tests by which hippuric acid may be

readily distinguished: (1) *Lücke's test:* Evaporate with excess of nitric acid; heat residue strongly, and an odour of hydrocyanic acid may be perceived. (2) It gives a brown precipitate with ferric chloride. (3) Heated with lime, it gives off benzin and ammonia. Hippuric acid may be formed from matters supplied in the food or from the decomposition of albuminous matters in the body. It is well known that it appears in the urine after the administration of benzoic acid, or of benzoates, or of articles of food containing benzoic acid, and this can be explained by assuming that it unites with glycocin, with the loss of a molecule of water, thus—

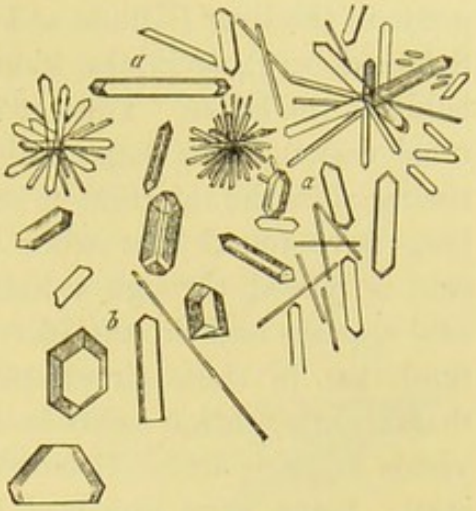


FIG. 38.—Hippuric acid. *a*, *a*, Prisms; *b*, crystals like those of the triple phosphate.

$$\underset{\text{Benzoic acid.}}{C_7H_6O_2} + \underset{\text{Glycocin.}}{C_2H_5NO_2} - H_2O = \underset{\text{Hippuric acid.}}{C_9H_9NO_3}$$

Marchand, after taking 30 grains of benzoic acid, found 39·2 grains of hippuric acid in the urine. It is also found in the urine after the ingestion of cinnamic and mandelic acids. It would appear also that this reaction is the type of others. Thus, such aromatic acids as toluilic, anisic, and salicylic acids unite in the body with glycocin to form toluric, anisuric, and salicyluric acids. Quinic acid is changed into hippuric acid in the organism, probably first into benzoic acid. The large amount of hippuric acid in the urine of herbivora has not received a satisfactory explanation. In this case it is not derived from benzoic acid or quinic acid, as both of these substances are usually absent from the food of herbivora. Shepard and Meissner have maintained that it is derived from the cuticular matter of plants, and from cellulose, and they state that the amount of hippuric acid is increased by a diet rich in such matter, such as the straw of cereals, whilst it is diminished by a diet of potatoes, carrots, or beet-root, substances rich in starch but containing little of the cuticular matter. These statements have not been unquestioned, and it has been pointed out that much may depend on the kind of animal, as the straw of oats increases hippuric acid in the urine of the ox, but not in that of the rabbit. (See Hippuric acid, Watt's *Dict. of Chem.*, 2nd supplement, p. 647.)

As hippuric acid is always found in small quantity in the urine even with an animal diet, it has been supposed that it may originate from the decomposition of proteid matter. It is conceivable that such a decomposition may give rise to benzoic acid and glycocolle; and, in

that case, hippuric acid would probably be formed. This union may occur in the liver (Kühne and Hallwachs), or in the tissues (Bunge and Schmiedeberg), or in the kidneys (Shepard and Meissner)—most probably in the latter. Thus, it has been found that after ligature of the renal vessels in the dog, no hippuric acid appears in the liver or in the tissues after the injection of benzoic acid. Again, Bunge and Schmiedeberg have found that when blood containing glycocolle and benzoic acid is passed through a kidney by an artificial circulation, hippuric acid appears both in the blood returning from the kidney and in the fluid that in these circumstances may exude from the ureter. No definite information exists as to the substance in the blood that thus yields hippuric acid. Coloured blood corpuscles and oxygen are necessary. Some have supposed that it may arise from the oxidation of tyrosin; and others, that a substance exists which may, in certain conditions, become urea, and, in other conditions, hippuric acid. (Beaunis.)

4. *Inosinic* or *Inosic acid*, $C_{10}H_{14}N_4O_{11}$, is a substance found in the mother liquor of the preparation of creatin from muscle juice. It is uncrystallizable; it is readily soluble in water, and alcohol precipitates it from its aqueous solution. The nature and properties of this substance have not been fully investigated.

5. *Cryptophanic acid*, $C_{10}H_{18}N_2O_{10}$, is said by Thudichum to exist in human urine. It is an amorphous, gummy mass, transparent and nearly colourless, readily soluble in water, and less soluble in alcohol. It is said to have feeble acid properties. (See *Journal of Chemical Society* [2] viii. 132; see also Beaunis, *Physiologie*, vol. i. p. 138.)

6. *Bile acids.* The bile of most animals contains the sodium salts of amido-acids of complex constitution. Of these acids, two are found in the bile of man, namely, glycocholic acid and taurocholic acid, and these in turn are related to two others, cholalic acid and choloidic acid.

(*a*) *Glycocholic acid*, $C_{26}H_{43}NO_6$, the *cholic acid* of Gmelin, exists in human bile as glycocholate of soda in amounts varying according to diet. An animal diet diminishes the quantity, while a vegetable diet has the contrary effect. It is therefore readily found in ox bile and in the bile of herbivora generally, and the amount is smaller in that of carnivora. When obtained from ox bile, it forms brilliant, colourless, transparent needles, sparingly soluble in cold water, readily soluble in hot water and in alcohol, glycerine, and acetic acid, whilst it is almost insoluble in ether. The taste is at first sweet, and afterwards intensely bitter. The alcoholic solution is dextro-rotatory when examined with the polariscope, $[a]_D = +29°$. By the action of potash, baryta, dilute sulphuric acid, or dilute hydrochloric acid, it takes up a

molecule of water, and then is decomposed into glycocin and cholalic acid, thus—

$$\underset{\text{Glycocholic acid.}}{C_{26}H_{43}NO_6} + H_2O = \underset{\text{Cholalic acid.}}{C_{24}H_{40}O_5} + \underset{\text{Glycocin.}}{C_2H_5NO_2}$$

Sodium glycocholate, $C_{26}H_{42}NaNO_6$, crystallizes in stellate needles (Fig. 39), is readily soluble in water, less so in alcohol, and is insoluble in ether. With polarized light $[a]_D = +25°{\cdot}7$. Hamersten states that the glycocholic acid of man differs slightly from that of the ox. The bile of sea fishes contains the glycocholates and taurocholates of potash, not of soda, and chiefly in the form of the soda salt.

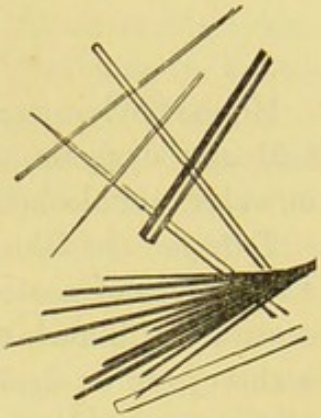


Fig. 39.—Glycocholate of soda.

(*b*) *Taurocholic acid*, $C_{26}H_{45}NO_7S$, exists in human bile and in the bile of carnivora; in small amount in the bile of herbivora. It is easily obtained from dog's bile, in the form of silky needles, deliquescent, and readily changed into an amorphous resinous mass. It is soluble in water and alcohol, forming intensely bitter solutions. The alcoholic solution gives with polarized light $[a]_D = +24°{\cdot}5$. Barium hydrate and acids give the following decomposition—

$$\underset{\text{Taurocholic acid.}}{C_{26}H_{45}NO_7S} + H_2O = \underset{\text{Cholalic acid.}}{C_{24}H_{40}O_5} + \underset{\text{Taurin.}}{C_2H_7NO_3S}$$

The same decomposition occurs in the presence of putrefying material, and in the intestine. (Witthaus.) *Taurocholate of soda*, $C_{26}H_{44}NaNO_7S$, is soluble in alcohol and in water, and is very difficult to crystallize. When the glycocholates and taurocholates exist together in an aqueous solution, they may be separated by dilute sulphuric acid and a small quantity of ether, which precipitates glycocholic acid alone.

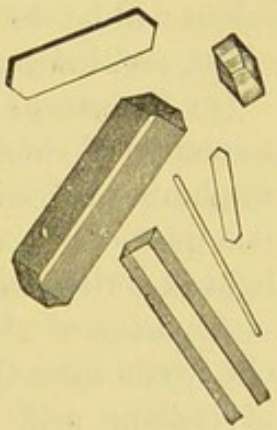


Fig. 40.—Cholalic acid.

(*c*) *Cholalic acid*, $C_{24}H_{40}O_5$, the result of the decomposition of the conjugate bile acids, as already explained, is found in the intestines and fæces in the form of large, shining, deliquescent crystals (Fig. 40), slightly soluble in water, and readily soluble in alcohol and ether. With polarized light, $[a]_D = +35°$.[1] By boiling with acids or heated to 200° C., cholalic

[1] Hoppe-Seyler gives for cholic acid + aq., 31°·2; for cholic acid alone, 50°·2; for sodium cholate dissolved in alcohol, 31°·4; potassium cholate, 30°·8; for sodium cholate in water, 26°; and for potassium cholate, 25°. (Jl. for Chem. lxxxix. 257; Bull. Soc. Chem. v. 622, quoted in Watt's *Dict. of Chem.* vol. vi. p. 342.

acid may lose one molecule of water, and become (*d*) *choloidic acid*, or (*e*) *dyslysin*. Thus—

$$\underset{\text{Cholalic acid.}}{C_{24}H_{40}O_5} - H_2O = \underset{\text{Choloidic acid.}}{C_{24}H_{38}O_4}$$

$$\underset{\text{Cholalic acid.}}{C_{24}H_{40}O_5} - 2H_2O = \underset{\text{Dyslysin.}}{C_{24}H_{36}O_3}$$

Hoppe-Seyler regards choloidic acid as merely a mixture of cholalic acid and dyslysin, the latter being a neutral resinous body, insoluble in water and alcohol, and only sparingly soluble in ether.

Tests for the bile acids. (*α*) *Pettenkofer's test.*—Add to the liquid a few drops of a strong solution of cane sugar and a few drops of concentrated sulphuric acid, and keep at a temperature of about 70° C., and a cherry-red or dark reddish-purple colour appears. The presence of nitrates and chlorates delays the reaction. Albuminoids, lecithin, oleic acid, cerebrin, phenol, turpentine, benzin, tannic acid, salicylic acid, morphia, codeia, amyl-alcohol, cod-liver oil, and camphor give a similar reaction. The red liquid produced by the reaction is dichroic, and gives two absorption bands between F and E. When examined by the spectroscope, the red liquid formed by the action of strong sulphuric acid on albuminoids is not dichroic, and those with oleic acid and amyl-alcohol show no absorption bands. Still, as stated by Witthaus, the spectroscopic appearances with different fluids are not sufficiently definite to serve as a check to the Pettenkofer reaction.

(*β*) *Bogomoloff's test.*—Evaporate an alcoholic solution to dryness; spread out the residue on a white plate, and wet with one or two drops of concentrated sulphuric acid and one drop of alcohol. Shades of colour will be shown from centre to circumference—yellow, orange, red, violet, and indigo.

(*γ*) *Strassburg's test.*—Dip a morsel of filter paper in fluid, then into a solution of cane sugar, allow it to dry, and let a drop of concentrated sulphuric acid trickle down a glass rod to the centre of the paper; in a quarter of a minute the spot will become translucent and transmit light of a violet colour.

Physiological characters of the bile acids.—From their constitution, one would infer that the bile acids are formed in the liver by the union of cholalic acid and taurin or glycocin. Glycocin and taurin, as already explained, may originate from the decomposition of albuminoids, and it is probable that cholalic acid has the same origin. This view receives some support from the similar behaviour of albuminoids, the bile acids, and cholalic acids towards sugar and concentrated sulphuric acid. Some have, on the contrary, supposed that the non-nitrogenous cholalic acid may be derived from fats. After their formation, the acids pass into

the intestine, and there they are partially decomposed into cholalic acid, choloidic acid, dyslysin, glycocin, and taurin, and some of these products are reabsorbed into the blood. The remainder is voided in the fæces. In the blood, or in the tissues, the bile acids and their derivatives are no doubt decomposed still further. At all events, when injected into the blood—in cases of biliary fistulæ in rabbits, by which it was possible to collect the bile—it was found that only a third or a fourth part of the acid injected appeared in the bile, the remainder disappearing. Injections of the bile acids into the blood cause destruction of red blood corpuscles, slowing of the pulse and of the respiratory movements, fall of arterial pressure, and lowering of the bodily temperature. Doses of from 2 to 4 grammes given to a dog, caused epileptiform convulsions, the passing of very dark-coloured urine, and death. The biliary acids may be detected in the blood and urine in jaundice and acute atrophy of the liver. Several important physiological questions relating to the action of the bile acids will be discussed in treating of the bile.

Chap. VIII.—NITROGENOUS BODIES CONTAINING NO OXYGEN.

1. *Trimethylamine*, $(CH_3)_3N$, occurs normally in human urine, and it has been found in the blood of the calf, in cod-liver oil, ergot, chenopodium, yeast, guano, herring-brine, and in many flowers. It is an oily fluid, having an odour of fish, boils at 9° C, is soluble in water, alcohol, and ether, and has an alkaline reaction. It is also found among the products of the decomposition of albuminous bodies.

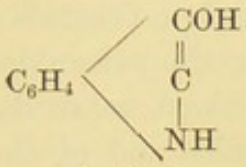
2. *Naphthylamine*, $C_{10}H_9N$, has been found among the oxidation products of albuminous matter (Schützenberger), and naphthalene ($C_{10}H_8$) in minute quantity has been detected in the urine. (Hoppe-Seyler.)

3. *Indol*, C_8H_7N, belongs to the group of aromatic bodies related to indigo; indeed, it may be regarded as the nucleus of the indigo group. Thus various plants yield indigo, or indigo-blue, indigotin, C_8H_5NO. This, by the action of reducing agents, becomes indigo-white, or indigogen, C_8H_6NO. Again, a third body, isatin, $C_8H_5NO_2$, is produced by the action of nitric or chromic acid on indigo,—$C_8H_5NO + O = C_8H_5NO_2$. The parent of these three bodies is indican, $C_{26}H_{31}NO_{17}$, which is a colourless substance existing in the plants that yield indigo. Indican, when boiled with acids, splits up into indigo-blue and a substance named indiglucin, $C_6H_{10}O_6$. Thus—

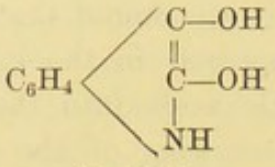
$$\underset{\text{Indican.}}{C_{26}H_{31}NO_{17}} + 2H_2O = \underset{\text{Indigo-blue.}}{C_8H_5NO} + 3\underset{\text{Indiglucin.}}{(C_6H_{10}O_6)}.$$

Now, isatin, $C_8H_5NO_2$, plus a molecule of water, H_2O, yields isatic

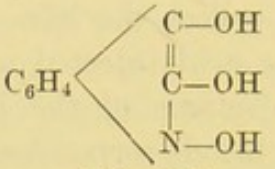
acid, $C_8H_7NO_3$, which is a hydroxyl derivative of indol, or trioxindol. Thus, trioxindol may be expressed $C_8H_4N(HO)_3$. Trioxindol by reduction yields dioxindol $C_8H_5N(HO)_2$, and oxindol $C_8H_6N(HO)$. Finally, Baeyer and Emmerling represent all these bodies by the following formulæ, in which the benzin nucleus, C_6H_4, plays an important part—



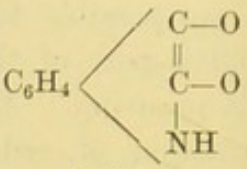
Oxindol.



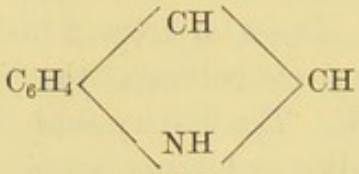
Dioxindol.



Trioxindol.



Isatin.



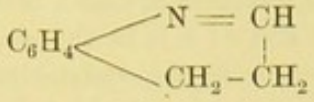
Indol.

Thus indol may be obtained from indigo by converting the indigo into isatin, dioxindol, and oxindol, and then reducing the oxindol by zinc dust. The relationship of these bodies may also be illustrated by putting their formulæ in series, thus—

Indol, - - - - C_8H_7N.	Isatin, - - - - $C_8H_5NO_2$.
Oxindol, - - - C_8H_7NO.	Indigo-white, - - C_8H_6NO.
Dioxindol, - - - $C_8H_7NO_2$.	Indigo-blue, - - - C_8H_5NO.
Isatyde, - - - $C_8H_6NO_2$.	

We pass from indol to indigo-blue by successive oxidations, and from indigo-blue to indol by successive reductions. It is of more importance, however, to observe that indol is also obtained by the decomposition of albumin through the agency of a ferment in the pancreatic juice. (Nencki and Kühne.) It appears as an oily fluid, which, when mixed with water, solidifies to a crystalline mass, and on recrystallization from pure water, crystals of pure indol are obtained, which melt at 52° C. Indol has a peculiar fæcal odour, like that of naphthylamine; it is readily soluble in alcohol and ether, and it acts as a very weak base. One of its characteristic properties is to give a reddish precipitate with dilute fuming nitric acid: this is soluble in alcohol, "and the alcoholic solution mixed with hydrochloric acid colours fir wood cherry-red, changing after a while to dirty brown-red." (Baeyer.) The history of indol, as a decomposition-product of albuminoids, prepares us for finding it in the intestines and in the fæces, as is the case, and it also exists in all putrefying albuminoids. Part of it thus formed in the intestine is evacuated and a portion may be changed into indican which is absorbed and finally excreted in the urine by the kidneys.

4. *Skatol*, C_9H_9N, or methyl-indol, $C_8H_6(CH_3)N$, may be represented by the formula—



It is found in human excrement along with indol and phenol, and it is also one of the products of the pancreatic digestion of albuminoids. The fæces of dogs contain no skatol, but only indol and an offensive volatile yellow oil, the nature of which is not known. It is said that a similar oil is met with in various fluids of the human body in pathological conditions. In small doses, skatol has no evident physiological action, but in large doses it produces disturbance of the nervous system and even tetanus. It appears in irregularly dentate shining plates, having a fæcal odour and melting at 94° C. Fuming nitric acid gives a white cloudy precipitate with its solutions, thus distinguishing it from indol.

5. *Pyrrol*, C_4H_5N, a substance produced when animal or vegetable matters containing nitrogen are subjected to destructive distillation, has been found in the fæces and in the urine, and probably arises in these circumstances from the decomposition of albuminous matter.

Chap. IX.—THE PIGMENTS.

Various pigments occur in the body, as in the blood, the bile, the urine, the skin, the choroid coat of the eye, and in muscular tissue. The parent of most of these is in all probability the pigment of the blood, hæmoglobin. One of the characteristic properties of pigments is that when white light is passed through a solution containing the pigment and then through a prism, part of the light will be absorbed, so that certain bands appear in the spectrum, termed absorption bands, or a portion of the spectrum may be entirely absorbed. The detection of these bands, the position of which in the spectrum is constant for each substance, has made the spectroscope a useful instrument for physiological research, and the study of the position of the bands, and of the way in which the spectrum of each substance may be modified by subjecting the substance to various chemical operations has added largely to our knowledge of the nature and relations of these pigments. It is therefore necessary at this stage briefly to describe the spectroscopic method of research so as to make intelligible the results obtained in this field of inquiry.

When white light is passed through a prism composed of crown or flint glass, or of a triangular bottle with glass sides containing the highly refractive substance, bisulphide of carbon, a continuous spectrum is obtained, showing the following

series of colours—red, orange, yellow, green, blue, indigo, violet; and if the light be that of the sun, the well known lines of Fraunhofer, with suitable arrangements, also become visible. The first Fraunhofer line A is in the red, and a little to the right and also in the red are B and C; D is in the orange-yellow; E in the green; F between blue-green and blue; G in the violet-blue; and H in the violet. The bright yellow line seen when the vapour of sodium is in the flame corresponds with the line D, and this line D is called sometimes the sodium line. The position of these fixed lines corresponds to a certain wave-length (λ) of light, and the wave-length is usually expressed in 10-millionths of a millimetre. Thus, the wave-

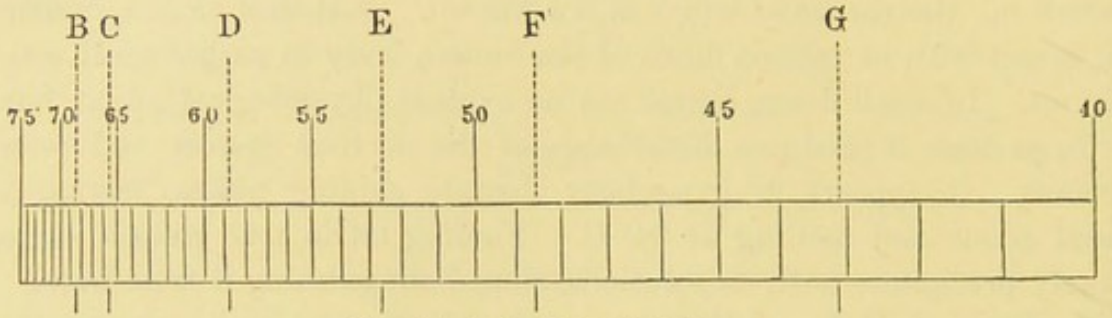


FIG. 41.—Scale of wave-lengths.

lengths of some of the chief fixed lines shown in the accompanying scale (Fig. 41) as determined by Ångstrom[1] are:—B, 6874; C, 6567; D, 5894; E, 5273; F, 4865; and

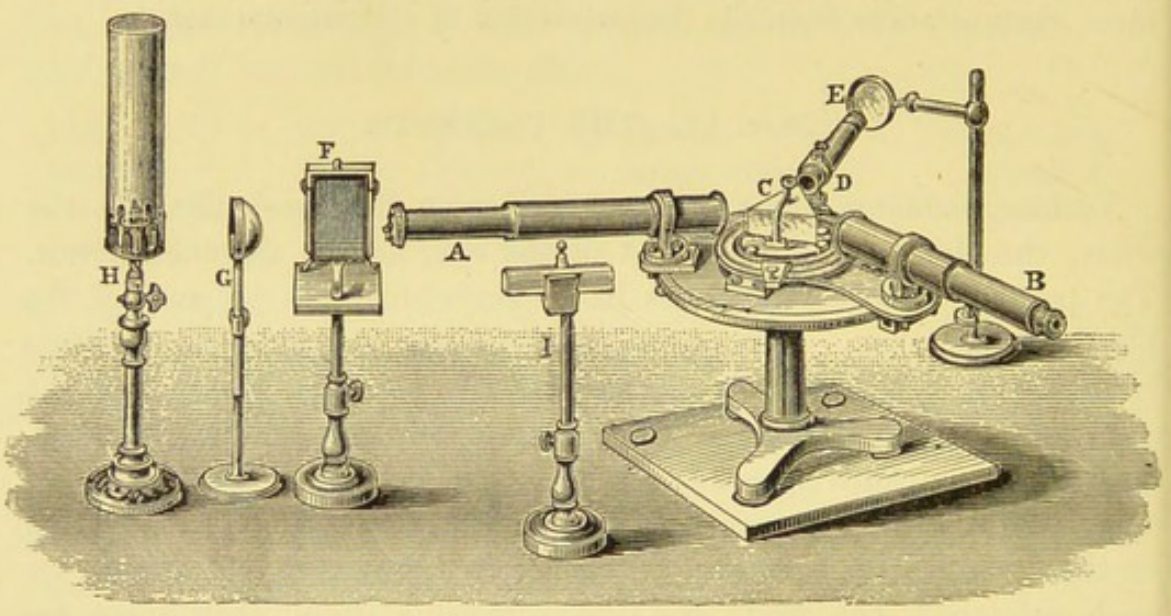


FIG. 42.—Arrangement of spectroscope. A, Collimator, with adjustable slit at left end of tube and collimating lens at right end; B, telescope, moving on graduated arc divided into degrees; C, prism or combination of prisms; D, tube for scale; E, mirror for illuminating scale; F, specially constructed vessel with parallel glass sides for holding fluid, the spectrum of which is under examination; G, lens for condensing light of Argand flame H. I, vessel for deep layer of fluid. (From a photograph taken for this work by Dr. MacMunn.)

G, 4310. In this scale, provided along with the micro-spectroscope of Zeiss of Jena, on the suggestion of Professor Abbe, the figures indicate wave-lengths in

[1] The wave-lengths given by Ångstrom in *Recherches sur le Spectre Solaire, Spectre Normal du Soleil*, p. 25, Upsala, 1868, are as follows: A, 7604; B, 6867; C, 6562; D, 5892; E, 5269; F, 4860; G, 4307. These are more correct than those given in the text. For physiological purposes it is only necessary to measure bands in millionths of a mm.

100,000ths of a mm., and each division indicates a difference in wave-length equal to 100,000th of a mm. = ·00001 mm. Thus take E: λ is seen to be = about 52·7 one hundred-thousandths of mm., that is 527 millionths, or with careful measurement 5273 ten millionths of mm.

The spectroscopes employed in physiological work (see Fig. 41) may be either such as are used by physicists, consisting of a prism, C, of flint-glass, or combination of prisms mounted on the centre of a table, a tube A, called the *collimator*, having an adjustable slit at one end, and a lens, the *collimating lens*, at the other; a *telescope*, B, for examining the spectrum, and a tube, D, by which an image of a scale photographed on glass, placed at E, is reflected from the prism into the eye of the observer looking through the telescope B. In using the instrument, a black velvet cloth is thrown over the prism and ends of the tubes, the light H is adjusted so as to illuminate the slit, the width of the slit is also carefully adjusted, and by careful focusing of the telescope, B, a well-defined spectrum will then be seen, having an illuminated image of the scale above or below it. If now a solution of permanganate of potash, in a vessel having flat glass sides, be placed in front of the slit, it will be found that the red and blue-violet parts of the spectrum are unaltered, but there are dark bands in the yellow and green, that is, the red and violet parts of the spectrum are transmitted, but portions of the rays producing yellow and green have been absorbed. Again, if a solution of chlorophyll is substituted for the permanganate, another kind of absorption spectrum is seen: "in the middle of the red a black band between B and C, three feeble absorption striæ in orange, yellow, and green, while the violet and indigo colours are entirely shaded." (MacMunn, *The Spectroscope in Medicine*, p. 6.) Such bands are termed *absorption bands*. To see the bands distinctly it is usually necessary to dilute the fluid and to examine it in a trough having parallel glass sides, or such an instrument as the *Hæmatoscope* of Hermann, seen in Fig. 43, may be employed.

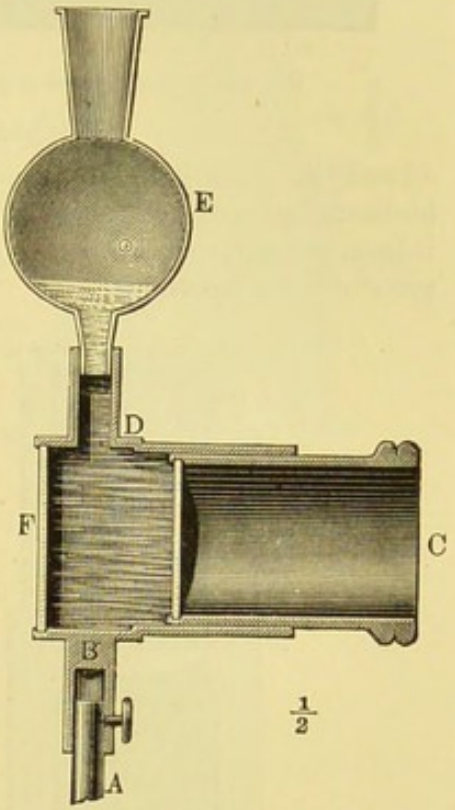


FIG. 43.—Hæmatoscope. F, A glass plate; C, piston-like tube having at its inner end a glass plate. By sliding C in or out of tube D, the capacity of vessel D B F is varied, and the thickness of the stratum of fluid is read off on a millimetre scale on the tube C. E, vessel for holding fluid to be examined. A, rod on which apparatus is fixed by a screw.

The spectroscope has also been adapted to the microscope. In the micro-spectroscope there is a compound prism consisting of two prisms of flint glass between three prisms of crown glass, united by means of Canada balsam. Thus, as seen in Fig. 44, there is sufficient dispersion, while the light continues in the same path.

Below the prisms there is a slit, the width of which may be regulated by a side screw, and the tube bearing the prisms may, by a rack and pinion movement, be brought near the slit or removed from it. Usually the micro-spectroscope is furnished with a side tube which admits the light to a small right-angled prism, which reflects it upwards into the eye of the observer through that portion of the slit which this right-angled prism covers. By this arrangement, on placing a small

glass tube containing the solution outside the hole in the end of the side tube, a second spectrum is seen which may be compared with the one obtained from the object under examination. Two such micro-spectroscopes are shown in Figs.

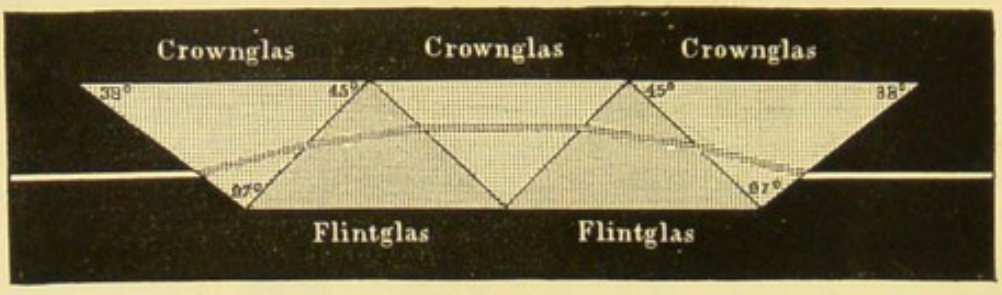


FIG. 44.—Arrangement of prisms in direct vision spectroscope. (Gscheidlen.)

45 and 46. In using the micro-spectroscope, a tube containing the fluid is placed horizontally on the stage of the microscope, and the light is transmitted through it from the mirror below the stage. The spectroscope is substituted for the eye-piece of the microscope.

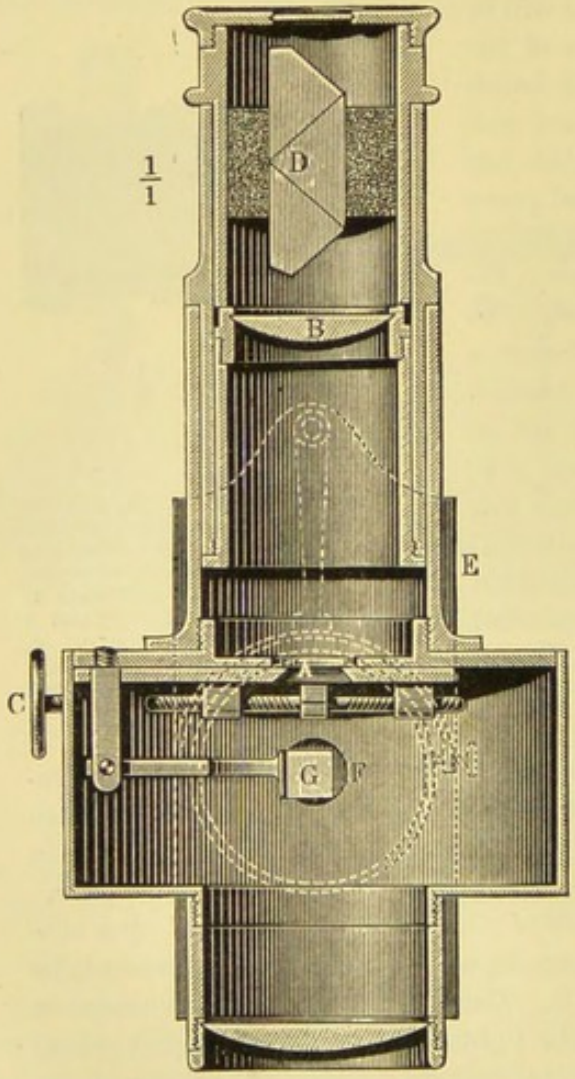


FIG. 45.—Micro-spectroscope. A, slit, adjustable by screw C; B, field glass; D, three prisms; E, movement for bringing prisms nearer to or farther away from the slit A; the outlines of a plate bearing the slit seen in dotted lines, F, G, are arrangements for increasing or diminishing breadth of slit. These arrangements are seen in Fig. 46.

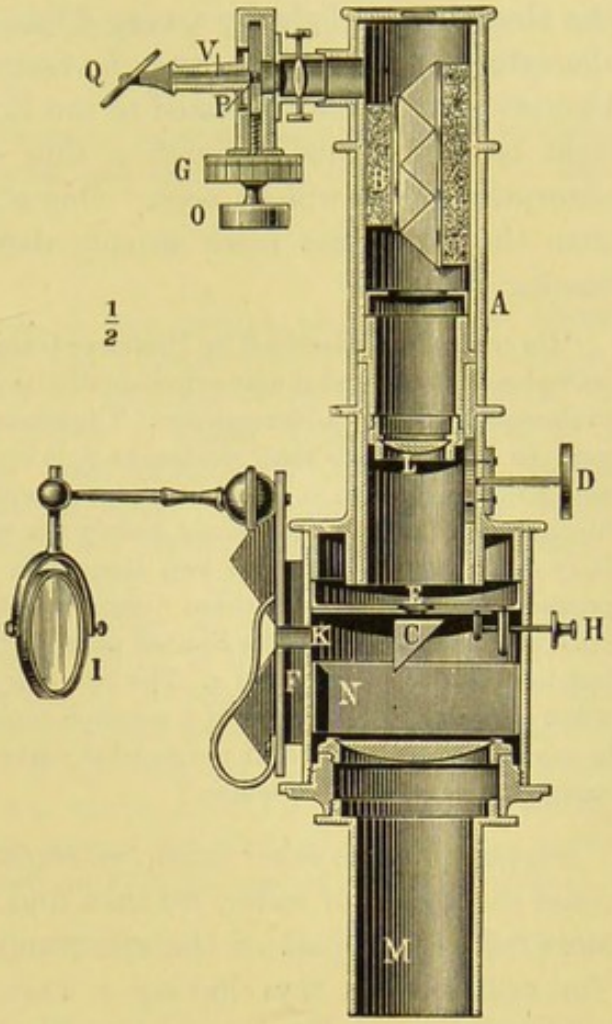


FIG 46.—Micro-spectroscope of Sorby. A, tube carrying prisms ; B, five prisms—three of crown and two of flint glass ; C, prism for obtaining a second spectrum by light entering at slit K reflected from mirror I through tube containing fluid held by spring clips ; H, screw and rod for regulating slit E ; L, lens ; M, tube for body of microscope. At upper end on left hand is the apparatus for obtaining an image of scale placed at P, movable by screws G and O, by light reflected from mirror Q passing along tube V.

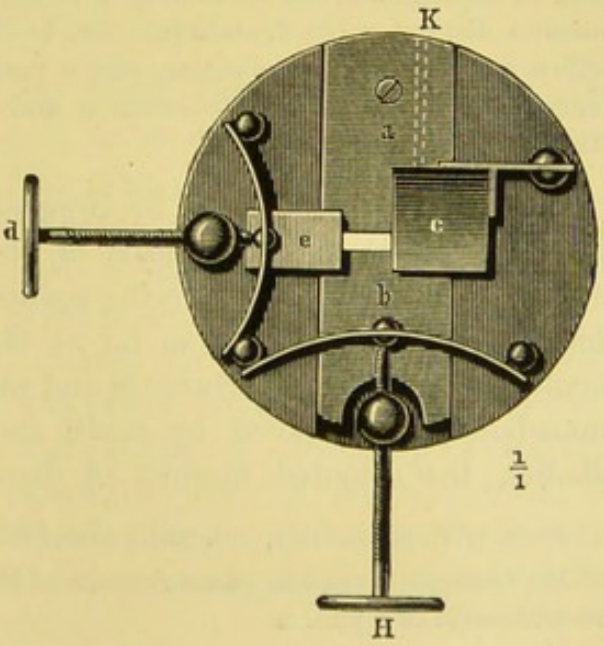


FIG 47.—View of plate K belonging to the micro-spectroscope seen in Fig. 45, and having slit narrowed by the plates a and b, moved by screw H, and shortened in length by plates e e, moved by screw d.

In 1862, Hoppe-Seyler noticed the remarkable spectrum produced by the absorption of light by a very dilute solution of blood. Immediately thereafter, the subject was investigated by Professor Stokes of Cambridge and communicated to the Royal Society in 1864.[1] If white light be transmitted through a thin stratum of blood, two distinct absorption bands will be seen. One of these bands next D is narrower than the other, has more sharply defined edges, and is undoubtedly blacker.

"Its centre," as described by Professor Gamgee (*op. cit.* p. 97), "corresponds with wave-length 579,[2] and it may conveniently be distinguished as the absorption band α in the spectrum of oxy-hæmoglobin. The second of the absorption bands, *i.e.* the one next to E, which we shall designate β, is broader, has less sharply defined edges, and is not so dark as α. Its centre corresponds approximately to W. L. 553·8. On diluting very largely with water, nearly the whole of the spectrum appears beautifully clear except where the two absorption bands are situated. If dilution be pursued far enough even these disappear; before they disappear they look like faint shadows obscuring the limited part of the spectrum which they occupy. The last to disappear is the band α. The two absorption bands are seen most distinctly when a stratum 1 cm. thick of a solution containing 1 part of hæmoglobin in 1000 is examined: they are still perceptible when the solution contains only 1 part of hæmoglobin in 10,000 of water."

Suppose, on the other hand, we begin with a solution of blood in ten times its volume of water, we then find that such a solution cuts off the more refrangible part of the spectrum, leaving nothing except the red, "or rather those rays having a wave-length greater than about 600 millionths of a millimetre." On diluting further, the effects, so well described by Professor Gamgee, are as follows—

"If now the blood solution be rendered much more dilute so as to contain 8 per cent. of hæmoglobin, on examining a stratum 1 centimetre wide, the spectrum becomes distinct up to Fraunhofer's line D (W. L. 589), *i.e.* the red, orange, and yellow are seen, and, in addition, also a portion of the green between *b* and F. Immediately beyond D, and between it and *b*, however (between W. L. 595 and 518), the absorption is intense."

These facts were observed by Hoppe-Seyler. Professor Stokes made the very important contribution of observing that the spectrum was altered by the action of reducing agents. Hoppe-Seyler had remarked that the colouring matter, so far as the spectrum was concerned, was unaffected by alkaline carbonates and caustic ammonia, but was almost immediately decomposed by acids, and also slowly by caustic fixed alkalies, the coloured product of decomposition being hæmatin, the

[1] *Proc. of Royal Society*, vol. xiii.; also *Philosophical Magazine*, 1864.

[2] Dr. Gamgee gives the measurements of the wave-lengths in millionths, not in ten-millionths of a mm.

spectrum of which was known. Professor Stokes was led to investigate the subject from its physiological interest, as may be observed on quoting his own words—

"But it seemed to me to be a point of special interest to inquire whether we could imitate the change of colour of arterial into that of venous blood, on the supposition that it arises from reduction."

He found that—

"If, to a solution of proto-sulphate of iron, enough tartaric acid be added to prevent precipitation by alkalies, and a small quantity of the solution, previously rendered alkaline by either ammonia or carbonate of soda, be added to a solution of blood, the colour is almost instantly changed to a much more purple-red, as seen in small thicknesses, and a much darker red than before, as seen in greater thickness. The change of colour which recalls the difference between arterial and venous blood is striking enough, but the change in the absorption spectrum is far more decisive. The two highly characteristic dark bands seen before are now replaced by a single band, somewhat broader and less sharply defined at its edges than either of the former, and occupying nearly the position of the bright band separating the dark bands of the original solution. The fluid is more transparent for the blue and less so for the green than it was before. If the thickness be increased till the whole of the spectrum more refrangible than the red be on the point of disappearing, the last part to remain is *green*, a little beyond the fixed line *b*, in the case of the original solution, and *blue* someway beyond F, in the case of the modified fluid."

From these observations, Professor Stokes was led to the important conclusion that—

"The colouring matter of blood, like indigo, is capable of existing in two states of oxidation, distinguishable by a difference of colour, and a fundamental difference in the action on the spectrum. It may be made to pass from the more to the less oxidized state by the action of suitable reducing agents, and recovers its oxygen by absorption from the air."

To the colouring matter of the blood, Professor Stokes gave the name of *cruorine*, and described it in its two states of oxidation as *scarlet cruorine* and *purple cruorine*. The name *hæmoglobin*, given to it by Hoppe-Seyler, is generally employed. When united with oxygen it is called oxy-hæmoglobin, and when in the reduced state it is termed reduced hæmoglobin, or simply hæmoglobin.

We are now in a position to discuss the properties of the various pigments, including their optical properties, which are of much physiological interest. The latter are well illustrated by Plate I., which is a chart of physiological spectra, specially prepared for this work by Dr. Charles A. MacMunn, of Wolverhampton, who has for many years made the application of the spectroscope to physiology and to practical medicine a special subject of study.

A.—The Pigment of the Blood and its Derivatives.

1. *Hæmoglobin*, *hæmatoglobulin*, *cruorin*, *hæmatocrystallin*, conveniently represented by the symbol **H**, is probably the most complex substance known. Its percentage composition varies slightly in different animals, as shown in the following table—[1]

Animal,	Dog.	Dog.	Guinea-Pig.	Squirrel.	Goose.	Horse.
Carbon,	54·15	53·85	54·12	54·09	54·26	54·87
Hydrogen,	7·18	7·32	7·36	7·39	7·10	6·97
Nitrogen,	16·33	16·17	16·78	16·09	16·21	17·31
Oxygen,	21·24	21·84	20·68	21·44	20·69	19·73
Sulphur,	0·67	0·39	0·58	0·40	0·54	0·65
Iron,	0·43	0·43	0·48	0·59	0·43	0·47
Phosphoric acid,	...	...	...	...	0·77	...
Author,	C. Schmidt.	Hoppe-Seyler.				Kossel.

Preyer, on the assumption that the molecule contains one atom of iron, has suggested the empirical formula, $C_{600}H_{960}N_{154}FeS_3O_{179}$, but no rational formula has been offered, and its molecular structure is unknown. It is the only proximate principle containing iron—about ·4 per cent.

In Professor Arthur Gamgee's well-known work on the *Physiological Chemistry of the Animal Body* (already quoted), pp. 85, 86, 87, 88, no fewer than eight methods of obtaining hæmoglobin are described, and I take the liberty of reproducing only two of these, Numbers IV. and VII., both of which, in my own experience, have yielded excellent results—

"IV. The defibrinated blood of the dog is mixed with its own volume of distilled water, and the diluted fluid is treated with one fourth of its volume of alcohol. The mixture is kept for twenty-four hours at a temperature of 0° C., or below. The crystals which separate are dissolved in as small a quantity as possible of water at 25° to 30° C., and, the solution being cooled to 0° C., a fourth of its volume of alcohol is added. It is better to place the fluid in a freezing mixture at a temperature of − 10° to − 20° C. for twenty-four hours. The whole fluid then becomes filled with a magma of crystals. The process of recrystallization may be several times repeated."

"VII. 500 c.c. of defibrinated dog's blood are treated with 31 c.c. of ether, and the mixture shaken for some minutes. It is then set aside in a cool place. After a period varying from twenty-four hours to three days the liquid has become converted into a thick magma of crystals. These may be separated by placing in

[1] Rollet, *Physiologie des Blutes*; Hermann's *Handbuch der Physiologie*, vol. iv.

tubes and using the centrifugal apparatus. The cakes of crystals thus obtained are mixed with water holding one fourth of its volume of alcohol in solution, and again centrifugalized. By repeating this process the crystals are obtained free from serum albumin. If requisite, the crystals are dissolved in water, and re-crystallized by the method mentioned under IV."

To obtain hæmoglobin crystals suitable for microscopical examination, the method of Gscheidlen, thus described by Dr. Gamgee, may be successfully adopted. "In order to obtain very large crystals of oxy-hæmoglobin for microscopic examination, Gscheidlen seals defibrinated dog's blood which has stood in the air for twenty-four hours, in narrow glass tubes (vaccine-tubes answer well), and keeps the tubes for some days at a temperature of 37° C. On opening these tubes and emptying their contents into a watch-glass, and allowing some time for evaporation to take place, there are formed crystals of extraordinary size." (Gamgee, *op. cit.* p. 88.)

Hæmoglobin exists in two conditions—(1) that of oxy-hæmoglobin, or hæmoglobin united with oxygen, HO; (2) reduced hæmoglobin, that is, oxy-hæmoglobin from which the oxygen has been removed, H. To form oxy-hæmoglobin, 1 gramme of dried hæmoglobin absorbs 1 c.c. of

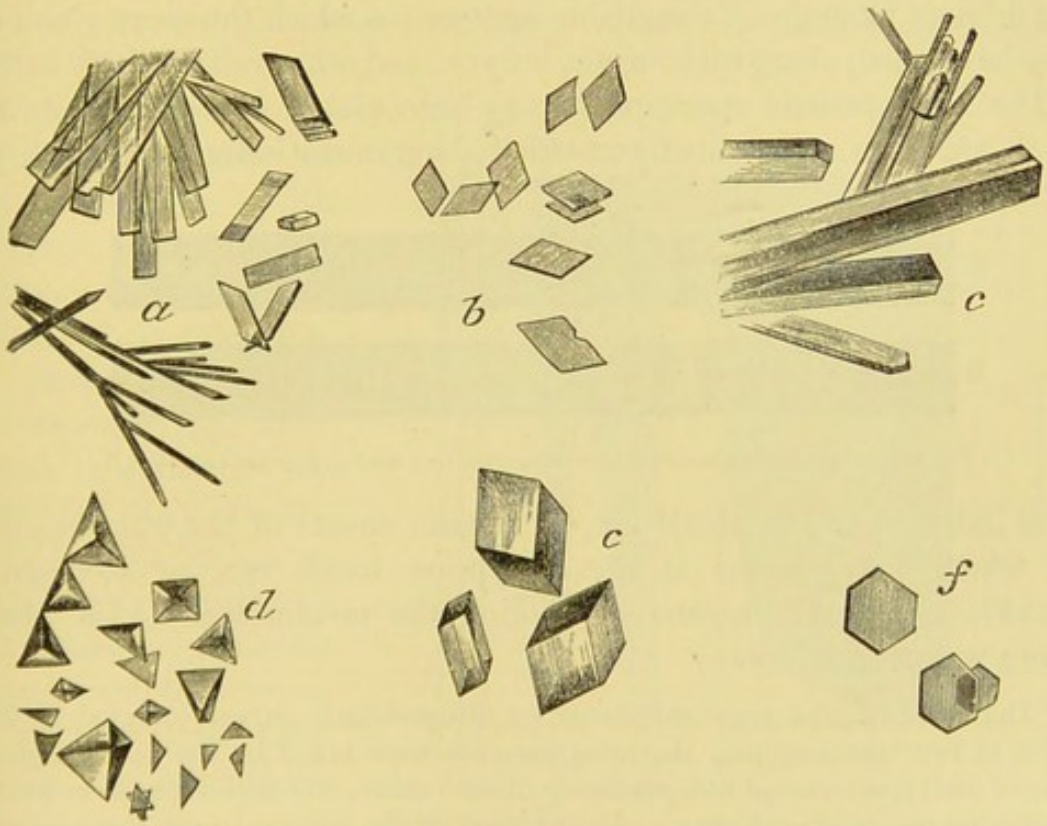


FIG. 48.—Crystals of oxy-hæmoglobin. *a*, *b*, *c*, and *c* (middle) show forms from human blood and that of the majority of mammals; *d*, tetrahedral crystals from blood of guinea-pig; *f*, hexagonal from squirrel's blood.

oxygen, and this oxygen may be expelled by the vacuum, heat, or reducing agents. HO from the blood of man and the majority of

animals crystallizes in rhombic prisms of a blood-red colour; that of the guinea-pig crystallizes in tetrahedra, and that of the squirrel in six-sided plates belonging to the hexagonal system (Fig. 48, *a*, *b*, *c*, *d*, *f*). It does not crystallize so readily from the blood of some animals as from others. Preyer thus classifies these varieties according to facility of crystallization: (1) Very difficult—calf, pig, pigeon, frog; (2) Difficult—man, ape, rabbit, sheep; (3) Easy—cat, dog, mouse, horse; (4) Very easy—rat, guinea-pig.

The crystals are readily soluble in water, and the solubility is in the inverse ratio of their facility of crystallization. Carbonates, phosphates, and borates of the alkalies, the alkalies themselves, serum, urine, solutions of urea, solutions of the bile acids, and glycerine dissolve oxy-hæmoglobin. Solutions of HO give a precipitate with potassium carbonate, but there is no precipitate with acetate of lead or with acetate of lead and ammonia, nor with nitrate of silver, although the last three reagents cause decomposition of the hæmoglobin. It is precipitated by alcohol. Solutions of hæmoglobin become muddy at a temperature of between 70° and 80° C., and the substance is decomposed by all agents that decompose albuminoid matters, being resolved into hæmatin, and one or more albuminoid coagulable matters (to which the name globulin may be applied) along with formic, butyric, and other volatile fatty acids.

The characteristic spectrum of oxy-hæmoglobin is seen in Plate I., sp. 2, and it is represented somewhat diagrammatically in Fig. 49, B.

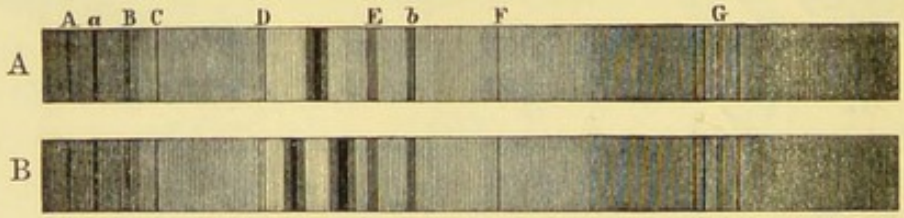


FIG. 49.—Diagram of spectrum of hæmoglobin A; and of oxy-hæmoglobin B.

I am indebted to Dr. MacMunn for measurements of the wave-lengths by which the position of the absorption bands can be accurately defined. As to the method of making the measurements, Dr. MacMunn writes as follows—

"The wave-lengths were calculated by interpolation curves adapted to the scales of two spectroscopes, the measurements were taken on the photographed scale of each spectroscope independently of each other, and then reduced to wave-lengths by means of each curve. If any marked discrepancy arose, the readings were repeated until this was corrected. The measurement of the edges of feeble bands must more or less depend on the "personal equation" of the observer, and may differ by a few millionths of a millimetre, in which they are calculated."

Dr. MacMunn, in his work on *The Spectroscope in Medicine*, p. 32, thus

describes the method of determining the wave-lengths by interpolation curves—

"A piece of paper ruled into square inches and tenths has a scale of wave-lengths ruled off along one edge, and the edge at right angles to this has a scale corresponding to the scale of the instrument marked on it. The value of the Fraunhofer lines on the scale of the spectroscope is observed, and by a reference to the above numbers, their value in wave-lengths, they are then marked in their proper place on the scale with + +. A curve is then drawn through these marks *as uniformly as possible.* When a band or bright line has to be mapped out, all that is necessary is to take its reading on the scale; then, knowing between what lines it is placed, we find its position on the curve, opposite which its wave-length is printed on the right hand edge."

The measurements for oxy-hæmoglobin in one millionths of mm. are as follows—

"In a concentrated solution the red rays only are transmitted, light is cut off completely at the red end at λ706, a shading extends to λ647; light is again completely absorbed at λ591, and a shading extends to λ610. In a more dilute solution one broad band embracing the two oxy-hæmoglobin bands becomes detached from about λ589 to λ520. Still more diluted, the first band extends from λ589 to λ564, with its darkest part from λ585 to λ569, and the second from λ555 to λ517, with its darkest part from λ552 to λ526. In a greater degree of dilution the first band reads λ587 to λ566, the darkest part being from λ583 to λ571, and the second from λ550·5 to λ523, the darkest part ranging from λ549 to λ529."

The character of the absorption spectrum of oxy-hæmoglobin, with different strengths of solution in a layer one centimetre in thickness, as measured by the hæmatoscope, is illustrated graphically by Rollet (*op. cit.*) in Fig. 50. Here the line *o x* has on it at the proper distances the chief Fraunhofer lines, whilst the figures along the right hand border are percentages of the amount of oxy-hæmoglobin present. Thus, with ·1 per cent. the two bands are narrow and pale, they gradually become broader until they fuse at nearly ·7 per cent., and at ·9 per cent. no light passes

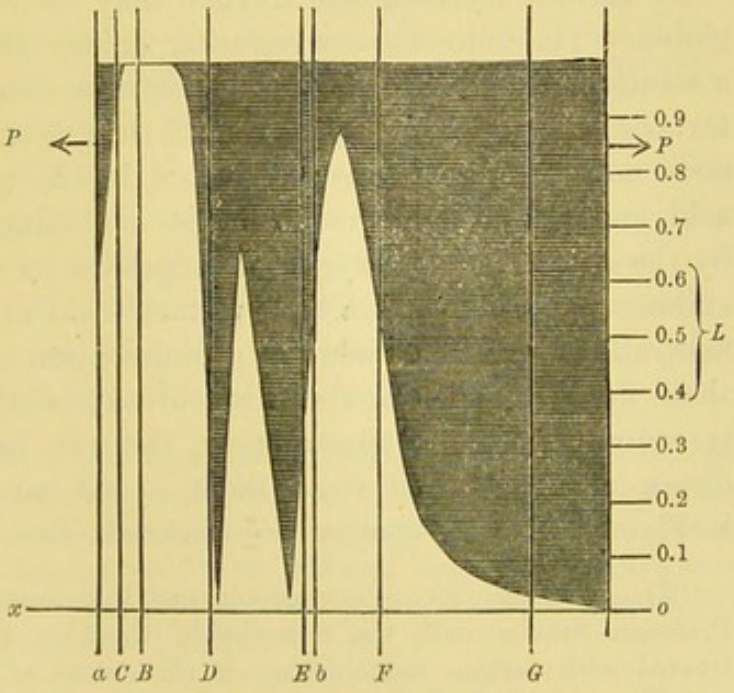


FIG. 50.—Graphic representation of the amount of absorption of light by solutions of oxy-hæmoglobin of different strengths. The shading indicates the amount of absorption of the spectrum.

except in the red. It will be observed also that from a little over ·6 per cent. to the top there is a gradually increasing, though slight, absorption of the low red.

Preyer has devised a spectro-colorometric method of estimating the amount of oxy-hæmoglobin by preparing a standard solution, having the absorptive power indicated in the diagram as on the level of the lines *PP*. This is the point at which the green is extinguished and only red rays are transmitted. The specimen of blood is diluted with water until it gives a similar spectrum (that is, to find the exact point at which the absorption of the green takes place), and then the percentage amount is estimated by the formula—

$$x = K\frac{(b+w)}{b},$$

in which K = the percentage of the standard solution, b = the amount of blood, and w = the amount of water, and x = the percentage required. Thus, suppose 2 c.c. of blood required 25 c.c. of water to give an absorption spectrum similar to that of the standard spectrum, the percentage of which is ·85; then

$$x = ·85\frac{(2+25)}{2} = 11·4 \text{ per cent. of oxy-hæmoglobin.}$$

By various methods the oxygen may be removed from oxy-hæmoglobin. The following are reducing fluids: (1) *Stokes' Fluid*. Thus, to a solution of sulphate of iron is added a small amount of citric or of tartaric acid, and then ammonia until the reaction is alkaline. Ammonia does not precipitate ferrous hydrate in the presence of the vegetable acid, and a clear light green solution is obtained, which becomes darker by the absorption of oxygen on exposure to the air; (2) a solution of stannous chloride, with a little tartaric acid and ammonia added until neutralization; (3) a solution of ammonium sulphide. When any of these fluids is added to a solution of oxy-hæmoglobin, and the solution is examined by the spectroscope, the two bands of oxy-hæmoglobin disappear and in their stead there is one band, seen in *A*, Fig. 49 and in Plate I. 3. The spectrum is thus described by Dr. Gamgee—

"The spectrum of oxy-hæmoglobin had been described by Hoppe-Seyler when Professor Stokes made the remarkable discovery that when diluted blood is treated with certain reducing agents the colour of the liquid and its spectrum undergo remarkable changes; the former loses its bright red and acquires a brown colour, while the green interspace which had existed between the absorption bands α and β of oxy-hæmoglobin disappears, and instead of the two bands there appears a single one, less deeply shaded, and with less finely defined edges, extending between D and E. The band we may distinguish as absorption band γ."

Dr. MacMunn states that "the broad hazy band extends from λ597 to λ535, and that the darkest part is from λ573 to λ542."

In Fig. 51 there is represented the amount of absorption with different percentages of reduced hæmoglobin, as in Fig. 50, showing oxy-hæmoglobin. It will be observed that the absorption band diminishes in breadth as the solution becomes weaker, the blue rays becoming more visible. With very weak solutions, the single broad band never breaks into two narrow ones, but gradually fades from view. With two weak solutions of equal strength, that of oxy-hæmoglobin may distinctly show the two bands, whilst that of reduced hæmoglobin shows no band. Further, the general appearance of the spectrum of reduced hæmoglobin differs from that of oxy-hæmoglobin. In the former less of the blue end is absorbed, and even in strong solutions some of the bluish-green rays pass through. This accounts for the difference of colour—the blood containing oxy-hæmoglobin being scarlet, whilst that containing reduced hæmoglobin is purple. The first kind of blood allows the red and orange-yellow rays to pass, and the second kind the red and blue-green rays. The difference is thus described by Professor Michael Foster—[1]

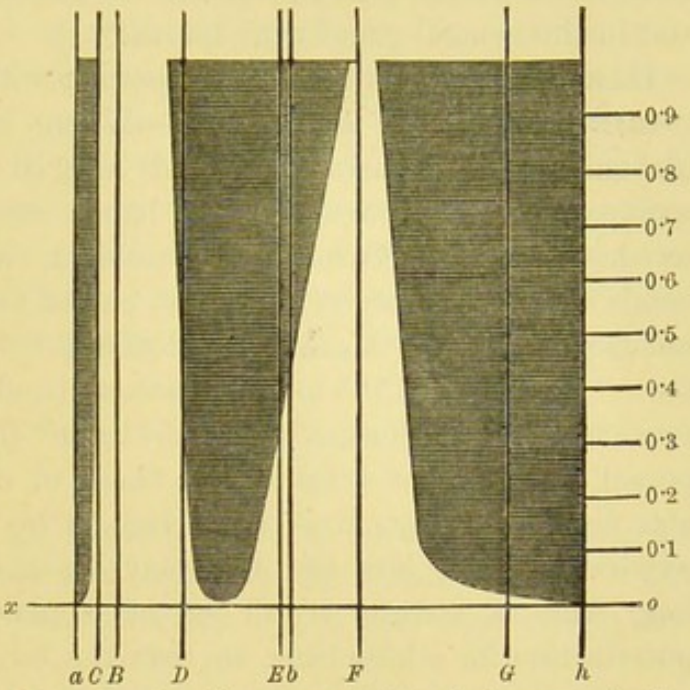


FIG. 51.—Graphic representation of the amount of absorption of light by solutions of reduced hæmoglobin of different strengths. The figures on the right border express percentages.

"In dilute solutions, or in a thin layer, the reduced hæmoglobin lets through so much of the green rays that they preponderate over the red, and the resulting impression is one of green. In the unreduced hæmoglobin or oxy-hæmoglobin, the potent yellow which is blocked out in the reduced hæmoglobin makes itself felt, so that a very thin layer of hæmoglobin, as in a single corpuscle seen under the microscope, appears yellow rather than red."

Hæmoglobin is widely distributed in the animal kingdom. Thus it has been found in the red blood corpuscles of all vertebrates except amphioxus (in which it is found in the plasma only); in the striated

[1] *Handbook for Physiological Laboratory*, by Klein, Burdon-Sanderson, Foster, and Lauder Brunton.

muscles of mammals and birds; in the cardiac muscles and very active muscles of other vertebrates; in *Ophiactis virens* (an echinoderm); in the blood of some of the annelidæ; in the fluid in the perivisceral cavity of the leech; in the larva of *Chironomus* (midge), although absent in other insects, and in myriapods and arachnids; in the blood plasma of certain crustaceans; in the blood of certain molluscs (*Planorbis*); in the muscular fibres of the pharynx of certain gastropods (*Lymnæus, Paludina*), and in the ventral ganglia of *Aphrodite*.

Hæmoglobin forms definite compounds with at least other two gases—

(*a*) *Carbonic-oxide Hæmoglobin.*—When blood is shaken up with carbonic oxide it assumes a red arterial colour, and if examined spectroscopically two absorption bands are seen similar to those of oxy-hæmoglobin. Careful measurement, however, shows that both bands are slightly nearer the violet end of the spectrum. The measurements given by Dr. MacMunn, calculated from Preyer's Map of Spectra, are: "First band, λ583 to λ564; second band, λ547 to λ529; or, in more concentrated solutions, λ583 to λ561 and λ547 to λ521."[1] This compound, which forms crystals like those of oxy-hæmoglobin, is remarkable for its stability; it is not acted on by those agents which reduce oxy-hæmoglobin, but the CO may be driven off by passing for a long time a stream of air or of a neutral gas. It also resists putrefaction for a long time, so that the two bands may be visible for months or years, whilst, when normal blood putrefies, the reduction of oxy-hæmoglobin occurs at once. (Gamgee, quoting Hoppe-Seyler.) Blood containing carbonic-oxide-hæmoglobin gives a cinnabar red precipitate with caustic soda, due to the formation of a compound of carbonic oxide with hæmatin. The gas CO can displace the O from oxy-hæmoglobin. It is well known that carbonic oxide, given off during the imperfect combustion of carbon, as occurs in charcoal stoves, is a powerful poison, soon destroying life. This it does by combining with the hæmoglobin of the blood, and thus interfering with the respiratory functions of that important body.

(*b*) *Nitric-oxide Hæmoglobin.*—When ammonia is added to blood, and then a stream of nitric oxide is passed through it, this compound is formed (Hermann), and it may be obtained in a crystalline form (isomorphous) with those of oxy- and carbonic-oxide-hæmoglobin, whilst it also has a similar spectrum. A stream of nitric oxide (NO) can displace carbonic oxide (CO) from the carbonic-oxide-hæmoglobin in blood, thus showing, as is well pointed out by Dr. Gamgee, that we have three compounds of hæmoglobin with gases, increasing in stability in

[1] Dr. Gamgee gives his own and the measurements of other observers at p. 105 of *Physiological Chemistry*, vol. i.

the following order: Oxy-hæmoglobin, carbonic-oxide-hæmoglobin, and nitric-oxide-hæmoglobin.

2. *Hæmochromogen.*—When hæmoglobin is acted on by acids or alkalies it decomposes into hæmatin, a substance containing the iron, and one or more albuminous bodies, to which the name globulin may be given. This, however, is not a direct decomposition, but, in the presence of oxygen, there is in the first instance oxidation, with the result of the production of an intermediate body, hæmochromogen. The oxidation occurs so quickly that the new body and its spectrum can only be recognized with special arrangements. If, however, hæmoglobin be decomposed in an apparatus[1] from which oxygen is excluded, by the action of alcohol containing sulphuric acid or caustic potash, a purple-red fluid (in alkaline solutions) is obtained. This is hæmochromogen, which, by oxidation, yields hæmatin. Hoppe-Seyler supposes it to have the composition $C_{34}H_{36}N_4FeO_5$, and gives the equation—

$$\underset{\text{Hæmochromogen.}}{2(C_{34}H_{36}N_4FeO_5)} + O_2 = \underset{\text{Hæmatin.}}{C_{68}H_{68}N_8Fe_2O_{10}} + 2H_2O.$$

It is really a product of the decomposition of hæmoglobin, although it is erroneously termed reduced hæmatin by some authors. The spectrum of this substance, seen in Plate I. 8, is thus described by Dr. MacMunn—

"Obtained from blood treated by rectified spirit and ammonia by adding sulphide of ammonium. The second band varies much in breadth, the first being much better marked. In concentrated solutions, the first band reads λ569 to λ542, and the second band λ535 to λ504. More diluted, first band, λ569 to λ542—darkest part, λ566 to λ549; second band, from edge to edge, λ536·5 to λ506. In a still more dilute solution, they read: First band, λ566 to λ547, and second band, λ535 to λ514. If we distinguish the readings of the extreme edge of each band from the darker parts, we get: First band, λ567·5 to λ547, and the darker part from λ564 to λ550·5; the second band, λ535 to λ514."

3. *Methæmoglobin.*—This is a substance intermediate between hæmoglobin and oxy-hæmoglobin, and it would appear that the oxygen it contains is more firmly united to hæmoglobin than in the case of oxy-hæmoglobin. It is produced when a solution of hæmoglobin has been exposed to the air for some time, when it becomes acid and loses its blood-red colour, acquiring a brownish tinge, and the spectrum, as given in Plate I. 4, shows two absorption bands like those of oxy-hæmoglobin and a new band in the red near *C*. This is the spectrum of an acid solution. If this be now rendered alkaline by the addition of ammonia, the band near *C* disappears, and another fainter band is

[1] Apparatus figured in Gamgee's *Physiological Chemistry*, p. 119.

seen before *D*. (See Plate I. 5.) On comparing the spectra 4 and 5 in Plate I., it will be seen also that the position of the band corresponding to the α-band of oxy-hæmoglobin, that is the one immediately to the right of *D*, is moved a little farther to the right in the spectrum of alkaline methæmoglobin. Dr. MacMunn thus describes these spectra—

Methæmoglobin.—Prepared by acting on an aqueous solution of sheep's blood with a solution of permanganate of potash : First band, λ647 to λ622—often another feebler band may be observed from λ610 to λ597 ; second band, λ587 to λ571 ; third band, λ552 to λ532; and a fourth band (?), λ514 to λ490. *Alkaline methæmoglobin.*—Prepared by adding a little ammonia to the previous solution : First band, λ610 to λ597 ; second band, λ587 to λ567·5 ; third band, λ552 to λ523."

The chief interest in methæmoglobin is connected with the facts that if the alkaline solution be shaken up with sulphide of ammonium, a solution is obtained giving the spectrum of reduced hæmoglobin, and if this latter solution be now shaken up with air, oxy-hæmoglobin is detected by its bands α and β. These facts were first observed by Dr. Arthur Gamgee in his well-known research on the action of nitrites on the blood, in which he showed that blood mixed with nitrites contained oxygen that was not removed by a stream of carbonic oxide, nor was given up to a vacuum, and he believed that this was owing to some kind of combination having taken place between the hæmoglobin and the nitrites. Later researches have supported the view that this nitrite-hæmoglobin is really methæmoglobin. This substance is not a hyperoxide of hæmoglobin, nor a per-oxyhæmoglobin, because, as shown by Hoppe-Seyler, it may be formed in circumstances in which oxidation cannot occur.

4. *Hæmatin*, $C_{34}H_{34}N_4FeO_5$ (or twice that formula), is formed along with globulin when hæmoglobin is decomposed. A solution may be readily made by adding acetic acid to blood. The blood becomes of a brownish colour, and if it now be shaken up with ether, the latter floats to the top and an ethereal solution of acid hæmatin is obtained. This may be used for examination with the spectroscope, or the ether may be evaporated, and the residue, hæmatin, washed with ether, alcohol, and water. When so obtained, it is a reddish-brown amorphous powder, having a metallic lustre, insoluble in water, alcohol, and chloroform, but soluble in acidulated alcohol and alkalies. The spectroscopic appearances of acid hæmatin and of alkaline hæmatin are given in Plate I. 6 and 7. With acid hæmatin four bands are seen, the 1st in the red between *C* and *D*; the 2nd, a narrow faint band close to *D*; the 3rd, a broad band between *D* and *E*; and the 4th, broad and faint, between *b* and *F*. If the fluid is made alkaline with ammonia, then we see the spectrum of alkaline

hæmatin, one broad absorption band near *D*, and a shaded dark part of the spectrum from a little to the left of *E* to midway between *E* and *F*, and complete absorption beyond this boundary. The exact details as to the position of the bands are thus given by Dr. MacMunn—

"(1) *Acid Hæmatin.* — A rectified spirit and sulphuric acid filtered extract from sheep's blood. The 1st band from λ647 to λ605, dark part λ641 to λ622; 2nd band, very feeble from λ593 to λ571; 3rd band, from λ550·5 to λ529; 4th band, from λ517 to λ488 or λ485. Prepared in the usual way by acting on blood with acetic acid, and shaking with ether, the ethereal solution gives bands with these measurements: 1st band, from λ656 to λ615—darker part λ647 to λ630; 2nd band, from λ597 to λ577; 3rd band, from λ557 to λ529; 4th band, from λ517 to λ488.

(2) *Alkaline Hæmatin* got by adding rectified spirit and caustic soda to blood and filtering. The whole band extends from λ630 to λ562—the darker part from λ619 to λ581. The purer form of alkaline hæmatin prepared from pure hæmatin gives a band, when examined in rectified spirit, from λ627·5 to λ562—darker part from λ619 to λ581."

A substance similar to hæmatin (entero-hæmatin) occurs in the bile of snails (Sorby), and in that of the limpet and cray fish (MacMunn).

5. *Hæmatoporphyrin* or *cruentin*, $C_{68}H_{72}N_8O_{12}$, is a body formed by the decomposition of hæmatin. When the latter is mixed with strong sulphuric acid, it is dissolved, and after filtering through asbestos, a clear purple-red solution is obtained. (Gamgee, *op. cit.*) If water be now added largely to this solution the greater part of the dissolved coloured substance is precipitated as a brown flocculent mass, and the amount of this is increased if alkalies be added to neutralize the acid. It is important to note that in this decomposition the whole of the iron of the hæmatin separates and is found in the solution as a ferrous salt. Thus—

$$\underset{\text{Hæmatin.}}{C_{68}H_{68}N_8Fe_2O_{10}} + 2\underset{\substack{\text{Sulphuric}\\\text{acid.}}}{(H_2SO_4)} + O_2 = \underset{\text{Hæmatoporphyrin.}}{C_{68}H_{74}N_8O_{12}} + 2\underset{\substack{\text{Sulphate of}\\\text{iron.}}}{(FeSO_4)}.$$

Hoppe-Seyler has obtained hæmatoporphyrin by the action of nascent hydrogen on hæmatin, and MacMunn has found the same substance by the action of sodium amalgam on hæmatin. The precipitate is hæmatoporphyrin. Like hæmatin, it may exist in either an acid or an alkaline solution, and the two solutions give different and characteristic spectra. Acid hæmatoporphyrin gives a broad dark band a little to the right of *D* (Plate I. 9), and a thin faint band to the left of *D*.[2] Alkaline hæmatoporphyrin (Plate I. 10) gives four bands: 1st, thin and narrow between *C* and *D*; 2nd, a broader band near to *D*; 3rd, another broad

[2] Gamgee states, "The first (solution in strong sulphuric acid) exhibits a pretty dark band immediately below *D* and a sharply-defined band nearly intermediate between *D* and *E*." (Gamgee, *Physiol. Chem.* p. 117.)

band near *E*; and 4th, a well marked dark band between *b* and *F*. The exact position of the bands is thus stated by Dr. MacMunn—

"(1) *Acid Hæmatoporphyrin.*—In concentrated solution in sulphuric acid the bands read: 1st band, λ607·5 to λ593; 2nd band, λ585 to λ536·5. In a weaker solution: 1st band, λ605 to λ593; 2nd band or shading, from λ585 to the next band which extends from λ567·5 to λ540. If the fluid be diluted by adding a little rectified spirit to the sulphuric acid instead of adding acid, the bands read: 1st band, λ610 to λ591—darker part from λ605 to λ593; 2nd band or shading, λ585 to λ567·5; and dark band, λ567·5 to λ540. For the bands of *pure* acid hæmatoporphyrin in rectified spirit and sulphuric acid the following are the measurements: 1st band, λ605 to λ591; 2nd, a shading from λ583 to λ564; 3rd band, λ564 to λ542.

(2) *Alkaline Hæmatoporphyrin.*—In solutions of this pigment, the edges of bands vary much according to concentration. The following may be taken to apply to a solution of mean concentration: 1st band, λ633 to λ612·5, and dark from λ630 to λ615; 2nd band, λ589 to λ564, and a shading before this which is most difficult to measure, and may be from λ605 to λ589; 3rd band, λ549 to λ529, which is the darker part; 4th band, λ518·5 to λ488."

MacMunn has found hæmatoporphyrin in the integument of *Uraster rubens* (star fish),[1] in the integument of *Limax flavus*, *Limax variegatus*, and in *Atrion ater* (slugs), in common earth worm, *Lumbricus terrestris*, in *Solecurtus strigillatus*, and he shows that polyperythrin, a pigment discovered by Moseley in various actiniæ and deep-sea polypes, is probably identical with hæmatoporphyrin. It is also found in the eggshells of some birds. A kind of hæmatoporphyrin also appears in the urine of cases of Addison's disease, of acute rheumatism, and other diseases.

Dr. MacMunn[2] has found in various common species of actinia, *A. mesembryanthemum*, *Bunodes crassicornis*, *Sagartia bellis*, etc., a colouring matter related to hæmoglobin, very similar to hæmochromogen, and convertible into hæmatoporphyrin.

6. *Hæmatolin*, $C_{68}H_{78}N_8O_7$, is another derivative of hæmatin described by Hoppe-Seyler. It is insoluble in sulphuric acid and in caustic lyes, thus differing from hæmatoporphyrin.

7. *Hæmatoidin.*—In old blood clots such as occur in the brain, in the *corpora lutea* of the ovary, and in the coagula of aneurisms, crystals of this substance are found in the form of regular or oblique rhombs or rhomboids (Fig. 52). The crystals are strongly refractive and translucent, of a brick-red or yellowish-red colour. They are insoluble in water, alcohol, ether, acetic acid, and the dilute acids and alkalies. Caustic potash gives a bright red at first and then the crystals dissolve.

[1] MacMunn, *Quart. Jour. of Micros. Science*, 1877. Also *Journal of Physiology*, vol. vii. See also MacMunn on the hæmatoporphyrin of *Solecurtus strigillatus*, *Jour. of Physiology*, vol. viii. No. 6.

[2] MacMunn, *Phil. Trans. of Royal Society*, 1885.

Concentrated acids and fuming nitric acid give a play of colours with crystals of hæmatoidin like the reaction of these acids with the colouring matter of the bile, the bodies gradually becoming brownish-red, green, blue, rose-coloured, and finally yellow. Hæmatoidin is also said to appear in a granular form. (Robin.)

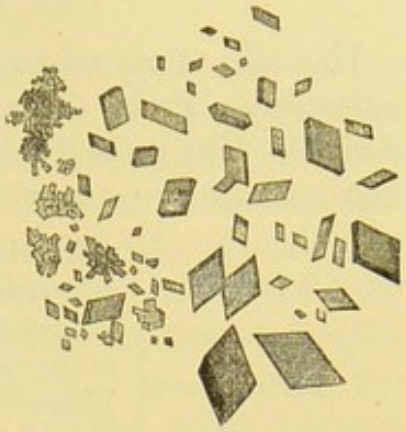


Fig. 52.—Crystals of Hæmatoidin

There has been much discussion as to the true nature of hæmatoidin, some asserting that it is identical with bilirubin, one of the pigments of the bile to be hereafter described, while many others—Holm and Staedeler—on the ground of differences in chemical reactions, and Preyer, from differences in the spectra, have denied the identity.[1]

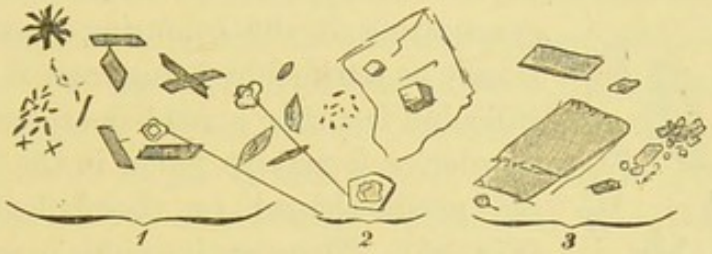


Fig. 53.—1. Hæmin crystals from man—whetstone form; 2. Crystals of common salt; 3. Hæmatoidin crystals from man, 500 diameters.

8. *Hæmin*, $C_{96}H_{142}Fe_3O_{18}$, is a derivative of hæmatin, the appearance of which is an important test for the presence of this substance, or in other words, of blood, in medico-legal inquiries. Sometimes termed Teichmann's crystals, they are rhombohedral plates or six-sided plates, of a brownish colour by transmitted light (Fig. 54). They are insoluble in water, soluble in hot alcohol, in hot ether, and in caustic potash. To obtain them, add to a drop of blood on a glass slide a few particles of common salt, and then a drop of glacial acetic acid; place the slide in a warm place, or heat cautiously over the flame of a spirit lamp, and then examine with the microscope. The crystals will then be found as represented in Fig. 53, probably mixed with a few crystals of common salt.

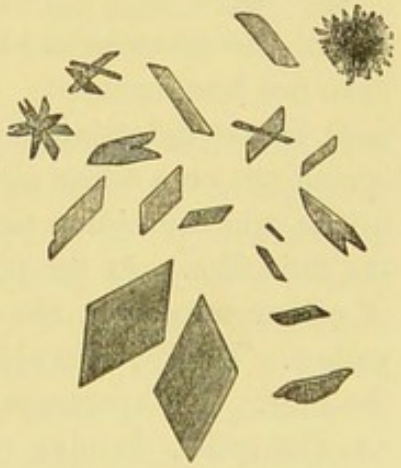


Fig. 54.—Crystals of Hæmin. The view in Fig. 53 is more like what one may readily obtain from a drop of blood.

[1] See on this point Gamgee's *Physiolog. Chem.* p. 120, and Wagner's *General Pathology*, p. 313.

B.—The Pigments of the Bile.

The bile of mammals contains two chief pigments, bilirubin and biliverdin. From these, certain other pigmentary bodies may be derived. The general relation of these pigments may be shown in the first instance by contrasting their empirical formulæ as follows—

	Bilirubin, - - - -	$C_{16}H_{18}N_2O_3$
	Biliverdin, - - - -	$C_{16}H_{18}N_2O_4$
Derivatives	Choletelin, - - - -	$C_{16}H_{18}N_2O_6$
	Bilifuscin, - - - -	$C_{16}H_{20}N_2O_4$
	Biliprasin, - - - -	$C_{16}H_{22}N_2O_6$
	Hydrobilirubin, - - -	$C_{32}H_{40}N_4O_7$

1. *Bilirubin*, $C_{16}H_{18}N_2O_3$, or $C_{32}H_{36}N_4O_6$, may be obtained from fresh bile by acidulating and then shaking up with chloroform. The latter dissolves the bilirubin, and on drawing off the chloroform solution and evaporating it the colouring matter remains. It may be purified by being treated with alcohol. Bilirubin is either a reddish-orange amorphous powder or it may be found in the form of dark red needle-shaped or rhombohedral crystals (Fig. 55). These are insoluble in water, slightly soluble in alcohol or ether, and readily soluble in chloroform, benzin, bisulphide of carbon, sulphuric acid, and the alkalies. Bilirubin, by oxidation, becomes changed into biliverdin and choletelin, and during the oxidation other transitory bodies are formed, some of which have not been isolated. On this fact depends Gmelin's *reaction or test*, used for the detection of the bile in the urine. A few drops of bile are spread out on a white porcelain plate, and a drop of fuming nitric acid (nitric acid containing nitrous acid) is allowed to fall into the middle of the fluid film. At the point of contact of the two fluids, beautiful rings of colour appear in the following order: green, blue, violet, red, and yellow. If biliverdin alone is present, the play of colours begins with the blue. The spectrum of the chloroform solution of bilirubin shows no absorption bands; only an absorption of the violet end of the spectrum to *b*. (See plate I. 13.) A solution that shows Gmelin's reaction, when examined spectroscopically, gives "a broad shading (probably composed of two distinct bands) in orange and yellow, and a black band extending from near *b* to beyond *F*. . . . In a very short time the shading in orange begins to fade, and at the time the oxidation process is completed and the colour of the solution has become

Fig. 55.—Crystals of bilirubin.

yellow, nothing but the band at *F* is left." (MacMunn, *op. cit.* p. 160.)

Whilst bilirubin has never been obtained artificially by the chemist from the decomposition of hæmoglobin, there are strong grounds for holding that in the body, and probably in the cells of the liver, this transformation occurs. The injection into the blood of substances that destroy the red blood corpuscles, such as large quantities of water, the bile acids, or ammonia, is followed by the excretion of bilirubin in the urine; therefore it is fair to infer that the increased amount of bilirubin has come from the breaking up of hæmoglobin. As, however, the direct injection of a solution of hæmoglobin into the blood does not cause an increase of urinary pigment, it is evident that the chemical transformations are not yet understood.[1]

2. *Biliverdin*, $C_{16}H_{18}N_2O_4$, the chief pigment in the bile of herbivora, may be made by exposing greenish bile for some time to the air. The addition of hydrochloric acid throws down green flaky masses, amorphous, insoluble in water, but soluble in alcohol. The alcoholic solution has a deep green colour, and on evaporation an amorphous residue is obtained, soluble in glacial acetic acid, and the evaporation of the acetic acid solution yields biliverdin. (Beaunis.) It is then found to be an amorphous powder, insoluble in water, ether, and chloroform, but soluble in alcohol and sulphuric acid. Biliverdin is undoubtedly formed by the oxidation of bilirubin.

A substance like biliverdin has been found by MacMunn in various actiniæ, and by Krukenberg in the shells of certain molluscs. MacMunn believes it may be looked on as excretory, being probably derived from other pigments like hæmoglobin, or from those he terms histohæmatins, pigments scattered through the tissues.

3. *Choletelin*, $C_{16}H_{18}N_2O_6$, is an oxidation product of biliverdin, or it may be regarded as the substance formed at the end of the oxidation process of bilirubin. It is prepared by passing nitrous vapours into an alcoholic solution of bilirubin. When the alcoholic solution is poured into water, flakes of choletelin "of the colour of ferric oxide" separate, and these, by evaporation, may be obtained as a dry powder. "Choletelin cannot be made to crystallize from any solvent. It dissolves very easily in the fixed alkalies, and their carbonates, also in ammonia, forming brown solutions, from which it is precipitated in flocks by acids. It is soluble in alcohol, ether, and chloroform. The alcoholic solution

[1] It is significant that urobilin can be obtained from hæmoglobin and hæmatin, and from bilirubin by the action of reducing agents. (See below.) Moreover, after blood has been extravasated in large quantity into the tissues, the urobilin of the urine is much increased.

is precipitated by water. With silver nitrate, it gives a precipitate only on the addition of ammonia. No perceptible reaction is produced by hydrogen sulphide, or by zinc and hydrochloric acid."[1] It does not give Gmelin's reaction, as it has been oxidized beyond that stage. The spectrum of a similar pigment, to which the bands in ox and sheep bile are due, is shown in Plate I. 12, and is thus described by Dr. MacMunn—

"*Cholohæmatin*, from bile of sheep. The bile is, after addition of absolute alcohol and acetic acid and filtering, agitated in a tap funnel with chloroform, which, after separation, shows this spectrum. On letting the chloroform stand for a day or so, a *urobilin* band also generally becomes visible. (1) The *bile itself*—1st band, centre at λ649 (not shown in plate); 2nd band, λ613 to λ585; 3rd band, λ577·5 to λ561·5; 4th band, λ537 to λ521·5 (?). Bands 2, 3, and 4 shown in plate.

"(2) *Chloroform solution*—1st band, λ654 to λ636; 2nd band, λ607 to λ580·5; 3rd band, λ572 to λ560; 4th band, λ536 to λ516. Bands 2, 3, and 4 shown in plate."[2]

4. *Bilifuscin*, $C_{16}H_{20}N_2O_4$, may be obtained from brown gall stones from the human being. By means of ether, cholesterin is removed from the powdered gall stone, and the powder is then treated with water and a little hydrochloric acid. It is then thoroughly washed with water to remove all the acid, next extracted with boiling ether to remove fatty acids, and finally boiled with absolute alcohol. On evaporating the alcoholic solution, bilifuscin appears as a dark brown powder, or a black brittle mass.[3] By a somewhat similar process, Simony has extracted it from human bile.[4] It does not give Gmelin's reaction. It is readily soluble in alcohol, glacial acetic acid, and alkalies; sparingly soluble in chloroform, but insoluble in water, ether, and dilute acids. The alcoholic solution absorbs the violet and indigo-blue parts of the spectrum.

5. *Biliprasin*, $C_{16}H_{22}N_2O_6$, is the name given by Staedeler to a green pigment obtained from gall stones. By exhausting the gall stones with ether, hot water, chloroform, and dilute hydrochloric acid, he obtained a brownish-green residue, from which chloroform dissolved a brown pigment (bilifuscin) and some bilirubin, whilst the undissolved residue gave to alcohol biliprasin. Its spectrum has not been studied.

[1] Watt's *Dict. of Chem.*, Bile Pigments, vol. vii. p. 189. Quoted from Maly and Heynsius and Campbell.

[2] See *Jour. Physiol.* vol. vi. Nos. 1 and 2, p. 24.

[3] Dr. Thudichum's *Chemical Physiology*, 1872.

[4] Watt's *Dict. of Chem.* vol. viii. p. 325.

6. *Bilicyanin*, a blue pigment, may be obtained by adding an alcoholic solution of bromine to bilirubin suspended in chloroform. On evaporation the liquid assumes a blue colour, and on complete evaporation "there remains a shining dark mass, which appears green when spread in a thin layer upon porcelain, but dissolves with a splendid blue colour in alcohol. It is less soluble in ether, but more freely in ether-alcohol." [1] It gives two or perhaps three absorption bands in the yellow and in the green.

7. Another blue colouring matter has been separated from the bile of man and of the ox, sheep, pig, dog, and cat by E. Ritter, to which no name has been given. Bile is shaken with chloroform till a yellow solution is obtained. This solution is treated with carbonate of soda till the yellow colour disappears. On neutralization with hydrochloric acid, two layers are formed, one the yellow chloroform solution and the other the blue pigment. This pigment is insoluble in acids and chloroform; it dissolves in alkalies, and when the alkaline solution is neutralized with acids and exposed to air, a brown precipitate is formed, which becomes blue in a few days or only after a month, thus differing from an alkaline solution of reduced indigo which instantly turns blue on exposure to the air.

8. *Bilihumin* is a name given by Staedeler to a humus-like residue left after exhausting gall stones with water, alcohol, ether, chloroform, and dilute acid successively. It is probably impure biliverdin and mucus.[2]

9. *Reducible Product of the Oxidation of the Bile Pigment.*—The following account of this substance from the paper on the subject by Stokvis is given in Watt's *Dictionary of Chemistry*, vol. vii. p. 189—

"The substance is formed as a secondary product in most cases of the oxidation of biliary colouring matter, whereby Gmelin's reaction is produced. It is colourless, or of a light yellow tint, and is soluble in water, alcohol, and dilute acids. It becomes of a beautiful rose-red colour when boiled with reducing agents in alkaline solutions. The red solution gives in the spectrum a tolerably broad absorption band in the green. In concentrated solutions (thick strata) the band begins close on the line *D* and extends to *b*. In dilute solutions (thin strata) it occupies only two thirds of the space between *D* and *E*, ending short of *E*. Shaking with air causes both the rose colour and the absorption band to disappear. This bye-product differs from the bile colouring matter and other oxidation products of the same, in being insoluble in chloroform and ether and in not forming

[1] Watt's *Dict. of Chem.* vol. vii. p. 188, quoting from Heynsius and Campbell. See also Dr. MacMunn, *op. cit.* p. 152.

[2] For an account of Dr. Thudichum's views as to the pigments of the bile, see his *Tenth Report of the Medical Officer of the Privy Council*, 1867; also his *Chemical Physiology;* also MacMunn's *Spectroscope in Medicine*, p. 154. Dr. MacMunn also gives an account of the spectra of the bile of various animals.

insoluble compounds with neutral or basic lead acetate. It is precipitated however by ammonia and basic lead acetate. This substance exists as such in the gall stones both of man and of the ox. It can be obtained from them by simply boiling with distilled water and extraction by dilute acids. It does not exist in fresh bile. It occurs in the urine of animals which have been starved for some days previously, in icteric urine, and in the urine of febrile diseases, such as small-pox, typhus, etc. It is not found in healthy urine. It seems to be present in the alimentary canal, although in direct experiments with different kinds of food little or none could be found. In alkaline solutions it soon loses its characteristic properties. Its occurrence in any liquid of neutral or acid reaction affords an indication of the previous existence of bile pigment therein. In applying the test, the liquid is to be precipitated with lead acetate, excess of lead removed by oxalic acid, and the filtrate concentrated and boiled with alkalies and a reducing agent. If no reduction takes place, and if the other tests for biliary colouring matter have given a negative result, their absence may be safely inferred."

C.—The Pigments of the Urine.

The urine contains at least two pigments, urobilin and indigo-blue, derived from indican. Thudichum describes a third, named urochrome, which by oxidation becomes uroerythrin.[1]

1. *Urobilin*, $C_{32}H_{40}N_4O_7$, is believed to be closely related to bilirubin, and it may be termed *hydrobilirubin*. When bilirubin is dissolved in dilute potash lye, and sodium amalgam added, air being excluded, no hydrogen is evolved, the dark brown fluid becomes lighter in colour, in the course of a few days becoming yellow or brownish-yellow, and then hydrogen is given off. Hydrochloric acid added to this solution separates a pigment of weak acid reaction, which is soluble in alcohol, slightly in water, readily in ammonia and fixed alkalies, and in ether, glacial acetic acid and chloroform. This substance contains about 1·5 per cent. less of carbon and about 1·5 per cent. more of hydrogen, and therefore it may be called *hydrobilirubin*. Thus—

$$\underset{\text{Bilirubin.}}{2(C_{16}H_{18}N_2O_3)} + H_2 + H_2O = \underset{\text{Hydro-bilirubin or urobilin.}}{C_{32}H_{40}N_4O_7}$$

Biliverdin with sodium amalgam behaves in a corresponding way. There is every reason to believe that in the intestines a portion of these bile pigments is changed into hydrobilirubin, which is then absorbed and excreted by the kidneys as urobilin. Urobilin is a dark reddish-brown powder, soluble in water and alcohol, less soluble in ether. It forms a reddish yellow solution in chloroform. With chloride of zinc and ammonia it gives a rose-coloured solution, with green fluorescence. This substance gives a characteristic spectrum, seen in Plate I. 14, which is thus described by Dr. MacMunn—

[1] This statement has however been disproved.

"Spectrum of urobilin from *normal* urine thus prepared: The urine is precipitated with neutral and basic acetate of lead, the precipitate separated by filtering, decomposed by rectified spirit containing sulphuric acid; this extract filtered, diluted with water, and agitated in a tap-funnel with chloroform; the chloroform separated off, filtered, evaporated, and the brownish residue redissolved in rectified spirit. *Febrile urobilin* differs from this in the greater shading and breadth of band and in other slight particulars. Stercobilin, found in fæces, also differs from it."

It will be seen that there is a distinct broad band a little to the left of *F*. In Plate I. 15 the spectrum is shown of the same solution with the addition of ammonia and chloride of zinc and filtered. Here the band is narrower and fainter, and less of the blue is visible. Dr. MacMunn gives the position of the absorption band, thus—"(1) Chloroform solution, λ 504 to λ 481; (2) Rectified spirit solution, λ 504 to λ 481; (3) with zinc, chloride and ammonia, λ 512·5 to λ 496." The band is specially well marked in febrile urines, but Dr. MacMunn asserts that urobilin is invariably present in normal human urine, although the quantity may be so small that the absorption band cannot be detected unless the urine has been subjected to the treatment with acetate of lead above described, or oxidized by means of permanganate of potash or bromine water. According to Disqué, there is a colourless form of urobilin in urine which may be called reduced urobilin, *i.e.* a *chromogen*, which gives no absorption spectrum. This, he says, may be readily changed into urobilin by oxidation,[1] either in the intestine or during the passage of the chromogen from the intestine to the kidneys. There is no strong evidence for the existence of this substance.

2. *Derivatives of Indican.*—By decomposition indican may give rise to leucin, volatile fatty acids, and a reddish colouring matter, *indigo-red*, the *indigrubin* of Schunck, the *urrhodin* of Heller. Sometimes, also, indican in the urine may give rise to *indigo-blue*. (See p. 109.) The uroxanthin of Heller appears to be indican. If the urine of cases of cancer of the liver, or of obstruction of the great intestine, be allowed to stand until putrefaction occurs, an irridescent pellicle forms, which yields small crystals of *indigotin*, and the fluid may become blue. Indigo-blue also may be obtained from most specimens of normal urine, and quite easily from the urine of the horse, by mixing the urine with an equal volume of strong hydrochloric acid, then adding a solution of hypochlorite of calcium till a blue colour appears, and, lastly, shaking up with chloroform. A blue chloroform solution of indigo-blue is thus obtained

[1] Ludwig Disqué. Ueber Urobilin. Zeitschrift für Physiol. Chemie, Band ii. 1878. Hoppe-Seyler has obtained urobilin by the action of tin and hydrochloric acid on hæmoglobin or hæmatin.—*Handbuch der Physiol. und Pathol. Chem. Analyse*, s. 214.

The spectrum of this solution is shown in Plate I. 16, and is thus described by Dr. MacMunn—

"Spectrum of *indigo-blue* from *normal* urine. The urine is boiled with about a third of its bulk of hydrochloric acid, cooled, and agitated with chloroform, which shows this spectrum, and also—although not here shown—a band at *F*, due to urobilin. The band before *D* is due probably only to the blue constituent, *indigo-blue*, whilst that after *D* is probably due to the *reddish* constituent, for this reason that the *bluer* the solution the deeper is the band before *D*, while the more it approaches violet the darker is the band after *D*. This test for the presence of indican will detect it when Jaffé's hypochlorite of calcium test fails. *Normal* urine can generally be made to show the presence of indican by its means."

3. *Urohæmatoporphyrin*, or *urohæmatin*, is a pigment found by Dr. MacMunn in the urine of cases of rheumatic fever and of Addison's disease. He believes it to be nearly related to the body prepared by Nencki and Sieber by the action of tin and hydrochloric acid on hæmatin. The alcoholic solution gives the following spectrum—"1st band, λ595 to λ587; 2nd band, λ576 to λ566; 3rd band, λ557 to λ541·5; and 4th band, λ503 to 482·5."[1]

4. *Urochrome*, a substance described by Dr. Thudichum is said to be an amorphous substance of a yellow colour, easily soluble in water, less so in ether, very dilute acids, and alkalies, and least of all in alcohol.[2]

5. *Uromelanin* is another dark pigment separated by Dr. Thudichum from urine by the action of strong sulphuric acid. Its existence as an independent pigment is more than doubtful.[3]

D.—Pigment of the Fæces.

In addition to the ordinary pigments of the bile, some of which are voided in the fæces, a variety of urobilin, to which the name *stercobilin* has been given, has been examined by Dr. MacMunn. He states that there is scarcely any difference between the urobilin found in the urine of febrile patients and the stercobilin of human fæces; but that there is a distinct difference in the spectrum of ordinary urobilin and stercobilin.[4] By covering fæces with absolute alcohol, chloroform, ether, rectified spirit, and sulphuric acid (1 in 15), and water, solutions were obtained which gave the following results with the spectroscope—[1] *Alcohol solution*, 1 broad band from λ506 to λ482·5; (2) with caustic

[1] *Cf.* C. le Nobel, *Archiv f. d. ges. Phys.* B. xl. 1887.

[2] Thudichum, *Brit. Med. Journal*, 1864.

[3] For an account of it see Watt's *Dict. of Chem.*, 1st supplement, p. 1120; and cf. Hoppe-Seyler, *Handbuch f. Physiol. und Path. Chem. Analyse*, Vierte Auflage.

[4] MacMunn, *Journal of Physiology*, vol. vi. Nos. 1 and 2.

soda, λ521·5 to λ505; (3) with zinc chloride alone, 1 band λ517·5 to λ501. [2] *Ether solution*, 1 band which, with acetic acid, extends from λ504 to λ482·5. [3] *Chloroform solution*, λ507 to λ486·5. (Compare this with the description of the spectrum of *normal* urobilin, given at p. 135.) On evaporating the chloroform solution in the water bath, Dr. MacMunn obtained a brown amorphous residue, which gave practically almost all the characters of febrile urobilin both as to solubility and spectrum. In none of the solutions did he detect unchanged bile pigments. If febrile urobilin and stercobilin were the same, then the amount of the former found in the urine would be the measure of the amount of the latter absorbed from the alimentary canal, a fact of considerable clinical importance. Dr. MacMunn thus sums up our knowledge of the relation of the urinary fæcal and bile pigments—

"It would appear that the stercobilin resulting from the putrefactive processes in the intestine, and accompanied by imperfectly changed biliary pigments, is taken up by the branches of the portal vein and carried into the liver, where it is probably again changed by the action of a ferment into a chromogen. A portion of this chromogen gets into the blood and is excreted by the urine as a chromogen. A portion may escape in the condition of biliary urobilin as such, and appear in the urine in a further oxidized condition or, owing to disturbance of circulation in the liver, a large portion of unchanged biliary urobilin may appear in the urine. Besides this, the urine under certain conditions may contain a pigment that has no biliary origin, and may be derived entirely from hæmatin; while, in certain diseased states a reduction product of hæmatin, having no connection with bilirubin or biliverdin, and closely related to hæmatoporphyrin, may appear in the urine and, to a great extent, if not altogether, may replace urobilin."[1]

E.—Pigments of the Tissues.

1. The *Histohæmatins* are a class of pigments probably first detected by Dr. Sorby, but elaborately examined and discussed by Dr. MacMunn,[2] found in the tissues of many animals, both invertebrate and vertebrate. Thus they have been found in the echinodermata, mollusca, arthropoda, and vermes, where they may be detected in the absence of hæmoglobin and its derivatives, which apparently they replace. With regard to the vertebrata, Dr. MacMunn says—"In vertebrata the search for histohæmatins in the organs and tissues is attended with difficulty, owing to the presence of hæmoglobin, but fortunately the bands of the histohæmatins can be recognized in the tissues or organs

[1] MacMunn, *Jour. of Physiology*, vol. vi. p. 39.

[2] MacMunn on Myohæmatin and the Histohæmatins. *Phil. Trans. of Royal Society*, part i. 1886.

when squeezed out to a degree of thinness which no longer allows the bands of hæmoglobin to be seen, and in most cases the blood-vessels can be injected with salt solution sufficiently to eliminate the influence of the circulating hæmoglobin." Dr. MacMunn has detected them in the stomach wall, liver, kidneys, and intestinal wall of various fishes; in the testes, liver, spleen, and stomach wall of the frog and other amphibians; in the liver, spleen, kidney, and intestinal wall of various reptiles; in the spleen, pancreas, liver, kidney, and gizzard of the common rock pigeon and other birds; in the liver, spleen, kidney, and stomach wall of the hedgehog, guinea-pig, rat, rabbit, dog, cat, pig, ox, and sheep. He also discovered a histohæmatin in the thymus gland of a child of ten months old, and in the spleen, liver, and kidney of man. A hæmochromogen was detected in the medullary portion of the human suprarenal body, and a histohæmatin in the cortex. No trace was discovered in nervous tissues in invertebrates or vertebrates. No histohæmatins have yet been isolated,[1] as they are probably united to a proteid, the compound being changed by the action of the various solvents used. Dr. MacMunn, however, has made the important discovery that they can be reduced in the tissues, and that the appearance of an absorption spectrum of bands indicates a *reduced* state of the histohæmatins, the "bandless" spectrum characterizing them in the oxidized condition. The histohæmatins are allied to the hæmochromogens, the spectra being changeable into one like that of hæmochromogen; and, as "their bands are intensified by alkalies and enfeebled by acids, intensified by reducing agents and enfeebled by oxidizing agents," they are capable of oxidation and reduction, and are therefore respiratory. They are concerned in the internal respiration of the tissues. The position of the bands is thus indicated by Dr. MacMunn—(1) *Stomach wall of cat*—blood free: "1st band, λ613 to λ593; 2nd band, λ569 to λ563; 3rd band, λ556 to λ551. (2) *Kidney of cat:* 1st band, λ613 to λ596·5; 2nd band, λ569 to λ563; 3rd band, λ556 to λ550."[2]

2. *Myohæmatin.*—This is a pigment, of a yellowish-red colour, also discovered by Dr. MacMunn in the muscles of certain beetles (*Hydrophilus, Dytiscus*), the common fly (*Musca vomitoria*), and other insects; in spiders, crustaceans, molluscs, fishes, amphibians, reptiles, birds and mammals.[3] He has also found it in the heart and voluntary muscles of man. "Its bands are seen with great distinctness in the *musculi*

[1] Although they can be got into solution by means of ether, see below, Myohæmatin.

[2] MacMunn, "Further Observations on Myohæmatin and the Histohæmatins," *Jour. of Physiol.* vol. viii. No. 2.

[3] MacMunn, *Phil. Trans. op. cit.* part i. 1886.

papillares of the human heart." By a process of freezing, combined with pressure, which apparently prevents the decomposition of the proteid compound in which myohæmatin exists, Dr. MacMunn[1] has obtained "a few drops of a reddish-yellow liquid," which gives the characteristic spectrum, mixed with the spectrum of oxy-hæmoglobin. On reducing the latter with ammonium sulphide, "through the thin hazy band of reduced hæmoglobin," the bands of myohæmatin were apparent. The spectrum of myohæmatin from the alar muscle of a meat fly is shown in Plate I. 11, and Dr. MacMunn remarks—"The spectrum is practically the same throughout the whole animal kingdom." He gives the position of the bands as follows—"(1) *Heart of hare:* 1st band, λ613 to λ600; 2nd band, λ569 to λ563; 3rd band, λ556 to λ550. (2) *Heart of rat:* 1st band, λ613 to λ596·5; 2nd band, λ569 to λ563 3rd band, λ556 to λ550."

F.—Luteins or Lipochromes.

In 1849, Dr. Thudichum[2] described a pigment which he obtained from the *corpora lutea*, from eggs, from butter, and from blood serum, to which he gave the name *lutein*. Considerable doubt has been thrown on the existence of all of these bodies, but a colouring matter giving absorption bands does exist in the *corpora lutea* and in the yolk of egg.[3] A yellow colouring stuff has been extracted by ether from the eyes of frogs after removal of the retinæ. It has two absorption bands between F and G, and it bleaches by sunlight. It has been called *lipochrin*, and is no doubt a lutein. The lutein pigment is held in solution in fatty matter. The name *lipochromes* has been given by Krukenberg to all animal pigments soluble in certain fat solvents and that give bands in the blue and violet. These are all *luteins*. They give in the solid state a blue or green colour with strong nitric and sulphuric acids, also generally with iodine dissolved in iodide of potassium. An immense number of lipochromes have been found not only among animals but also among plants. Carotin, $C_{18}H_{24}O$, may be taken as a typical lipochrome. None so far has been found to contain nitrogen.

[1] MacMunn has obtained myohæmatin in solution from the pectoral muscles of pigeons by covering the blood-free muscles (finely divided) with ether for some days, when a red juice exudes, which shows the bands of modified myohæmatin. *Jour. of Physiol.* vol. viii. No. 2, 1887.

[2] Thudichum, *Proc. Roy. Soc.* xvii. 253. The yellow colouring matter was first separated from ovary of cow by Piccolo and Lieben and called *hæmolutein*. An ethereal extract of corpora lutea boiled with potash has excess of water added, when small shining dichroic crystals are deposited.

[3] MacMunn on "Animal Chromatology," *Proceed. of Birmingham Philosoph. Society*, vol. iii. 1883.

G.—Chromophanes.

These are colouring matters that have been separated by Kühne from the cones of the retina. Three such bodies have been isolated and their spectra examined.[1] "A large number of eyes (50 to 300) of doves or hens are bisected so as to cut off the anterior segments; the vitreous humour being removed, the posterior segments of the eyes are placed at once in absolute alcohol; as soon as possible the alcohol is poured away and the eyes are thoroughly exhausted with ether. On evaporating the ether, a fiery-red fat is obtained which is dissolved in hot alcohol and saponified by the action of caustic soda, water being used to replace the alcohol as it evaporates. The hard soap which separates from the mother liquor is well dried and treated successively with petroleum ether, then with ether, and lastly with benzol, which dissolve in order—*chlorophane*, *xanthophane*, and *rhodophane*." The probable function of these colouring matters in connection with the sense of vision will be discussed fully in treating of that sense. All that is here necessary is to describe shortly the spectra of these pigments.

a. Chlorophane is a greenish-yellow pigment and gives the absorption bands seen in Fig. 56.

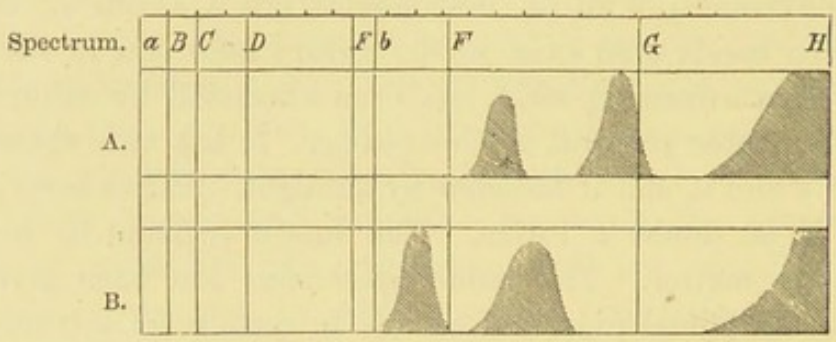


Fig. 56.—Spectrum of Chlorophane—A, dissolved in ether or in petroleum ether; B, dissolved in bisulphide of carbon.

b. Xanthophane is slightly soluble in petroleum ether, readily soluble in alcohol, ether, and carbon disulphide, and gives the spectrum seen in Fig. 57.

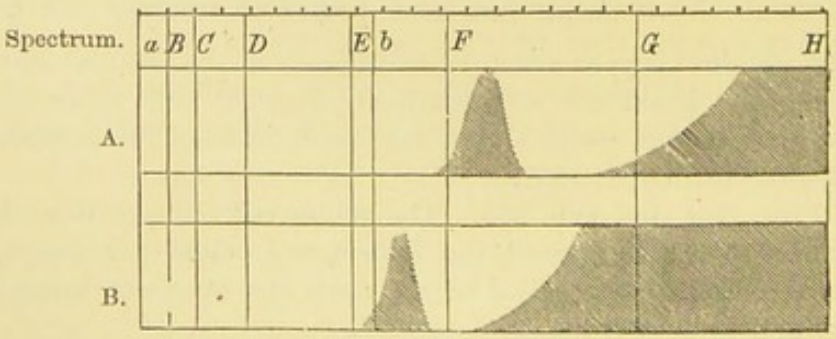


Fig. 57.—Spectrum of Xanthophane—A, dissolved in ether; B, dissolved in bisulphide of carbon.

[1] Gamgee's *Physiological Chemistry*, vol. i. p. 460.

c. Rhodophane is insoluble in petroleum ether or carbon disulphide, is soluble in oil of turpentine, in alcohol acidified with acetic acid, and in benzol, and it shows the spectrum given in Fig. 58.

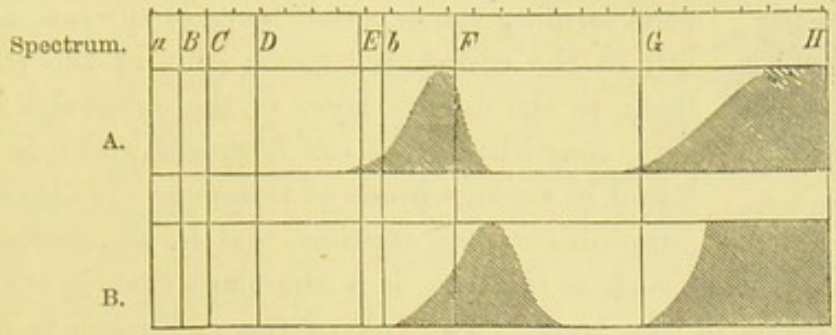


FIG. 58.—Spectrum of Rhodophane—A, dissolved in benzol; B, dissolved in oil of turpentine.

d. Rhodopsin or visual purple is a colouring matter associated with the rods of the retina, and sensitive to light. Kühne discovered that it is soluble in "crystallized bile" thus prepared—

"Colourless crystallized bile is obtained by evaporating ox-bile to dryness in the water bath, after mixing it thoroughly with much animal charcoal. The perfectly dry residue is treated with absolute alcohol, and a large excess of ether is added to the filtered solution; by this means the salts of the bile acids are precipitated and ultimately acquire a crystalline structure. The precipitate, which consists of sodium glycocholate and taurocholate is termed 'crystallized bile.'"[1]

As this substance is destroyed by the action of actinic light, care must be taken to open the eyes of frogs in a chamber lit by a sodium flame, and the retinæ are moistened with about 1 c.c. of a 2 per cent. solution of the bile salts, and gently shaken for about one hour. A reddish-purple fluid is thus obtained which is a solution of rhodopsin. Light causes it to pass from purple, "through red and orange, to yellow, before becoming colourless." Thus "visual purple" is converted into "visual yellow" and the latter then fades away. The spectra of the two conditions are shown in Fig. 59.

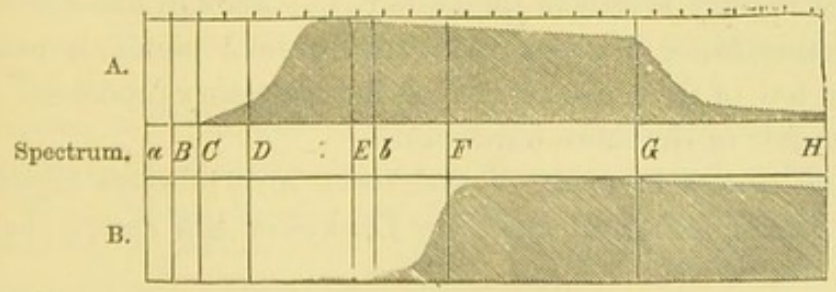


FIG. 59.—Spectrum of Rhodopsin—A, unaltered by light, "visual purple"; B, altered by light, "visual yellow."

[1] Gamgee's *Physiological Chemistry*, vol. i. p. 464.

H.—Black Pigment.

A black pigment named *Melanin* occurs in the hexagonal epithelial cells forming the external layer of the retina (Fig. 60) in the connective tissue cells of the choroid itself, in the deeper layer of the epidermis of the skin, constituting the *rete Malpighi*, and it is often found in various kinds of tumours. It also occurs in the skin of fishes, amphibians, and reptiles, and in the feathers of birds. In amphibians, such as the frog, it is abundant even in the deep tissues and organs of the body. The ink of the cuttle-fish is of the same nature; but there is no doubt that quite different colouring matters have been included under the name melanin, which only possess one attribute in common, viz., blackness. Some of these pigments are non-nitrogenous, *e.g.* from birds and *Sepia* (cuttle-fish). It is insoluble in water, acids, alcohol, and ether. It is imperfectly dissolved in boiling caustic potash, a brown fluid being formed, thus distinguishing it from the particles of carbon sometimes found in lung tissue, which are quite insoluble in boiling caustic potash. The percentage composition varies from C, 51·7 to 58·3; H, 4·02 to 5·09; N, 7·1 to 13·8; and O, 22·03 to 35·44.[1] The pigment of the choroid is said to contain a small amount of iron.

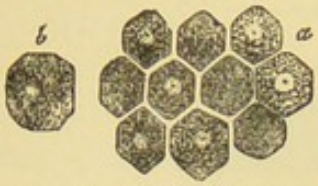


Fig. 60.—Pigment cells.

I.—Other Animal Pigments.

To attempt to give the characters of the numerous pigments existing in animals, more especially among the invertebrata, that have been described by Moseley, Ray Lankester, Krukenberg, MacMunn, and others in recent years, is quite beyond the aim of this work. Only a few are alluded to, specially with the view of pointing out the wide range of many of these pigments, and the remarkable resemblance they bear to some of the pigments already described as relating to man and the higher animals. This branch of inquiry is still in its infancy, but enough has been done to show the resemblance between plant and animal pigments and the fact that not a few of them are concerned in respiratory processes. The following are a few of the more important.[2]

1. *Chlorophyll* occurs in many of the lower invertebrates (*Spongilla*, *Hydra*, *Paramecium*), and Professor Ray Lankester has shown that the

[1] Gamgee, *Physiological Chemistry*, vol. i. p. 304.

[2] The inquirer wishing to study these pigments may refer to MacMunn's papers already frequently referred to, which are not only critical and full of the results of original inquiry, but also give many references to the literature of the subject.

corpuscles are part of the animal itself and not unicellular algæ leading an independent existence, as Brandt supposed.[1] It has also been found in the blood of caterpillars[2] and in the elytræ of cantharides beetles.[3] Dr. MacMunn has found in many animals (more especially in alcoholic extracts of the "liver and other appendages of the intestine answering to it"), a substance having a spectrum similar to that of the chlorophyll of plants, to which he gives the name *entero-chlorophyll*.[4] He has also recently found chlorophyll in several salt water sponges.[5]

2. *Hæmocyanin* is a blue pigment found in the blood of many invertebrates. The blood of many molluscs and arthropods becomes bluish in colour after exposure to the air (*Helix*, *Unio*, *Limulus*, *Octopus*, *Sepia*, *Scorpio*, *Cancer*, *Homarus*, *Portunus*, *Maja*, *Squilla*, etc.). MacMunn found no absorption bands in that of *Helix pomatia*, *Helix aspera*, *Paludina vivipara*, *Lymnæus stagnalis*, *Homarus vulgaris*, *Cancer pagurus*, *Carcinus mænas*, and *Astacus fluviatalis*.[6]

3. *Chlorocruorin* is a green pigment discovered by Professor Ray Lankester in the blood of *Sabella ventilabrum* and *Siphonostoma*,[7] which is capable of being oxidized and reduced by ammonium sulphide like hæmoglobin. MacMunn has figured the spectrum of the blood of *Sabella* and of *Serpula contortuplicata* in the paper referred to,[6] and shown its resemblance in some respects to that of hæmochromogen.

4. *Echinochrome* is a brown pigment found in the perivisceral cavities of *Echinus* (Schäfer, P. Geddes, and MacMunn), and also in *Strongylocentrotus lividus* by MacMunn, who first described its spectrum. When exposed to the air it becomes deeper in colour. Dr. MacMunn remarks with regard to the spectra figured in the paper—"A comparison of these spectra shows how remarkably unstable echinochrome is. It is on this instability that its usefulness as a respiratory substance depends."

[1] Ray Lankester on the Chlorophyll Corpuscles and Amyloid Deposits of Spongilla and Hydra—*Quart. Jour. of Micros. Science*, vol. xxii. N.S. See also MacMunn on the Spectroscope in Biology—*Proceed. of Birmingham Philosophical Society*, vol. v. part 1, and *Quart. Jour. Micros. Science*, vol. xxvii. p. 573 *et seq.*

[2] Poulton, The essential nature of the colouring of Phytophagous Larvæ—*Proc. Roy. Soc.* 1885.

[3] Pocklington, *Phar. Jour. Trans.* vol. iii. ; MacMunn, *Brit. Ass. Report*, 1883.

[4] MacMunn, Further observations on Enterochlorophyll and Allied Pigments—*Phil. Trans. Royal Society*, part 1, 1886. See also MacMunn's Notes on the Chromatology of *Anthea cereus*—*Quart. Jour. of Micros. Science*, March, 1887.

[5] MacMunn, *Proc. Physiol. Soc.* March, 1887.

[6] MacMunn, Chromatology of Blood of some Invertebrates—*Quart. Jour. of Micros. Science*, 1885.

[7] Ray Lankester, *Jour. of Anat. and Physiol.* 1868.

5. *Pentacrinin*, a pigment discovered by Professor H. N. Moseley[1] in species of *Pentacrinus* dredged by H.M.S. "Challenger." It exists either as a purple or a red pigment, and gives a spectrum of three bands, "one intensely black covers *D*, the second is between *D* and *E*, and the third, a broad dim one, stretches from *b* to *F*." Its spectra have been mapped and measured by MacMunn (*Proc. Phil. Soc. Birming. op. cit.*), and he remarks—"It may be concluded, however, that Moseley's purple pentacrinin does bear a most remarkable resemblance to plant pigments. It also bears a resemblance to *bonellein*, the colouring matter of *Bonella viridis*, which had long been taken to be a chlorophyll, and which Professor Lankester thinks may also be present in *Chætopterus*."

6. *Actiniohæmatin* is a colouring matter found in *Actinia mesembryanthemum* by MacMunn, which can be changed into hæmochromogen and hæmatoporphyrin. It is not actiniochrome, a pigment widely distributed in actiniæ, chiefly in the tentacles. In *Sagartia parasitica*, a special pigment is found not identical with either actiniohæmatin or actiniochrome, and in *Anthea cereus*, *Bunodes balii*, and *Sagartia bellis*, a mixture of plant-like pigments is met with containing chlorofucin (an algal pigment), and derived from the "yellow cells" in the tentacles.[2]

7. *Turacin* is a pigment obtained from the feathers of the Cape lory, which is said to give a spectrum like that of blood. (Church.)

8. *Tetronerythrin* is a pigment found by Hoppe-Seyler in the rose-coloured rings round the eyes of certain birds. Merejkowski[3] has found it in 104 species of invertebrates and fishes. This body is of great interest inasmuch as it is said to perform the function of hæmoglobin as a carrier of oxygen to the tissues. In many invertebrates it takes the place of hæmoglobin, and this no doubt accounts for its wide distribution.

9. *Crustaceorubin*[4] is the name given by Moseley to a pigment he found in some deep-sea decapod crustacea.

10. *Cochineal*, from the insect *Kermes cacti*, and *Lac-dye*, from *Coccus laccæ*, give no characteristic absorption spectrum. Carminic acid, from carmine, gives a spectrum somewhat resembling that of hæmoglobin.

11. *Aphidein* is a pigment obtained by Sorby from *Aphides*. It gives an absorption spectrum, and it appears to be a true respiratory pig-

[1] Moseley, *Quart. Jour. of Micros. Science*, 1877.

[2] MacMunn, Notes on the Chromatology of Anthea cereus—*Quar. Jour. of Micros. Science*, vol. xxvii. N.S., p. 573-590.

[3] It would appear that under the name "tetronerythrin" several lipochromes of a reddish colour have been included.

[4] Probably identical with tetronerythrin.

ment. Sorby shows that aphidein is related to cochineal on the one hand and to hæmoglobin on the other.

12. *Aplysiopurpurin* is a purple colouring matter obtained by Moseley from the *Doris* (nudibranch). It also exists in *Aplysia*, the sea-hare.

13. *Tyrian purple* is the dye obtained from species of *Purpura* and *Murex*, and is a secretion of a layer of epithelium, "which is placed between the gills and the hind gut in the mantle cavity."

Conclusions Regarding the Pigments.

The previous discussion of the nature and properties of the pigments enables us to appreciate their physiological significance and to understand in some measure the part they play in the animal body. In most, if not in all, animals there is one chief pigment such as the hæmoglobin in the blood of the higher groups, and the entero-chlorophyll, hæmocyanin, chloro-cruorin, echinochrome, or tetronerythrin[1] of many of the lower. This chief pigment is mainly if not wholly concerned in respiration, by its property of combining readily with a certain amount of oxygen and of giving this up to a reducing agent. Thus it receives oxygen from the air or from the air dissolved in the water in which the creature lives, and it conveys this oxygen to the living tissues. These tissues practically reduce it, and it returns to its non-oxygenated condition. In this process, however, it is not a stable substance. It undergoes decomposition, becoming modified into histohæmatins that permeate the tissues, probably entering into their very substance and still exercising a respiratory function. May it not be possible that these histohæmatins act intermediately as regards the hæmoglobin (or its representative) of the blood and the oxygen-seeking living tissue? Again, the hæmoglobin may be resolved in the liver cells, or in the intestinal canal, or in the kidneys, and probably in other organs, into simpler bodies which then appear as the pigments of the various excretions, waste-products, in short, formed in the chemical operations of the body. Such are probably the pigments of the bile, of the urine, and of the fæces, bodies of no physiological value, and voided as useless, at all events in the higher animals. In some of the lower animals, however, such waste-pigments may not be useless, but if innocuous they may be stored up in the epidermal tissues, thus staining the covering of the body so as to suit the habits of the animal, or perhaps adorning it with beautiful colours that serve some useful purpose in the circumstances of its life. Lastly, certain pigments have a more specialized function. Such are the pigments in the eye-spots of many of the humbler forms, and the pigments of the rods and cones of

[1] With regard to the latter, it has never yet been shown that it is affected by reducing agents.

the retina. These are sensitive to light, and by the chemical changes occurring in them under the influence of light the ends of the optic nerve fibres may possibly be stimulated. The recent researches into the nature of pigments are of profound interest as giving us an insight into some obscure processes, and there is little doubt that after the comparative study of pigments has progressed much farther than it has yet done even more important generalizations may be possible than those I have ventured to make. Nor is the subject devoid of practical importance. The physician by studying the amount and nature of the urinary pigments, for instance, may obtain valuable information as to the metabolism occurring in the liver and the changes happening in the bowel, and it is likely that a more careful study of the pigments in some pathological conditions, such as Addison's disease, with the peculiar bronzing of the skin, may throw light on the nature of these obscure affections.[1]

Chap. X.—THE NON-NITROGENOUS MATTERS.

These include the alcohols, the fats, the carbo-hydrates, and certain acids belonging to the acetic acid, the glycollic acid, the oxalic acid, and the oleic acid series of non-nitrogenous organic acids.

1. The Alcohols.

1. *Ethylic Alcohol*, $C_2H_5 . HO$, common alcohol, is said by Bechamp and Rajewski to be formed in small quantities in the body, so that it may be detected in the urine even in the absence from the food and drink of all fermented liquors. In these circumstances it must be formed by an alcoholic fermentation in the body, either in the intestinal canal, or in the tissues, most probably in the former.

2. *Cholesterin*, or *Cholesteric Alcohol*, $C_{26}H_{44}O . HO$, a crystalline substance in the bile, and forming the chief constituent of gall stones, is regarded by chemists as an alcohol belonging to the series $C_nH_{2n-8}O$. It was first obtained by Conradi, so long ago as 1775, from gall stones. It exists in the bile, and it has been obtained from blood, urine, nervous matter, yolk of egg, seminal fluid, red blood corpuscles, white blood corpuscles, milk, the spleen, the contents of the intestines, the meconium, the fæces, tubercular and cancerous deposits, cataracts of the eye, and atheromatous blood vessels. It has also been found in peas, fixed oils, fat of wheat, glutin, fat of rye, barley, maize seeds, young shoots of roses, and in yeast. To obtain it pure, pulverize biliary calculi, extract with

[1] On this subject see Dr. MacMunn's suggestive paper in the *British Medical Journal*, Feb. 4th, 1888, entitled, "On Addison's Disease and the Function of the Suprarenal Bodies."

boiling ether, distil off the ether, dissolve the residue in alcohol, and allow the solution to cool. The crystalline mass in the solution is heated for some time with alcohol containing a little caustic potash, and on cooling, a crop of crystals is obtained. These are washed with alcohol, and if it is desired to get them pure, they may be re-dissolved in and recrystallized from ether. The crystals are odourless and tasteless. They then appear as rhombic plates, often with one obtuse angle awanting (Fig. 61). Cholesterin is insoluble in water, in alkalies and dilute acids; slightly soluble in cold, but very soluble in hot alcohol, and in ether, acetic acid, glycerine, benzol, petroleum, chloroform, and solutions of the bile acids. Anhydrous cholesterin fuses at 145° C., and solidifies at 137° C. Its specific gravity is 1·046. Solutions of cholesterin with polarized light give $[a]_D = -31°{\cdot}6$. Hot nitric acid oxidizes it so as to form cholesteric acid, α, $C_8H_{10}O_5$, a substance also produced by the oxidation of the bile acids. (Witthaus.) By oxidation with potassium permanganate, three acids may be obtained: cholesteric, β, $C_{26}H_{42}O_4$; oxycholesteric, $C_{26}H_{42}O_5$; and dioxycholesteric, $C_{26}H_{42}O_6$. (Latschinoff.) There are two good tests for cholesterin: (*a*) If the substance is bruised with a few drops of sulphuric acid, and a drop or two of chloroform then added, a blue-red or violet colour is produced, which changes to green on exposure to air; and (*b*) when a mixture of two volumes of sulphuric acid with one volume of ferric chloride is evaporated upon cholesterin a violet colour appears. When sulphuric acid is added to a solution of cholesterin in chloroform the upper fluid becomes blood-red or purple red and the under liquid has a green fluorescence. (Salkowski.)[1] A substance, isomeric with cholesterin (*isocholesterin*), has been obtained by E. Schulze from sheep's wool.

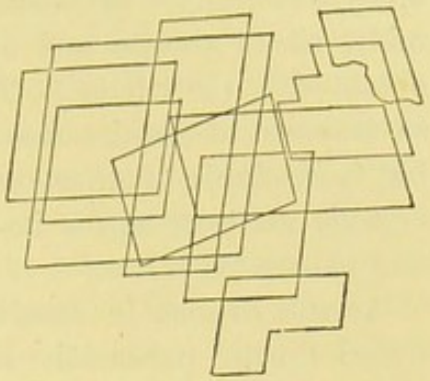


FIG. 61.—Crystals of cholesterin.

The mode of origin of cholesterin in the body has not been clearly made out. Whether it is formed in the tissues generally, or in the blood, or in the liver, is not known, nor has it been determined conclusively that it is derived from the decomposition of albuminous or even of nervous matter. It is also doubtful if we can regard it as a waste-substance of no use in the body, as its presence in the blood corpuscles, in nervous matter, in the egg, and in vegetable grains of various kinds, points to a possible function of a histogenetic or tissue-forming character.

3. *Glycerine*, $C_3H_5 . (OH)_3$, is a triatomic alcohol existing in the fats

[1] See Watt's *Dict. of Chem.* vol. vii. p. 329.

of the body, which, as will presently be seen, are its ethers. It also exists in palm and other vegetable oils, and it is one of the by-products of the alcoholic fermentation. The chemist has been able to build it up synthetically "by heating for some time a mixture of allyl tribromide, silver acetate and acetic acid, and saponifying the triacetin so obtained." (Witthaus.) The properties of glycerine are so well known as scarcely to require description. It is soluble in water and alcohol, but is insoluble in ether and chloroform. It is a good solvent for many organic matters, as, for instance, the ferments of the salivary, gastric, and pancreatic juices.

As will be seen in describing the digestive process, glycerine is produced during pancreatic digestion. The fat of the food is decomposed by the action of the pancreatic ferment, and glycerine is set free. Some have held that this free glycerine is instantly absorbed into the blood, and that in the blood it is quickly decomposed into simpler substances. That the process is rapid is evident from the fact that no free glycerine can be detected either in the bowel or in the blood, and even after the injection of glycerine into the blood, there are none of the products of its oxidation (formic acid, acetic acid, propionic acid, etc.). The amount of carbonic acid, however, is increased, and consequently it has been supposed that it is decomposed into carbonic acid and water. Others have contended that glycerine may contribute to the formation of glycogen, the carbo-hydrate of the liver. A diet rich in glycerine undoubtedly increases the amount of glycogen in the liver, but it is not easy to follow the steps of the process by which this may be accomplished. Thus "Ploesz has found in the urine of animals to which glycerine has been given a reducing body analogous to glucose, but differing in having no action on polarized light." (Beaunis.) This body is not glucose, but has been supposed to be an aldehyde of glycerine, $C_3H_6O_3$, and by the combination of two molecules of this substance, with the elimination of water, glycogen may be formed. Thus—

$$\underset{\text{Aldehyde of glycerine.}}{2C_3H_6O_3} - H_2O = \underset{\text{Glycogen.}}{C_6H_{10}O_5}$$

Again, it has been supposed that the glycerine may not be absorbed, but may be decomposed in the intestine into various acids, and in particular propionic acid, which may then form propionates voided in the fæces. It is not likely that the whole of the glycerine is thus disposed of. A more probable theory is that of Beneke, who suggests that as by the action of the gastric juice phosphoric acid is set free from the phosphates of the food, this free acid may unite with the free glycerine to form the important substance, phospho-glyceric acid, which again is concerned in

the formation of lecithin. (Beaunis.) On the whole, I am of opinion that the evidence is in favour of the view that glycerine contributes to the formation of glycogen.

4. *Phenol*, C_6H_5 . OH, phenic acid, carbolic acid, has been found in small quantities in the urine and fæces, and is probably the result of pancreatic digestion. Staedeler has discovered it in the urine of the cow. When found in urine, it is not in the free state, but in the condition of phenol-sulphuric acid or phenol-sulphate of potash, $C_6H_5O.SO_2.OK$. The simultaneous ingestion of phenol and of sulphuric acid, or of a sulphate, is said to increase the amount of the phenol-sulphates, and to cause a disappearance of the ordinary sulphates. The phenol of the urine may have originally been formed in the intestinal canal, but it is not improbable that some substances introduced into the body may be transformed directly into phenol. This peculiar combination of phenol and sulphuric acid is the type of a number of bodies to which Baumann has given the name of sulpho-conjugated acids. Phenol-sulphate of potash may be decomposed by acids and by heat into phenol and sulphuric acid. Many other compounds of a similar kind exist, such as thymol, pyrocatechin, pyrogallic acid, nitro-phenol, amido-phenol, sulpho-pyrocatechic acid, and chresol-sulphuric acid. (Beaunis, p. 115.)

2. The Fats.

Animal fats exist during life in a liquid form, contained in small cells lying in the meshes of connective tissue, from which the fluid may be expelled by pressure (Fig. 62). The oils thus obtained have been termed *palmitin*, *stearin*, and *olein*. Stearin is the most consistent of the three, while olein is fluid at ordinary temperatures. Human fat is formed chiefly of a mixture of palmitin and olein, and contains only a very small quantity of stearin.

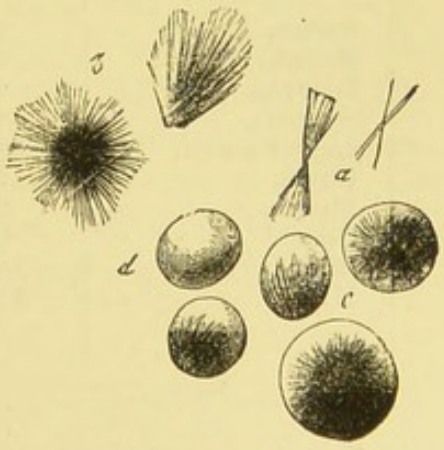


FIG. 62.—On the cooling of fat, say after death, the fatty matters may assume the forms of groups of needle-shaped crystals. *a*, Single needles; *b*, larger groups of the same; *c*, groups of needles within fat cells; *d*, a fat cell quite free from them.

Fats consist chemically of a combination of the triatomic alcohol known as *glycerine*, $C_3H_8O_3$, with a fatty acid. Their structure may be best comprehended by supposing that they are built on the type of three molecules of water, in which three atoms of hydrogen are replaced by three equivalents of the monatomic

radicle of a fatty acid and the remaining three by the triatomic radicle glyceryl. Thus—

$$\underset{\text{Radicle.}}{C_{16}H_{33}} - H + O = \underset{\text{Radicle of palmitic acid.}}{C_{16}H_{31}O}$$

$$\underset{\text{Radicle.}}{C_{18}H_{37}} - H_2 + O = \underset{\text{Radicle of stearic acid.}}{C_{18}H_{35}O}$$

$$\underset{\text{Water.}}{(H_2O)_3} \quad \underset{\text{Radicle of palmitic acid.}}{C_{16}H_{31}O} \quad \underset{\text{Glyceryl.}}{C_3H_5} \quad \underset{\text{Palmitin.}}{\left\{(C_{16}H_{31}O)_3C_3H_5\right\}O_3} \text{ or } C_{51}H_{98}O_6$$

$$\underset{\text{Water.}}{(H_2O)_3} \quad \underset{\text{Radicle of stearic acid.}}{C_{18}H_{35}O} \quad \underset{\text{Glyceryl.}}{C_3H_5} \quad \underset{\text{Stearin.}}{\left\{(C_{18}H_{35}O)_3C_3H_5\right\}O_3} \text{ or } C_{57}H_{110}O_6$$

$$\underset{\text{Olein.}}{\left\{(C_{18}H_{33}O)_3C_3H_5\right\}} O_3 . 3H_2O \text{ or } C_{57}H_{104}O_6 + 3H_2O$$

A fat may thus also be considered as a compound ether, or, in other words, as consisting of palmitic, oleic, and stearic glycerides, variously mixed together.

Moleschott found in the body of a man of thirty years of age, weighing 63·65 kilogr., about 1566 grammes, that is, 2·5 per cent. of the body weight. Burdach gave an estimate of 5 per cent., but it is evident the percentage of fat will vary much in different individuals. The following table from Gorup-Besanez[1] gives the percentage of fat in the chief organs and fluids of the human body—

Fluids.	Percentage of Fat.	Tissue or Organ.	Percentage of Fat.
Sweat, - -	·001	Vitreous humour,	·002
Saliva, - -	·02	Cartilage, - -	1·3
Lymph, - -	·05	Bone, - - -	1·4
Synovia, - -	·06	Crystalline lens, -	2·0
Liquor amnii, -	·06	Liver, - -	2·4
Chyle, - -	·2	Muscles, - -	3·3
Mucus, - -	·3	Hair, - - -	4·2
Blood, - -	·4	Brain, - -	8·0
Bile, - - -	1·4	Egg, - - -	11·6
Milk, - - -	4·3	Nerves, - -	22·1
		Adipose tissue, -	82·7
		Marrow of bone,	96·0

1. *Tripalmitin*, $C_3H_5(O . C_{16}H_{31}O)_3$, exists in most animal and vegetable fats, and especially in palm oil. It occurs in the form of crystalline plates, very soluble in ether, and sparingly soluble in hot alcohol. It melts at 50° C., and solidifies at 46° C.

2. *Tristearin*, $C_3H_5(O . C_{18}H_{35}O)_3$, is the chief constituent of ordinary

[1] Gorup-Besanez, *Physiologischen Chemie*, 1867, p. 149.

animal fats. It is, in the pure state, a hard crystalline solid, readily soluble in ether, and soluble in boiling, but scarcely soluble in cold, alcohol.

3. *Triolein*, $C_3H_5(O \cdot C_{18}H_{33}O)_3 \cdot 3H_2O$, is the principal constituent of all fats that remain fluid at ordinary temperatures, and in the pure condition it is a colourless, tasteless, odourless oil, soluble both in alcohol and in ether.

4. *Trimargarin*, $C_3H_5(O \cdot C_{17}H_{33}O)_3$, has been obtained artificially as a crystalline solid. By this name is sometimes meant a mixture of tripalmitin and tristearin.

5. *Tributyrin*, $C_3H_5(O \cdot C_4H_7O)_3$, is found in butter. It is a pungent liquid, and when it decomposes, butyric acid is set free.

6. *Trivalerin*, $C_3H_5(O \cdot C_5H_9O)_3$, exists in seal oil, and in the fat of some marine mammalia. It is identical with the *phocenine* of Chevreul. (Witthaus.)

7. *Tricaproin*, $C_3H_5(O \cdot C_6H_{11}O)_3$; *Tricaprylin*, $C_3H_5(O \cdot C_8H_{15}O)_3$; and *Tricaprin*, $C_3H_5(O \cdot C_{10}H_{19}O)_3$, are found in milk, butter, and cocoa-butter.

Fats are either derived from the fat in food or formed in the body. Their origin, function, and ultimate destination will be fully discussed in treating of nutrition. It is sufficient here to say that they contribute largely by oxidation to the production of heat, and that they also are concerned in histogenetic processes.

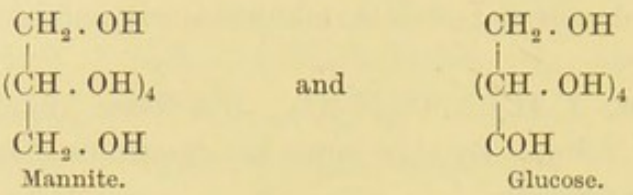
3. The Carbo-Hydrates.

The name of this important group indicates that they may be regarded as composed of carbon with hydrogen and oxygen in the proportions that form water. Thus, glucose is $C_6H_{12}O_6$, or $C(H_2O)_6$. This is, however, a very imperfect view of their chemical constitution, as there are chemical considerations which point to some of the carbohydrates being aldehydes, alcohols, or ethers. They are classified as follows, the + or − sign indicating that they are dextro- or lævo-rotatory as regards polarized light—

1. Glucoses.	2. Saccharoses.	3. Amyloses.
$n(C_6H_{12}O_6)$	$n(C_{12}H_{22}O_{11})$	$n(C_6H_{10}O_5)$
+ Glucose.	+ Saccharose.	+ Starch.
(Dextrose).	+ Lactose.	+ Glycogen.
Chondroglucose.	+ Maltose.	+ Dextrin.
− Lævulose.		Cellulose.
Mannitose.		Gums.
+ Galactose.		
Inosite.		

(1) The Glucoses.

These may be regarded as the aldehydes of mannite, and they contain in their rational formula the group of atoms, COH, characteristic of aldehydes. Thus—



Mannite. Glucose.

1. *Glucose, glycose, grape sugar, diabetic sugar, dextrose,* $C_6H_{12}O_6$, exists in fruits, in honey, in the liver, thymus, heart, lungs, blood, and muscles, and in the saliva, sweat, fæces, blood, and urine of persons suffering from *diabetes mellitus.* Human blood contains on an average about ·09 per cent. (Bernard.) It has also been found in the fluid of the amniotic and allantoic sacs of herbivora, and in the urine of the fœtal calf and fœtal sheep. Glucose may be crystallized from an aqueous solution in white spheroidal masses containing 1 molecule of water, and from alcohol in transparent anhydrous prisms. It is soluble both in hot and in cold water, and in alcohol. With polarized light, the result is $[a]_D = +52°{\cdot}85$. A solution of glucose heated with an alkali gives a brown or yellow colour, from the formation of glucic and melassic acids. In alkaline solutions, glucose reduces salts of silver, bismuth, mercury, and copper, in the case of the first three, the metal being precipitated, whilst cupric are reduced to cuprous compounds, with the separation of cuprous oxide.

The following are the chief reactions of glucose by which its presence may be detected, or its amount in any fluid determined—(1) *Moore's test.* Heat with an equal quantity of solution of caustic potash, and a yellow colour is produced, and, if a large amount of sugar is present, the colour is brown. In the latter case, there may be a molasses-like smell. (2) *Trommer's test.* Add to the solution a few drops of a weak solution of sulphate of copper, then a little of a solution of caustic potash; the latter precipitates hydrated cupric oxide, which is soluble in excess of caustic potash, if sugar be present. Then heat to near boiling, and a deposit appears of cuprous oxide, which may be yellow, orange, or red, according to the amount of cuprous oxide reduced. If a large amount of sugar be present along with only a small quantity of cupric oxide, on heating, the blue fluid may become yellow, without precipitation of cuprous oxide, the latter being held in solution by the excess of glucose. (Witthaus.) (3) *Fehling's test.* A solution known as "Fehling's solution" is thus prepared—I. Dissolve 51·98 grammes of pure

crystals of sulphate of copper in 500 c.c. of water; II. Dissolve 259·9 grammes of pure crystals of Rochelle salts (tartrate of soda and potash) in 1000 c.c. of a solution of caustic soda having a specific gravity of 1·12. When required for use, 1 volume of I. is mixed with 2 volumes of II. Keep in the dark in a stoppered bottle, having the stopper paraffined. To apply the test, boil 4 or 5 c.c. of Fehling's solution in a test tube; if it remain unaltered, add solution containing glucose drop by drop, and the fluid will become green, and a red or yellow precipitate of cuprous oxide is obtained. The reaction is precisely the same as in Trommer's test. In both we have an alkaline solution of cupric oxide along with glucic and melassic acids, formed by the action of the caustic alkali on the sugar, and, as these acids have a strong affinity for oxygen, they rob the cupric oxide of oxygen, reducing it to cuprous oxide, which, being insoluble in an alkaline fluid, is precipitated. (4) *Böttger's test.* A few cubic centimetres of the solution of glucose are mixed in a test tube with an equal volume of a solution of sodium carbonate (1 part of crystallized carbonate of soda and 3 parts of water), a few grains of subnitrate of bismuth are added, and the mixture boiled. In the presence of sugar, bismuth is thrown down by reduction as a brown or black powder. (5) *Picric acid.* To a solution of glucose add a few drops of a solution of picric acid, heat, then add a drop or two of caustic potash, and a brownish-red colour is obtained. Other substances give a similar reaction, such as creatinin. (6) *Fermentation.* Mix a solution of glucose with yeast in a large test tube, and invert the test tube so as to have the mouth immersed in a solution of glucose; keep at a temperature of 25° C. for ten or twelve hours, and a quantity of gas, carbonic acid, will collect in the test tube, and alcohol will be formed in the fluid. (7) *Mulder-Neubauer test.* A solution of glucose is rendered faintly blue with indigo solution, and "faintly alkaline with sodium carbonate, and heated to boiling without agitation." (Witthaus' *Chemistry*, p. 219.) It turns violet and then yellow without agitation, but if it is shaken the blue colour is restored. (8) *Saccharimeter.* A solution of glucose rotates the plane of polarization to the right.[1]

It is often important to determine quantitatively the amount of glucose or diabetic sugar present in such a fluid as the urine. The following are the methods to be relied on.

(1) *Saccharimeter* or *Polarimeter.*—Add to the fluid containing glucose a solution of acetate of lead, and filter. Place the filtered fluid in the

[1] The precautions to be observed in applying any of the above reactions for the detection of sugar in the urine are not here detailed, as these are the special applications of general methods which can be best understood and appreciated in the clinical work of the hospital.

tube of the saccharimeter, and take half a dozen readings of the angle of deviation. The mean will represent the angle of rotation, and the percentage of sugar is determined by $p = \frac{a}{52{\cdot}85} \times l$, in which p = the weight in grammes of glucose in 1 c.c. of fluid, a = the angle of deviation, and l = the length of the tube in decimetres. The numbers 52·85 indicate the specific rotatory power of glucose = $[a]_D$.

A very ingenious spectropolarimeter has been invented by E. v. Fleischl for the determination of the amount of glucose in a solution. It is specially adapted for the estimation of diabetic sugar in urine. One of its chief advantages is, that it avoids the difficulty of forming a judgment as to the identity of two coloured surfaces, as has to be done with one of the usual instruments. (See p. 69, Fig. 24.)

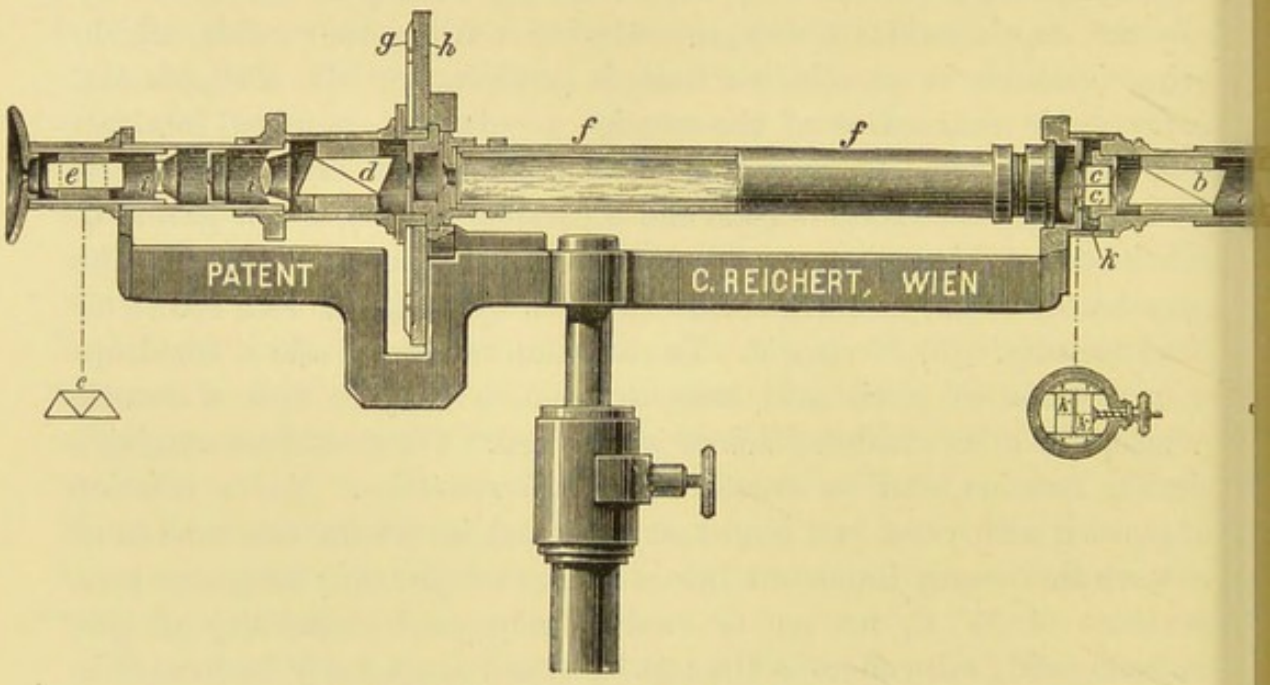


FIG. 63.—Spectropolarimeter of E. v. Fleischl.

A lamp or gas flame is placed opposite the end of the instrument shown to the right of the figure. The light passes first through a Nicol's prism *b*, the Polarizer, then through two small quartz plates *cc*, so placed that the bisecting plane in which they meet lies horizontally. The one quartz plate is dextro-, whilst the other is lævo-rotatory, and the thickness of each plate, 7·75 to 8 mm., is such that the green rays between the Fraunhofer lines *E* and *b* of the spectrum are circularly polarized through an angle of 90°, the one set passing through the upper quartz to the left, and the other through the lower quartz to the right. The light then continues onwards through a tube *ff*, 177·2 mm. in length, containing 15 c.c. of the solution, and having flat glass plates to close it at each end. It then passes through a second Nicol *d*, the Analyzer, with its principal axis horizontal, like that of the first Nicol *b*, and it will be evident that the light between *E* and *b*, extinguished by circular polarization through an angle of 90° by the quartz plates, will not pass through this second Nicol. Finally, the light passes through a direct vision spectroscope *e*. To give sharpness of definition to the spectrum thus obtained, there is a movable vertical slit at *k*, immediately in front of the quartz

plates *c* and *c*. Suppose then that the tube *ff* contained water or was empty, or suppose it not to be in its position, and a black cloth to be thrown over the part of the instrument at *ff* so as to exclude light, on looking through a small telescope carrying the prisms *e*, two beautifully distinct horizontal spectra are seen, one over the other, and separated by a very thin dark line. Each spectrum, however, shows a dark band in the green, owing to the extinction of that part of the spectrum by the right and left-handed rotation of the green rays by the quartz plates through 90°, and the two bands are exactly over each other, so that the lower edge of the one touches the upper edge of the other. The Analyzer is fixed in a tube carrying an arm bearing a vernier *g*, so that it can be rotated round the surface and near the border of a circular metal disc seen in section at *h*, and this disc is graduated into degrees. The two bands in the spectra exactly coincide when the zeros of the vernier and of the scale correspond. If now the tube *ff* containing the solution of sugar is interposed, the two bands are seen to be shifted—the one to the right or the other to the left, according to the direction of rotation by the substance under examination. If the substance be glucose, which is dextro-rotatory, then the band corresponding to the dextro-rotatory quartz plate will be pushed to the right, and the extent of the movement is measured by rotating the Analyzer through the number of degrees required to cause the zero points again to coincide, and the two bands are made also exactly to coincide as to vertical position. Suppose then, that to produce this effect, a rotation of 6° is necessary, then the fluid in the tube contains 6 per cent. of sugar, that is 6 grammes in 100 cubic centimetres. The scale of degrees is such that each degree corresponds to 1 per cent. of sugar in the given length of column of fluid in the tube *ff*. The green rays are chosen for exclusion, because they are the most suitable in the case of urine, which absorbs the violet rays (those having the greatest amount of rotatory dispersion), and, as already explained, the exclusion of these rays is effected by the determinate thickness of the quartz plates. It would have been equally easy, by altering the thickness of the quartz, to exclude the red rays ; but, as their rotatory dispersion is small, the instrument would not then have been so sensitive, or the blue rays, but in that case the bands would not have been so distinct. This instrument gives results to within $\frac{1}{10}$ per cent. of sugar, and as it can be quickly applied without the use of monochromatic light, and as it gives the percentage amount without any calculation, it is specially adapted for the wants of medical men.

(2) *Sir William Robert's method.*—Determine the specific gravity of the fluid at 25° C., add yeast, maintain at 25° C. till fermentation stops ; again take the specific gravity, and each degree of diminution of specific gravity represents ·2196 gramme of sugar in 100 c.c. (or 1 grain to the ounce). This method is well suited for the determination of sugar in urine.

(3) *Volumetric process by Fehling's solution.*—The strength of Fehling's solution is such that to reduce the cupric to cuprous oxide in 20 c.c. ·1 gramme of glucose is required. The solution of glucose is diluted with nine times its bulk of water and placed in a burette. In a porcelain dish 20 c.c. of Fehling's solution are mixed with 40 c.c. of water and 2 c.c. aq. ammonia and heated to boiling under the burette. The fluid

in the burette is allowed to mix with the blue fluid in the porcelain dish, and as the cupric oxide is reduced to cuprous oxide, the blue colour gradually disappears. When the blue colour has entirely gone, the number of c.c. of the fluid in the burette is read off. This represents the number of c.c. containing ·1 gramme of glucose, as ·1 gramme is required to precipitate all the cuprous oxide in 20 c.c. of Fehling's solution. To control the observation, filter the fluid in the porcelain dish and divide into three portions. To the first add a few drops of acetic acid and treat with a solution of ferrocyanide of potassium. If the reduction has not been complete, and any free cupric oxide still exists, a reddish-brown colour is obtained. In that case, enough of the solution in the burette has not been added. This may be corroborated by acidulating the second portion with a drop of hydrochloric acid and a few drops of ammonium sulphide, which will give a black colour with any free cupric oxide. To the third portion, in the event of the first two giving no reaction, add a few drops of Fehling's solution and boil; the absence of any yellow colour will show that there is no excess of glucose, that is, that we have not added too much of the fluid from the burette. Suppose 40 c.c. of fluid in burette has been used and that it was diluted with 9 times its bulk of water. This would represent 4 c.c. of fluid containing ·1 gramme of glucose, that is 2·5 per cent.

(4) *Pavy's Gravimetric method.*—In a gravimetric process the cuprous oxide is carefully collected, dried, and weighed, but this is a matter of great difficulty, and even in the hands of skilful manipulators is wanting in precision. It occurred to Dr. Pavy to dissolve the precipitated cuprous oxide and then to throw the copper down in the metallic form by galvanic action upon a platinum surface, and lastly to weigh the copper thus deposited. The process is fully described in Dr. Pavy's *Croonian Lectures on Points connected with Diabetes*, pp. 41-49 (1878), and he sums up its theory as follows—

"When sugar is boiled with the copper solution the change occurring stands in the relation of one atom of the former to five atoms of cupric oxide. One atom of sugar is oxidized by, or reduces, five atoms of cupric oxide. This is the foundation of the action involved in the operation of the test, and the calculation of the amount of sugar present is made accordingly. Taking 63·4 as the atomic weight of copper, and 180 as that of glucose ($C_6H_{12}O_6$), 317 parts of copper will stand equivalent to 180 parts of glucose. Thus one part of copper corresponds to ·5678 of glucose, and in calculating the amount of sugar in the blood analysis, the weight of copper deposited has only to be multiplied by ·5678 to give its equivalent in glucose. The quantity of sugar in the amount of blood taken for analysis being thus determined, the required information is supplied for expressing the proportion for 1000 parts."

The glucose found in the alimentary canal is derived from the

conversion of starch into glucose by the action of the salivary and pancreatic juices. This glucose is absorbed and carried to the liver, where it is changed into glycogen, the peculiar amylose found in that organ and in many of the tissues. Part of the glucose in the bowel may, by fermentations, be changed into lactic or butyric acids— $C_6H_{12}O_6 = 2(C_3H_6O_3)$, lactic acid, or $2(C_6H_{12}O_6) = 3(C_4H_8O_4)$, butyric acid.

The physiological importance of glucose will be shown in discussing the glycogenic function of the liver in relation to nutrition. By its oxidation into carbonic acid and water, heat will be produced. There is also evidence to show that the consumption of glucose is one of the chemical phenomena connected with muscular action, and with the activity of protoplasm generally.

2. *Chondro-glucose* is a substance formed by the action of nitric acid upon cartilage. It reduces cupric oxide to cuprous oxide and is said to be fermentable, producing melitose, a kind of sugar obtained from manna.

3. *Lævulose*, uncrystallizable sugar, is found in the intestine as the result of the inversion of cane sugar by the action of a ferment, when cane sugar is changed into equal parts of dextrose (glucose) and lævulose. It has been discovered in the blood, urine, and muscles. Lævulose is uncrystallizable, very soluble in water, insoluble in alcohol, and it reduces copper salts. With polarized light, it has a powerful lævo-rotatory action, $-[a]_D = -106°$.

4. *Mannitose*, or *Mannite*, is a yellow uncrystallizable sugar, like glucose in its general character, but having no action on polarized light.

5. *Galactose* is formed by the action of dilute acids on lactose, or milk sugar. With polarized light, $[a]_D = +83{\cdot}33$. Nitric acid converts it into mucic acid.

6. *Inosite*, *Muscle sugar*, is a sugar found in muscles (specially the muscle of the heart), in the kidneys, liver, lungs, pancreas, spleen, supra-renal bodies, brain, spinal cord, testes, blood, and in urine. (Beaunis.) Professor Külz asserts that it exists even in normal urine; it certainly is found in the urine of diabetes, and sometimes in that of uræmia. It forms long clear shining crystals (Fig. 64) with two molecules of water of crystallization, and "usually arranged in groups having a cauliflower-like appearance." (Witthaus.) Inosite is readily soluble in water and with difficulty in alcohol,

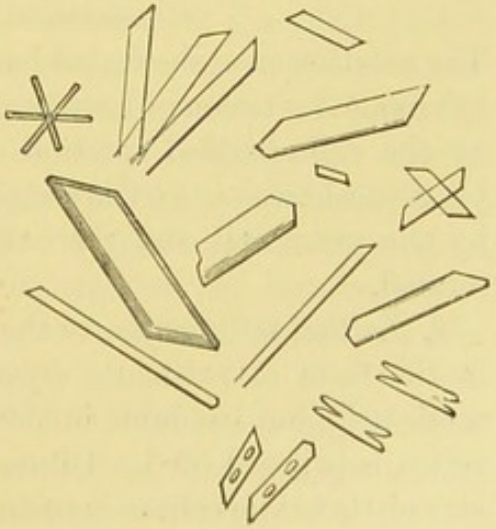


FIG. 64.—Crystals of inosite.

and is insoluble in absolute alcohol and ether. It has no action on polarized light. It is not fermentable. In the presence of inosite, hydrated cupric oxide is dissolved in excess of caustic potash, but on boiling the blue fluid no reduction occurs, as with glucose. The following reaction has been said to distinguish inosite, but it is difficult of application and does not give good results with inosite in a fluid containing other organic matters. *Scherer's test.* Evaporate the fluid with a drop or two of nitric acid on a platinum plate nearly to dryness; moisten the residue with ammonium hydrate; add a drop or two of a solution of chloride of calcium and again evaporate to dryness—a rose-pink colour may appear.

The origin of inosite, in addition to what may be introduced in the food, and its physiological uses are unknown.

(2) The Saccharoses.

These are regarded as condensed glucoses, inasmuch as they are formed by the combination of two molecules of a glucose with the loss of one molecule of water. Thus—

$$C_6H_{12}O_6 + C_6H_{12}O_6 - H_2O = C_{12}H_{22}O_{11}.$$

1. *Cane sugar*, beet sugar, does not occur in the body, but in relation to nutrition and to other carbo-hydrates it is important to notice some of its properties. Only those not familiar to everyone are here alluded to. It does not reduce cupri-potassic solutions in the cold, but it may do so with prolonged heating, with excess of alkali. Aqueous solutions with polarized light give $[a]_D = +73°{\cdot}8$. When boiled with water, it is converted into dextrose and lævulose. Thus—

$$\underset{\text{Saccharose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Lævulose.}}{C_6H_{12}O_6}.$$

The solution now shows left-handed rotation with polarized light as the left-handed rotation of lævulose, $[a]_D = 106°$, is only partially antagonized by the right-handed rotation of dextrose, $[a]_D = 52°{\cdot}85$. With active yeast saccharose is first inverted by a special soluble ferment produced by the yeast cell, and then there is fermentation of the glucose thus formed.

2. *Lactose*, milk sugar, as the name implies, occurs in milk. It exists in the form of prismatic crystals, soluble in hot or cold water, and in acetic acid, but insoluble in alcohol and ether. With polarized light the action is $[a]_D = +59{\cdot}3$. Dilute mineral acids change it into galactose, and nitric acid oxidizes it to mucic and oxalic acids. It reduces Fehling's solution. When a solution of lactose is mixed with yeast the alcoholic fermentation goes on slowly, and during putrefaction lactose produces lactic acid.

When introduced into the alimentary canal, lactose is changed into galactose (a glucose) which is then absorbed. With regard to the origin of lactose in the cells of the mammary gland, there are diverse opinions as to whether it may be formed from glucose taken in the food or produced from starch. The injection of glucose into animals during lactation has given unreliable results, and it cannot be said that the amount of lactose in the milk of such animals was sensibly increased. On the suppression of lactation, sugar may appear in the urine but it has not been satisfactorily determined whether it is glucose or lactose.

3. *Maltose* is a sugar formed during the glucose-forming action of the salivary and pancreatic ferments and of diastase on starch. It crystallizes like glucose, but differs from that sugar in being less soluble in alcohol and in exerting a dextrogyratory power three times as great.

(3) The Amyloses.

These may be regarded as the anhydrides of the glucoses.

$$\underset{\text{Glucose.}}{C_6H_{12}O_6} - H_2O = \underset{\text{Starch.}}{C_6H_{10}O_5}.$$

They are readily changed by the action of ferments and by weak sulphuric acid into glucose. Chemists hold that the formulæ of the amyloses should be expressed as a multiple of $C_6H_{10}O_5$, that is $n(C_6H_{10}O_5)$.

1. *Starch* does not exist in the human body, but forms an important element of food. A solution gives with polarized light $[a]_D = +216°$. Heat causes starch grains to swell and burst, and at 200° C. it is changed into dextrin. It is also changed into dextrin by heating with water at 160° C., and prolonged heating changes it into glucose. Starch is also changed into glucose by hydrochloric and oxalic acids. It is insoluble in alcohol, ether, and cold water. Cold water causes the starch grains to swell enormously and to become glutinous, forming starch paste or hydrated starch. If then boiled with water a solution of starch is obtained. A dilute solution of iodine gives a violet-blue with starch, and if to a solution of starch made blue with iodine, a solution of a neutral salt is added there separates a blue flocculent deposit of the so-called iodide of starch. As will be seen in the description of digestion, starch is converted into sugar by the action of the salivary and pancreatic juices.

2. *Glycogen* is a form of animal starch found in the liver, placenta, cartilage cells, pus cells, colourless blood corpuscles, muscular tissue, and in embryonic tissues generally. It may be prepared from the liver by either of the two following methods: (1) *Claude Bernard's method.* The liver is cut into small pieces and thrown into boiling water; the fragments are then bruised in a mortar for a quarter of an hour in a very

little water. This matter is then submitted to pressure and the fluid obtained is mixed with animal charcoal and filtered. The opalescent filtrate is mixed with five times its volume of alcohol of 38° to 40° strength, and the glycogen is precipitated. The precipitate is collected and washed with alcohol, and to obtain the glycogen pure it is boiled in a concentrated solution of caustic potash and is then reprecipitated with alcohol. The alcoholic precipitate is freed from the caustic potash by being washed with distilled water. (2) *Brücke's method.* The liver is thrown into boiling water. When hard it is bruised in a mortar with a little water, and the bruised matter is heated gently for half an hour in a little more water. The milky fluid is then poured off and is replaced by water. This is again boiled and the fluid again poured off repeatedly until the water has an opalescent tint. These washings are collected, cooled, and filtered, and acetic acid and potassio-iodide of mercury are added so as to form a precipitate. This is filtered, and the filtered liquid is heated with alcohol which precipitates the glycogen. This is then collected, washed with alcohol, and purified by the caustic potash method above described. The quantity may also be estimated by Brücke's method, by weighing the amount of the precipitate, or the glycogen may be converted into glucose by the action of weak acids, and the glucose thus formed may be fermented. Brücke's method gives the best results both qualitatively and quantitatively.

When so obtained, glycogen is a snow white, odourless, tasteless powder, soluble in water, and insoluble in alcohol and ether. Its solutions with polarized light are dextrogyrous to about three times the extent of those of glucose. In the presence of glycogen, hydrated cupric oxide is soluble in caustic potash, but no reduction occurs on boiling. Iodine gives with glycogen a wine-red colour. When boiled with nitric acid, oxalic acid is formed. The ferments of the salivary glands, and of the pancreas, glycerine-extracts of the liver, pancreas, muscle, lung, and brain, and dilute mineral acids change it into glucose.

The physiological importance of glycogen will be discussed in treating of the glycogenic function of the liver in its relation to nutrition.

3. *Dextrin* is a substance obtained by subjecting starch to a dry heat of 175° C., or by heating starch with dilute sulphuric acid to 90° C., or by the action of the diastase in infusion of malt upon hydrated starch. It is a slightly yellowish powder, soluble in water, forming mucilage, and its solutions with polarized light give $[a]_D = +138°{\cdot}88$. Dextrin is said to occur in the blood and in muscle, and when a solution is injected into the blood it is changed into glucose.

4. *Cellulose* is the basis of vegetable tissues. (See Vines' *Physiology of Plants*, Lect. i.) A variety of cellulose has been found by Schäfer in

the mantles of *Pyrosomidæ*, *Salpidæ*, and *Phallusia mamillaris*. The reactions of this substance leave no doubt as to the identity of animal and vegetable cellulose.[1] A similar substance is said to exist in the skin of the silkworm.[2]

4. The Non-Nitrogenous Organic Acids.

These are acids formed in the body or introduced in the food, and usually united with bases. Representatives of four groups of these organic substances occur in the body, belonging to the acetic acid series, the glycollic acid series, the oxalic acid series, and the oleic acid series.

(1) Acids related to the Monatomic Alcohols or Acetic Acid Series.

These are a series of acids, all monobasic, and related to a series of radicles, CH_3, C_2H_5, etc., each member of the series differing from the one before it by an additional increment of CH_2. They may be regarded as formed on the type of water in which the radicle of the acid is substituted for an atom of hydrogen, or there is a substitution of O for H_2 in the radicle, and this altered radicle is then united to hydroxyl—

$$\underset{\text{Ethyl.}}{C_2H_5} - H_2 + O = \underset{\text{Radicle of acetic acid.}}{C_2H_3O} \; : \text{ then } : \begin{matrix} H \\ H \end{matrix}\Big| O \qquad \begin{matrix} C_2H_3O \\ H \end{matrix}\Big| O$$

Acetic acid.

Another view is that they are derived from the primary monatomic alcohols by the substitution of O for H_2 in the group $CH_2 . OH$—

$CH_3 - CH_2 - CH_2 - CH_2 . OH$, Normal butyl alcohol.
$CH_3 - CH_2 - CH_2 - CO . OH$, Normal butyric acid.

The annexed series (see page 162) is sometimes termed the *fatty acid series*, because the higher substances, such as palmitic, margaric, and stearic acids, exist in fats. (See Fats, p. 150.) The following are the characters of the chief members of the group.[3]

Formic acid, CH_2O_2, is a colourless liquid of strong and piquant odour; volatile at 100° C. without residue; is not precipitated by the nitrate of mercury; heated with concentrated sulphuric acid, it decomposes into water and carbonic oxide, $CH_2O_2 = CO + H_2O$.

Acetic acid, $C_2H_4O_2$, transparent foliated crystals; changes at 17° C. to a colourless fluid, of a piquant characteristic odour, and of a very acid taste; volatile without residue. It is not precipitated by perchloride of iron, but if we saturate the acid with ammonia, the liquid becomes dark-

[1] Schäfer, *Annales Chem. Pharm.* clx. 312.
[2] De Lucca, *Comptes Rendus*, lii. 102, lvii. 43.
[3] From the Appendix to Beaunis' *Physiologie Humaine*, vol. ii. p. 1441.

The following are the chief members of the series having the general formula $C_nH_{2n}O_2$—[1]

RADICLE.		ALCOHOL.		ACID.		Boiling-point of Acid in deg. C.	WHERE FOUND.
Methyl,	CH_3,	Formyl,	$CH_3 . HO$,	Formic,	$CHO . HO$,	100	In blood, urine, bile, sweat, muscle juice; in red ants, and poisonous fluids of many insects.
Ethyl,	C_2H_5,	Ethyl,	$C_2H_5 . HO$,	Acetic,	$C_2H_3O . HO$,	119	Result of acetous fermentation.
Propyl,	C_3H_7,	Propyl,	$C_3H_7 . HO$,	Propionic,	$C_3H_5O . HO$,	140	In fermentation and in putrefactive products.
Butyl,	C_4H_9,	Butyl,	$C_4H_9 . HO$,	Butyric,	$C_4H_7O . HO$,	160	Milk, sweat, muscle juice, urine, contents of intestines, etc.
Amyl,	C_5H_{11},	Amyl,	$C_5H_{11} . HO$,	Valerianic,	$C_5H_9O . HO$,	175	In putrefactive products; occasionally, urine and fæces in cases of typhus, variola, etc.
	C_6H_{13},	Caproyl,	$C_6H_{13} . HO$,	Caproic,	$C_6H_{11}O . HO$,	198	Butter, cheese, etc.
	C_7H_{15},		$C_7H_{15} . HO$,	Œnanthylic,	$C_7H_{13}O . HO$,	212	Action of nitric acid on castor oil.
	C_8H_{17},		$C_8H_{17} . HO$,	Caprylic,	$C_8H_{15}O . HO$,	236	Butter, cocoa-nut oil.
	C_9H_{19},		$C_9H_{19} . HO$,	Pelargonic,	$C_9H_{17}O . HO$,	260	Oil of geranium.
	$C_{10}H_{21}$,		$C_{10}H_{21} . HO$,	Capric,	$C_{10}H_{19}O . HO$,	—	Butter, cocoa-nut oil.
	$C_{12}H_{25}$,		$C_{12}H_{25} . HO$,	Lauric,	$C_{12}H_{23}O . HO$,	—	Cocoa-butter, vegetable fats.
	$C_{14}H_{29}$,		$C_{14}H_{29} . HO$,	Myristic,	$C_{14}H_{27}O . HO$,	—	Butter, vegetable oils, spermaceti.
	$C_{16}H_{33}$,	Cetyl or Ethal,	$C_{16}H_{33} . HO$,	Palmitic,	$C_{16}H_{31}O . HO$,	—	Palm oil, animal and vegetable fats.
	$C_{17}H_{35}$,	Ceryl hydrate,	$C_{17}H_{35} . HO$,	Margaric,	$C_{17}H_{33}O . HO$,	—	Said to be a mixture of 90 per cent. of palmitic and 10 per cent. of stearic acid.
	$C_{18}H_{37}$,		$C_{18}H_{37} . HO$,	Stearic,	$C_{18}H_{35}O . HO$,	—	Animal fats especially; also in some vegetable oils.
	$C_{20}H_{41}$,		$C_{20}H_{41} . HO$,	Arachic,	$C_{20}H_{39}O . HO$,	—	Butter, pea-nut oil.
	$C_{22}H_{45}$,		$C_{22}H_{45} . HO$,	Benic,	$C_{22}H_{43}O . HO$,	—	
	$C_{25}H_{51}$,		$C_{25}H_{51} . HO$,	Hyænic,	$C_{25}H_{49}O . HO$,	—	
	$C_{27}H_{55}$,		$C_{27}H_{55} . HO$,	Cerotic,	$C_{27}H_{53}O . HO$,	—	
	$C_{30}H_{61}$,	Mericyl hydrate,	$C_{30}H_{61} . HO$,	Melissic,	$C_{30}H_{59}O . HO$,	—	

[1] In many cases no names have been given by chemists to certain hypothetical bodies in the series.

red (acetate of iron); white crystals are precipitated by the protonitrate of mercury.

Propionic acid, $C_3H_6O_2$, is a colourless liquid, of an odour like that of acetic acid; volatile at 142° C.; soluble in water; from an aqueous solution, chloride of calcium precipitates it in oily drops. Treated with alcohol and sulphuric acid it gives out an odour of fruit, due to the formation of propionate of ethyl. The propionate of sodium is much more soluble than the acetate.

Butyric acid, $C_4H_8O_2$, is a colourless liquid, of the odour of vinegar, or of rancid butter when it is impure, and soluble in water, alcohol, and ether; volatile at 160° C.; it is precipitated from concentrated solutions by chloride of calcium in oily drops. Heated with alcohol and sulphuric acid, it gives butyrate of ethyl, having an odour of strawberries.

Caproic acid, $C_6H_{12}O_2$, is a colourless liquid, oily, of the odour of perspiration; volatile at 202° C.; almost insoluble in water; miscible in alcohol and ether in all proportions; the caproate of baryta dissolves in 12 parts of cold water.

Caprylic acid, $C_8H_{16}O_2$, is an oily liquid of the odour of perspiration; crystallizes at 12° C.; insoluble in water; miscible in alcohol and ether in all proportions; the caprylate of baryta is soluble in 125 parts of cold water.

Capric acid, $C_{10}H_{20}O_2$, solid, of the odour of perspiration; fusible at 70° C.; slightly soluble in water; miscible in alcohol and ether in all proportions; the caprate of baryta is almost insoluble in cold water.

Palmitic acid, $C_{16}H_{32}O_2$, in crystalline masses, inodorous, insipid; fusible at 62° C.; insoluble in water, soluble in alcohol, very soluble in boiling alcohol, ether, and chloroform.

Stearic acid, $C_{18}H_{36}O_2$, a crystalline mass, white, inodorous, insipid; fusible at 69° C.; insoluble in water, less soluble in alcohol than palmitic acid, soluble in boiling alcohol, ether, and chloroform. Stearate of lead is insoluble in ether.

All of these acids may be changed by oxidation to a lower term or member of the series, and ultimately into carbonic acid and water. Thus—

$$\underset{\text{Propionic acid.}}{C_3H_6O_2} + O_3 = \underset{\text{Acetic acid.}}{C_2H_4O_2} + CO_2 + H_2O\,; \quad \underset{\text{Acetic.}}{C_2H_4O_2} + O_3 = \underset{\text{Formic.}}{CH_2O_2} + H_2O + CO_2\,;$$

$$\underset{\text{Formic.}}{CH_2O_2} + O = CO_2 + H_2O.$$

It would appear also that in fermentations the higher fatty acids may produce acids lower in the series simply by decomposition, without oxidation, and thus, in the decomposition of a fat (when it is rancid), we may find propionic, acetic, and formic acids. Probably such de-

compositions occur in the intestinal canal, and, the free acids uniting with bases, form salts which appear in the fæces.

(2) Acids related to the Diatomic Alcohols, or Glycols.

These are derived from diatomic alcohols, or glycols, by the substitution of 1 atom of oxygen for 2 of hydrogen, just as the acetic acid series is derived from monatomic alcohols. But the glycols contain two groups of $CH_2 . OH$, each of which may yield an acid. Thus—

$CH_2 . OH$	$CH_2 . OH$	$CO . OH$
$CH_2 . OH$	$CO . OH$	$CO . OH$
Ethene glycol.	Glycollic acid.	Oxalic acid.

Such acids are diatomic, and the hydrogen in each of the hydroxyls may be replaced by a radicle. There is thus a double series of these acids.

(a) *The Glycollic Acid Series*, $C_nH_{2n}O_3$.

These are formed by the partial oxidation of the corresponding glycol. Thus—

$CH_2 . OH$	$+ O_2$	$=$	$CH_2 . OH$	$+ H_2O$
$CH_2 . OH$			$CO . OH$	
Glycol.			Glycollic acid.	

From one point of view, *carbonic acid* belongs to this series, being the lower homologue of glycollic acid, from which, however, it differs in being dibasic. The physiological importance of this acid will be considered under Nutrition and Respiration. The remaining terms of the series are as follows—

Glycollic acid, - - -	$C_2H_4O_3$	
Lactic acid, - - -	$C_3H_6O_3$	Sour milk, contents of stomach, intestine, and in many organs.
Ethyleno-lactic acid, - -	$C_3H_6O_3$	Muscle juice.
Butylactic or oxybutyric acid,	$C_4H_8O_3$	
Oxyvaleric acid, - -	$C_5H_{10}O_3$	
Oxycaproic acid, - -	$C_6H_{12}O_3$	

1. *Glycollic acid*, $C_2H_4O_3$, forms deliquescent needle-shaped crystals, very soluble in water, in alcohol, and in ether. It does not occur in the body, but it is of physiological importance in its relation to glycolle or glycocin, one of the substances occurring in glycocholic acid. (See pp. 90 and 106.) Thus, glycocolle is derived from glycocollic acid by the substitution of the radicle NH_2 for one of the hydroxyls of the latter—

$CH_2 . OH$	$CH_2 . NH_2$
$CO . OH$	$CO . OH$
Glycollic acid.	Glycocolle.

2. *Lactic acid*, $C_3H_6O_3$, exists in two, if not three, isomeric conditions of physiological importance. First, there is the variety of lactic acid regarded chemically as *ethyleno-lactic acid*, as it contains the group CH_2, thus, $\begin{matrix} CH_2 \cdot OH \\ | \\ CO \cdot OH \end{matrix}$, to distinguish it from the other two varieties, which are called *ethylidene lactic acids*, as these contain the group CH_3, thus, $\begin{matrix} CH_3 \\ | \\ CH \cdot OH \\ | \\ CO \cdot OH \end{matrix}$. The ordinary lactic acid of fermentation (as in sour milk), and the variety of lactic acid found in muscle juice, to which the names of sarcolactic acid or paralactic acid have been given, belong to the ethylidene division, whilst muscle juice also yields the other kind, ethyleno-lactic acid.

a. Ordinary lactic acid (optically-inactive ethylidene lactic acid) is a syrupy liquid very soluble in water, alcohol, and ether. It is readily oxidized into formic and acetic acids. It may occur in gastric juice, in chyme, in sour milk, in the blood of leucocythæmia and in pus, and it is usually associated with alkaline bases, forming lactates. Ordinary lactate of zinc ($Zn(C_3H_5O_3)_2 + 3H_2O$) differs from sarcolactate of zinc in the amount of the water of crystallization.

b. Sarcolactic or *paralactic acid*, sometimes also called optically-active ethylidene lactic acid, occurs in muscle juice, and can be separated from it in the form of a salt of zinc ($Zn(C_3H_5O_3)_2 + 2H_2O$). It differs from the two isomers in having an influence on polarized light, its solutions being dextro-, whilst its salts are lævo-rotatory. Thus, with the solution $[a]_D = +3°{\cdot}5$; with the zinc-salt solution $= -7°{\cdot}6$; and with the lime salt ($2[Ca(C_3H_5O_3)_2] + 9H_2O$) solution $= -3°{\cdot}8$. Oxidation converts it into formic and acetic acids. Sarcolactic acid, as already mentioned, is found in muscles. Lactic acid also is met with in many organs, but whether it is of this variety or not has not been determined.[1]

c. Ethyleno-lactic acid is also found in muscle juice; like paralactic acid it forms a salt of zinc containing two molecules of water of crystallization, and when oxidized by chromic acid it yields malonic acid.

Lactic acid may be formed in the intestinal canal by fermentation, or it may be formed in the muscles. In the muscles, no doubt, it is derived from glycogen, glucose, or inosite. As it does not appear except in small quantities in the excretions, it is no doubt rapidly oxidized in the blood or tissues, being ultimately resolved into carbonic

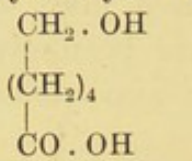
[1] As to the mode of preparing it, see Gamgee's *Physiological Chemistry*, vol. i. p. 361.

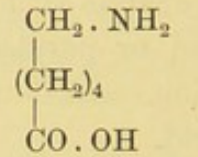
acid and water. The injection of solutions into the blood is followed by an increase of the carbonates in the urine, and one can follow the steps of the deoxidation process thus—

$$\underset{\text{Lactic acid.}}{2(C_3H_6O_3)} + O_2 = \underset{\text{Butyric acid.}}{C_4H_8O_2} + 2CO_2 + 2H_2O\ ;\quad \underset{\text{Butyric acid.}}{C_4H_8O_2} + O_3 = \underset{\text{Propionic acid.}}{C_3H_6O_2} + CO_2 + H_2O,$$

and then from propionic downwards, as given in the preceding account of the oxidation of the fatty acids.

3. *Oxycaproic acid* or *leucic acid*, $C_6H_{12}O_3$, although it has not been found in the body, merits attention on account of its relation to leucin. (See p. 91.) Leucin may be regarded as oxycaproic acid, in which NH_2 is substituted for hydroxyl. Thus—


Oxycaproic acid.


Leucin.

(β) *The Oxalic Acid Series*, $C_nH_{2n-2}O_4$.

These are obtained from the glycols by complete oxidation, and as they are dibasic they may form two series of salts. The following constitute the group—

Oxalic acid,	$C_2H_2O_4$	-	Urine, etc.
Malonic acid,	$C_3H_4O_4$	-	Identical with nicotic acid in tobacco.
Succinic acid,	$C_4H_6O_4$	-	Urine, spleen, thyroid, thymus.
Adipic acid,	$C_6H_{10}O_4$	-	Action of nitric acid on oleic acid, suet, etc.
Pimelic acid,	$C_7H_{12}O_4$	-	Action of nitric acid on oleic acid, wax, sper maceti, etc.
Suberic acid,	$C_8H_{14}O_4$	-	Action of nitric acid on cork, fats, etc.
Azalic acid,	$C_9H_{16}O_4$	-	Action of nitric acid on fats.
Sebacic acid,	$C_{10}H_{18}O_4$	-	Action of nitric acid on fats.
Roccellic acid,	$C_{17}H_{32}O_4$	-	From the lichen *Roccella tinctoria*.

The most important of these physiologically are oxalic and succinic acids.

(1) *Oxalic acid*, $C_2H_2O_4$, occurs in the urine in the form of oxalate of lime, more especially after ingestion of the leaves of the wood sorrel (*oxalis acetosella*), rhubarb, cinchona, or other herbs containing it. It abounds in the urine of herbivora. In the pure state it occurs in the form of white crystals, having an acid taste, soluble in water and alcohol, and decomposed into carbonic acid and carbonic oxide by the action of sulphuric acid. The most important salt is oxalate of lime, which appears in the urine in the form of beautiful octahedral crystals or bodies of a dumb-bell form. (See Fig. 65.)

FIG. 65. — Octahedral crystals of oxalate of lime.

Whilst oxalic acid may be introduced in food, there can be no doubt that it is also formed in the body itself, and in this connection it is important to observe that it may be formed by the decomposition both of non-nitrogenous and nitrogenous matter. Thus it may be produced by the oxidation of many organic substances, alcohol, glycol, sugar, glycerine, etc., and by the decomposition of albuminoids. Experiment indicates that it may arise from the incomplete oxidation of uric acid. Thus the injection of uric acid and of urates into the blood increased the amount of oxalate of lime in the urine. (Wöhler and Frerichs quoted by Beaunis.) The process by which this may take place has been already explained in treating of uric acid (see p. 99), and it is sufficient to say here that uric acid may be supposed to yield urea, allantoin, and oxalic acid, and that allantoin may be further resolved into oxalic acid. It is a well known clinical fact that in persons consuming a rich nitrogenous diet, and by insufficient exercise arresting or hindering efficient oxidation, the urine frequently contains deposits of uric acid and of oxalate of lime alternately.

2. *Succinic acid*, $C_4H_6O_4$, has been detected in urine after the use of asparagus and of various fruits containing malic acid and asparagin, and in many of the tissues and organs, such as the spleen, thymus, thyroid, and in the pathological fluids of hydrocele and ascites. It appears in large clear prisms or hexagonal plates, acid in taste, soluble in water, and slightly soluble in ether and cold alcohol. When oxidized, it yields butyric acid.

As to its origin, Meissner and Koch have shown that the artificial digestion by gastric juice of malate of lime and of asparagin yields succinic acid. As after a meal rich in asparagin and malates there is not much of the succinic acid eliminated by the bowels or kidneys, it is probably reabsorbed and oxidized in the blood, and resolved into carbonic acid and water, passing through the intervening stages of butyric, propionic acid, etc.

$$\underset{\text{Succinic acid.}}{C_4H_6O_4} = \underset{\text{Propionic acid.}}{C_3H_6O_2} + CO_2, \text{ etc., or}$$

$$\underset{\text{Succinic acid.}}{C_4H_6O_4} + H_2 = \underset{\text{Butyric acid.}}{C_4H_8O_2} + O_2.$$

(3) Acids related to the Allylic Alcohols.

A number of bodies belongs to this series in which the carbon of the hydrocarbon is non-saturated, thus differing from the hydrocarbons related to the monatomic and diatomic alcohols. Thus the formula of allylic alcohol is $\left.\begin{matrix}C_3H_5\\H\end{matrix}\right| O$, in which it is evident that two atomicities are

unsatisfied. Related to such alcohols are the acids of the acryllic series in the same manner as the fatty acids are related to the monatomic alcohols. Thus—

C_2H_5	$\left.\begin{matrix}C_2H_5\\H\end{matrix}\right\vert O$	$\left.\begin{matrix}C_2H_3O\\H\end{matrix}\right\vert O$	C_3H_5	$\left.\begin{matrix}C_3H_5\\H\end{matrix}\right\vert O$	$\left.\begin{matrix}C_3H_3O\\H\end{matrix}\right\vert O$
Ethyl.	Ethylic alcohol.	Acetic acid.	Allyl.	Allylic alcohol.	Acryllic acid.

The substances having the general formula $C_nH_{2n-2}O_2$ belonging to this group are—

Acryllic acid, -	$C_3H_4O_2$	- By oxidation of *acrolein*, an aldehyde produced by the action of sulphuric acid on glycerine.
Crotonic acid, -	$C_4H_6O_2$	- Obtained from croton oil.
Angelic acid, -	$C_5H_8O_2$	- In angelica root, chamomile flowers, croton oil.
Oleic acid, -	$C_{18}H_{34}O_2$	- Fats.

Oleic acid, $C_{18}H_{34}O_2$, as already shown, page 150, occurs in the glyceric ether olein, one of the common fats. It is an oily liquid, above 14° C.; yellow, inodorous, insipid; insoluble in water, soluble in alcohol, ether, and chloroform. At 4° C. it becomes a crystalline mass, having a beautiful pearly appearance. When heated in air it is decomposed with formation of hydrocarbons, volatile fatty acids, and sebacic acid. (Witthaus.) Nitric acid oxidizes it, forming volatile fatty acids and also acids belonging to the oxalic acid series, adipic, suberic, etc. It dissolves the fatty acids. Oleic acid has been found in small quantity in the blood and bile, and in considerable amount in the intestine.

Chap. XI.—THE GASES.

The gases of the body consist of oxygen, nitrogen, carbonic acid, hydrogen, carburetted hydrogen, and sulphuretted hydrogen. These gases exist either free in certain cavities of the body or are dissolved in certain fluids.

(1) Free Gases.

1. *Oxygen* is found in the pulmonary passages and in the intestinal tube. The oxygen of the lungs comes directly from the atmosphere of the air; while that of the intestinal tube, which is in small quantity, is no doubt introduced with food and drink. Oxygen may be simply dissolved in the fluid, or it may be in a state of combination with hæmoglobin. (See p. 119.)

2. *Nitrogen* is also found in the lungs and in the intestinal canal. According to Chevreul, the great intestine contains usually more than the small intestine, a fact which would indicate that a portion of the nitrogen may be derived from other sources than atmospheric air.

The following tables chiefly compiled by Beaunis[1] show the amount of the gases in various fluids, the gas being reduced to the volume they would occupy at 0°C and 760 mm. pressure—

TABLE I.—QUANTITY OF GAS, IN CUBIC CENTIMETRES, CONTAINED IN 100 CUBIC CENTIMETRES OF THE FLUID.

NAME OF GAS.	2 Arterial Blood.	2 Venous Blood.	5 Serum.	3 Lymph.	4 Fluid from Ascites.	2 Bile of Dog.*	2 Bile of Dog.†	2 Saliva of Dog.	2 Urine.	2 Serum of Muscle.	1 Albumin of Egg.	1 Pus.	2 Milk.
Carbonic acid, -	34·3	40·50	50·00	46·90	142·00	7·36	73·81	74·93	18·09	15·40	66·76	75·28	10·00
Nitrogen, - -	1·8	1·8	2·00	1·67	21·10	0·78	0·52	0·92	1·21	4·90	3·77	2·50	1·05
Oxygen, - -	22·2	8 to 12	1·47	0·13	0·14	0·00	0·26	0·65	0·10	0·09	2·31	...	0·11
Hydrogen, -	...	...	...	...	...	...	...	...	...	...	...	5·16	...
Total, - -	58·3	56·8 (average.)	53·47	48·70	163·24	8·14	74·59	76·50	19·40	20·39	72·84	82·94	11·16

TABLE II.—QUANTITY OF GAS, IN CUBIC CENTIMETRES, CONTAINED IN 100 CUBIC CENTIMETRES OF TOTAL GAS.

Carbonic acid, -	58·3	83·55	94·54	96·30	87·00	90·33	98·95	97·93	93·20	74·30	91·66	90·77	89·51
Nitrogen, - -	·3	2·77	3·15	3·43	12·92	9·67	0·70	1·20	6·23	25·20	5·17	3·01	9·42
Oxygen, - -	38·0	13·88	2·31	0·27	0·08	0·00	0·35	0·87	0·50	0·50	3·17	...	1·07
Hydrogen, -	...	...	...	...	...	...	...	...	...	...	...	6·22	...

* Dog fed with an animal diet. † Dog fed with vegetable diet.

1, 1, Matthieu and Urbain; 2, 2, 2, 2, Pflüger; 3, Hamersten; 4, Planer; 5, Probably old and decomposing.

3. *Hydrogen* has been found in small quantity in the air of expiration and also in the intestinal canal; it is said to increase in the great

[1] Beaunis, *Physiologie Humaine*, vol. i. p. 71.

intestine during a milk diet and to reach a minimum during a meat diet. Its origin in the intestine is probably due to fermentation.

4. *Carbonic acid* also exists in the lungs and intestinal canal. In the lungs it is derived from the blood, and in the intestinal canal it may come partly also from this source, but it is no doubt chiefly due to chemical decomposition. The gas may be simply dissolved or it may be loosely united to the carbonates and alkaline phosphates.

5. *Carburetted hydrogen* has been found in the great intestine, where it is said to exist to the amount of from 5 to 10 per cent. It is increased by a leguminous, and falls to a minimum during a milk, diet. It is the result of decomposition.

6. *Sulphuretted hydrogen* is occasionally found in the intestinal canal and results from the decomposition of the albuminous constituents of the food or from biliary matters, both of which contain sulphur.

Chap. XII.—THE CHEMICAL REACTIONS IN THE LIVING ORGANISM.

Hitherto we have been describing the substances obtained by chemical processes from dead animal matter, or from the secretions or excretions of the body. It is manifestly a much more difficult task to attempt to follow the chemical reactions which occur in the living body, and it may be at once admitted that much of our knowledge of such phenomena is based upon inferences collected from the general facts observed by the physiological chemist when he analyzes the substances obtained from the body, and studies how they are affected by oxidizing and deoxidizing agents, into what simpler substances they may be decomposed, or what more complex substances may be formed by their combination. We are still ignorant of the exact phenomena and conditions of those obscure chemical processes which occur in living tissue, and which appear to be absolutely necessary for the manifestation of vital action, inasmuch as it is impossible to submit living tissues to the ordinary processes of chemical analysis, even on a microscopic scale, without destroying their vitality.

The principal chemical reactions, however, may probably be classed into the great divisions of oxidation, decomposition, reduction, synthesis, and fermentation.

A. Oxidation.

Oxidation is the most common chemical reaction in the living organism. The albuminates, fats, and carbohydrates, by union with oxygen, form a series of compounds, somewhat simpler in chemical composition, and lower in molecular weight than themselves. From

these, by a continued process of oxidation, bodies still less complex in chemical composition are formed, and so on in successive stages until the elements which at first existed in albuminates now appear under the form of urea, carbonic acid, and water, and those of the carbohydrates and fats as carbonic acid and water. At present it is impossible to demonstrate the successive phases through which albumin may have to pass, but there is little doubt that many of these have been observed by chemists in the laboratory. Thus, by oxidation, albumin has been resolved into leucin, tyrosin, glycocin, and fatty acids; uric acid into urea, allantoin, oxalic acid, and carbonic acid; guanin into xanthin, oxaluric acid, and urea; creatin into sarcosin and urea, and fats into the whole series of fatty acids. It has also been found that the introduction into the living body of one or other of these substances has been followed by the appearance in the secretions of an increase of the materials into which this body may be resolved. Thus the introduction of uric acid increases the amount of urea and of oxalate of lime excreted.

The oxygen of the air introduced by respiration is the agent which effects the oxidations; but it is remarkable that, while in the laboratory these oxidations only take place at a high temperature and with very powerful oxidizing agents, such as permanganate of potash or nitric acid, in the living condition they occur at the temperature of the body and with much greater rapidity. To explain this, it has been assumed that the oxygen of the tissues is in the condition of ozone, which is known to have strong oxidizing powers even at comparatively low temperatures. Thus, at the temperature of the blood, and more especially in the presence of alkalies, or of alkaline carbonates, fats, glucose, and organic acids may be oxidized by ozone, but not by ordinary oxygen. (Gorup-Besanez.) No proof, however, has been offered that ozone, O_3, exists in the blood; indeed, its presence has been expressly denied by Schönbein. But oxygen in the nascent state, O, that is at the instant it has been set free from ozone, O_3, or from ordinary oxygen, O_2, or from any chemical substance containing it, is well known to have powerful oxidizing properties, and it has been suggested that it is thus liberated in some of the chemical processes occurring in the body. It has also been pointed out by Hoppe-Seyler, Pflüger, and others that the heat formed by molecular oxidation is quickly carried away, and that the mean temperature of the tissues is no direct measure of the heat produced in them. Thus, a high temperature will be associated with the oxidation of an atom of carbon or an atom of hydrogen, in the body as out of it, but in the body the heat so produced is quickly diffused.

Much discussion has also taken place as to whether oxidations occur

in the tissues or in the blood. There can be no doubt that oxidations occur chiefly, if not wholly, in the tissues. As first shown by Spallanzani, and corroborated by many modern physiologists (Valentin, Liebig, Claude Bernard, Hermann, and Paul Bert), living tissues consume oxygen and produce carbonic acid. On the other hand, blood left to itself in a vessel produces carbonic acid and consumes oxygen, but this is the beginning of putrefactive changes. A stream of oxygen passed through fresh blood does not increase the amount of carbonic acid given off which would be the case if oxidation occurred in that fluid. Pyrogallic acid, which is readily oxidized, if injected in considerable quantity into the blood, quickly appears in the urine, showing that it has passed through unchanged. But living tissues immersed in blood use up its oxygen and produce carbonic acid. The fact that nitrites are converted into nitrates, sulphites and hyposulphites into sulphates, and that organic acids, such as malates and tartrates, are changed into carbonates when passed through the body, would at first sight indicate that oxidations may occur in the blood; but even these changes would appear to depend on the living tissues, as lactate of soda in contact with blood remains unaltered, but if injected into a vein in a living animal, it is quickly decomposed, with the formation of carbonates, which then appear in the urine.

One must avoid entertaining too mechanical a view of the so-called oxidations in living matter. There is no such phenomenon as the direct union of oxygen with carbon or with hydrogen, as in burning these in the laboratory. If this were the case, there would be oxidations strictly so called, and we would find a constant relation between the amount of oxygen used and the amount of the combustion products. No such parallelism can be traced, nor, in many cases, can even the products of combustion be estimated quantitatively. Thus, one is led to infer that oxidations are one stage of complicated processes, possibly analogous to those of fermentation or putrefaction. On the oxidation processes occurring in the body, animal heat, mechanical work, and innervation largely depend.

B. Reduction.

The phenomena of reduction, which are so important in the life of a plant, do not occur so frequently in the animal body. The formation of fat from carbohydrates is an example: the carbohydrates lose oxygen, and become transformed into fats. The formation of indol, of trimethylamine, and the conversion of benzoic acid into quinic acid, of iodates and bromates into iodides and bromides, and of indigo-blue into indigo-white, during the passage of these substances through the body,

are no doubt examples of reduction. It is likely also that reductions and oxidations may form part of the same process, or the one process may be carried through before the other. Thus, malic acid by reduction becomes succinic acid, which is then oxidized into carbonic acid and water.

C. Decomposition.

By decomposition we mean the splitting up of an organic substance into two or more chemical compounds, the combined molecular weight of which is exactly equal to the molecular weight of the first substance. Thus, taurocholic acid may divide into choloidic acid and taurin—

$$\underset{\text{Taurocholic acid.}}{C_{26}H_{45}NSO_7} = \underset{\text{Taurin.}}{C_2H_7NSO_3} + \underset{\text{Choloidic acid.}}{C_{24}H_{38}O_4}$$

Occasionally, by dehydration or the removal of water, a simpler body may be formed. Thus, with the aid of heat, cholalic acid may be converted into dyslysin and water, and creatin into creatinin and water—

$$\underset{\text{Cholalic acid.}}{C_{24}H_{40}O_5} = \underset{\text{Dyslysin.}}{C_{24}H_{36}O_3} + 2H_2O$$

$$\underset{\text{Creatin.}}{C_4H_9N_3O_2} = \underset{\text{Creatinin.}}{C_4H_7H_3O} + H_2O$$

Sometimes a molecule of water may be removed, and the residue may then split up into simpler compounds. Thus, oxalic acid may be divided into carbonic acid, carbonic oxide, and water—

$$\underset{\text{Oxalic acid.}}{C_2H_2O_4} - H_2O = CO_2 + CO$$

It would appear in some instances that a compound must combine with water before splitting up. This occurs in the saponification of fats, and in the decomposition of glycocholic acid into cholalic acid and glycocin—

$$\underset{\text{Glycocholic acid.}}{C_{26}H_{43}NO_6} + H_2O = \underset{\text{Cholalic acid.}}{C_{24}H_{40}O_5} + \underset{\text{Glycocin.}}{C_2H_5NO_2}$$

In like manner, creatin may be resolved into urea and sarcosin, and urea into carbonic acid and ammonia—

$$\underset{\text{Urea.}}{CH_4N_2O} + H_2O = \underset{\text{Ammon. Carbonate.}}{CO_2 + 2NH_3}$$

A special form of decomposition is *dissociation* where a compound resolves itself into two or more simpler bodies under altered physical conditions. Thus, oxy-hæmoglobin in the diminished pressure of a partial vacuum, and with the aid of heat, gives up its oxygen, and resumes it when the conditions return to those of ordinary atmospheric pressure and a temperature of 15° C. Donders has suggested that a dissociation process may occur in respiratory exchanges. (See Respiration.)

D. Synthesis.

The formation of organic substances by synthesis in the living animal body is still very imperfectly understood, but it is interesting to observe that many nitrogenous organic compounds have been formed synthetically by the chemist in the laboratory. Thus urea, hippuric acid, glycocin, taurin, sarcosin, creatin, glucose, and oxalic, lactic, succinic, benzoic, propionic, acetic, and formic acids have been formed artificially; but as yet it has been impossible to prepare the higher members of the series. It is probable that in the living body more of the nitrogenous compounds are formed by analytical than by synthetical processes. One well-known example of a synthetical process is the formation of hippuric acid after the introduction of benzoic acid with food or medicine. In these circumstances, benzoic acid unites with glycocin to form hippuric acid, which makes its appearance in the urine—

$$\underset{\text{Benzoic acid.}}{C_7H_6O_2} + \underset{\text{Glycocin.}}{C_2H_5NO_2} = \underset{\text{Hippuric acid.}}{C_9H_9NO_3} + H_2O$$

In some cases there may be a simple union of this kind, but in most the process is probably much more complicated. Thus, a complex substance may be decomposed or oxidized, and the simpler products are then used to build up another complex body. For example, the ingestion of toluol is followed by the appearance of hippuric acid in the urine. Here toluol by oxidation becomes benzoic acid, and this combining with glycocin produces hippuric acid. Many organic acids must thus be built up, by the union of various substances with glycocin. Again, when taurin is given to a living animal, tauro-carbamic acid appears in the urine, by the union of the taurin with cyanic acid. Again, aromatic bodies unite with sulphuric acid to produce conjugated sulpho-acids. Thus, phenol is followed by the appearance of phenol-sulphate of potash or soda, cresol by cresolsulphate, etc. There may also be in the first instance oxidations, such as benzol into phenol, anilin into amidophenol, and then, as explained, phenol gives rise to phenolsulphate.

Syntheses play an important part in building up the complex bodies existing in living matter, and we may consider that substances so all-important as fats, lecithin, and other compounds existing in nervous matter, hæmoglobin itself, and albuminous bodies, are thus formed. How such processes are accomplished is not known, nor must we suppose that there is only one way by which a complex chemical substance may be formed. It has been conjectured that the elimination of water plays an important part in synthetic operations, and that the bodies

thus formed may be regarded as anhydrides of substances produced by the combination of the simpler bodies. Much of our knowledge on these points is still obscure, but it is remarkable that the triumphs of chemical science are in the synthetic production of complex organic bodies, and it is not unlikely that each successive step in this direction will lead to a better understanding of the similar processes occurring in the body in the upbuilding of its tissues.

E. Fermentation.

Certain substances have been long known to possess the property of exciting chemical changes in matters with which they come into contact. Such substances have been named by chemists *ferments*, and the process is known as *fermentation*. As fermentation plays a very important part in nature, and as in recent times it has been supposed to be the explanation of many processes occurring in the body, and to account for the origin of not a few forms of disease, namely, those included under the generic name of zymotic, it is necessary to refer to it here somewhat in detail.

Chap. XIII.—FERMENTATION.

The processes included under the name fermentation are so important as to demand careful study, more especially in these days when they are known to be of wide application to many phenomena of the living body, and also to some of the phenomena of disease. Not a few transformations occurring in the body, more especially during the process of digestion, are of the nature of fermentations; and it is highly probable that changes of a similar kind are involved in nutrition. It is now also generally acknowledged that fevers and epidemic diseases owe their origin to the action of fermentive agents, and that as we gather knowledge of the natural history of these agents, their conditions of vitality, their mode of growth, their reproductive processes, and the substances they produce, we may hope either to suppress them or to confine their action within narrow limits. Further, the study of fermentation throws a flood of light on many of the general phenomena of biology in the lower plants and animals, and lays a sound foundation or the appreciation of physiological processes, as these occur in the higher animals and in man.

When a fluid ferments, it becomes clearer or more muddy, as the case may be, and it usually froths and gives off a gas. This is well seen if the sweet juice of a plant, such as grape juice, is exposed to the fair. The clear fluid becomes turbid, a froth gathers on the surface

owing to the evolution of gas, the temperature of the fluid rises, and a slimy deposit gathers at the bottom of the vessel. These physical changes are also known to be associated with chemical changes. Alcohol and various other substances are now found in the fluid, and the gas given off is carbonic acid, whilst the sugar has been used up, so that at the end of the process it has entirely disappeared. Further, there is a change of a biological character, for if the slimy deposit at the bottom of the vessel be examined with the microscope, it will be found to contain numerous minute unicellular organisms, various species probably of *Torulæ* or *Saccharomycetes*, which were very few in number at the beginning of the process. Some of these phenomena must have been known from the most distant times, indeed since men began to drink the fermenting juice of the grape, or wine, but the one that specially caught their attention was the effervescence or frothing, and as it resembled the bubbling of the surface of a boiling fluid, such a process was called a fermentation (from *fervere*, to boil). The practice of distillation, by which the alcohol was separated from the rest of the fluid, was known as early as the 8th century, and in the 13th century the alchemists succeeded in removing the water from the "spirits of wine," thus obtaining absolute alcohol. It was not, however, until about 1600 that Van Helmont showed that the gas given off in fermentation was the same gas as was obtained by the combustion of charcoal, or what we now term carbonic acid. Then, it was ascertained that the production of the alcohol and carbonic acid was at the expense of the sugar in the fluid; and Lavoisier attempted to strike a balance between the amount of sugar at the beginning of the process and the amount of alcohol and carbonic acid at its close. As was to be expected, Lavoisier always found that he could not account for the whole of the sugar, because he did not know all the substances formed in the alcoholic fermentation, although he erroneously supposed acetic acid to be one of them. Gay-Lussac repeated these quantitative experiments and came nearer to the truth, but still there was a discrepancy. In 1847, Schmidt, of Dorpat, showed that succinic acid and glycerine were formed; and, at last, Pasteur established that, from 100 parts of cane sugar, equal to 105·4 of grape sugar, there were formed 51·1 of alcohol, 49·4 of carbonic acid, ·7 of succinic acid, 3·2 of glycerine—in all, 104·4—and he supposed that about 1 per cent. was taken up by the yeast itself. Even in this case the balance is not correct; but, making allowance for errors of experiment, it is sufficiently near to show that in the process of fermentation the sugar is resolved into a number of chemical substances. Such, then, is what we may term the chemical side of the problem.

Even the early observers could not fail in observing that, if pure

grape juice is boiled and kept in a well-corked bottle to exclude the air, fermentation does not take place. Pure solutions of cane sugar or of grape sugar do not ferment under these circumstances, whilst impure, unfiltered, unboiled solutions readily undergo fermentation. This disposes of the conjecture one might make, that the atoms forming the sugar are simply rearranged so as to produce alcohol, carbonic acid, glycerine, succinic acid, and other bodies, just as the atoms of cyanate of ammonium, NH_4CNO, may be rearranged so as to form urea, CH_4N_2O. Something evidently must be added to the grape juice to cause fermentation. As well put by Professor W. Dittmar,[1] an analysis of yeast does not reveal the existence of any substance capable of effecting this remarkable transformation. Take the most recent analysis of yeast by Schützenberger. An extract of yeast in boiling water showed —(1) a considerable quantity of phosphates ; (2) a large quantity of gum (arabin) convertible by nitric acid into mucic acid ; (3) leucin and tyrosin, to the former of which a sulphuretted compound obstinately adhered ; (4) carnin, xanthin, guanin, hypoxanthin, and sarcin. It is remarkable how similar this analysis is to that of an animal substance, but none of these chemical compounds can be supposed to effect the transformation of sugar into those formed in the alcoholic fermentation. By slow stages, however, the part played by the yeast came to be recognized. So long ago as 1680, Leeuwenhoek, with his simple microscope, saw that in fermenting fluids there were minute globular or ovoid particles. This was the first observation of the yeast-cells, and for many years they received little or no attention. In 1838, however, Schwann and Cagniard de la Tour demonstrated the vegetable nature of these yeast cells, and showed that they grew and multiplied in saccharine solutions, and for the first time it was asserted that fermentation in some way depended on the action of living things.

Previous to 1838, Berzelius put forward the theory that the action of the yeast is what is called catalytic (*κατα*, *λυσις*, separation), or a mere action of presence, similar to that of platinum-black on peroxide of hydrogen, causing the latter to give up an atom of oxygen. This theory was no explanation. Another view, first proposed by Liebig in 1848, is that there is no necessary connection between the fermentive process and the development of living organisms, and that the organisms may simply produce a substance, the molecular vibration of which may cause a rearrangement of the atoms of the substance undergoing fermentation. This is a modification of the mechanical theory of Berzelius above described. The splitting up of sugar into carbonic acid and alcohol, by the action of the yeast plant, he places side by side with the decompos-

[1] W. Dittmar, article "Fermentation" in the *Encyclop. Britann.* 9th edit.

ition of anhydrous acetic acid into acetone and carbon dioxide (a change brought about by heat), and with the change of an aqueous solution of cyanogen gas into oxamide, which is brought about by the action of the merest trace of aldehyde. Just as the vibration produced by the aldehyde determines the rearrangement of the atoms of cyanogen and water so as to constitute oxamide, so Liebig regards the rearrangement of the atoms of sugar as the result of a vibration produced by the chemical changes which take place in some unstable substance produced by the yeast-plant. The growth of the yeast-plant is, according to this idea, indirectly connected with the process of fermentation. "It is possible," he says, "that the physiological process stands in no other relation to the process of fermentation than that, by means of it, a substance is formed in the living cell, which, by an action peculiar to it—resembling that of emulsin on salicin or amygdalin—determines the decomposition of sugar and other organic molecules. In such a case, the physiological action would be necessary for the production of this substance, but would be otherwise unconnected with the fermentation properly so called."[1]

For many years this theory held its ground owing to the prestige of its illustrious author, and fermentation was held to be largely a chemical process. But facts came to light that again concentrated the attention of men of science on its biological aspect.

It had been shown by Gay-Lussac that clean grapes or boiled grape juice introduced into the Torricellian vacuum of a barometer tube kept free from fermentation for any length of time, but that if a single bubble of air were admitted fermentation soon appeared. Years afterwards, in 1838, in his celebrated experimental inquiry, Schwann repeated Gay-Lussac's experiment, and showed that if the air bubble were admitted to the vacuum through a red-hot tube then fermentation did not occur. Clearly then it was something in the air that caused fermentation, and this something was destroyed by heat. Further, it was noticed from time to time by many observers that active fermentation was always accompanied by an apparent growth of the yeast, and also that the process could be influenced by various physical and chemical conditions. Thus a temperature of from 20° to 24° C. was most favourable to it; it was arrested at 60° C., and boiling destroyed the power of fermentation. On the other hand, it was arrested by freezing, but on careful thawing, the process was resumed. Chemical substances, also, such as alcohol, bichloride of mercury, sulphuric acid, and sulphurous acid, impeded or arrested it.

The next great step was made when Schwann endeavoured to identify

[1] Article "Fermentation," in Supplement to Watt's *Dictionary of Chemistry*, vol. vi. p. 612.

the processes of fermentation and putrefaction. In the 17th century, Stahl, with that curious insight that gives his writings almost a prophetic character, said that fermentation and putrefaction were essentially the same, and he explained both by disturbances in the molecules of the fermentive or putrefactive body. Schwann, however, full of views about the origin of the tissues from cells, and of speculations as to spontaneous generation, investigated putrefactive fluids with a knowledge of the existence in these fluids of vibrios and other living organisms. He found that if a putrescible fluid is boiled and excluded from the air no putrefaction occurs, but that if air is subsequently admitted, putrefactive changes soon ensue. Obviously then the air has to do with putrefaction. But if the air were previously passed through red-hot tubes there was then no putrefaction, and this warranted the conclusion that it was not the air itself but something in the air that caused putrefaction. He also made the important observation that the chemical substances affecting fermentation and putrefaction varied in their mode or degree of action. Thus white arsenic and corrosive sublimate, which poison both plants and animals, stop both putrefaction and fermentation, whilst nux vomica, which poisons animals but not plants, prevents putrefaction, whilst it does not arrest the vinous fermentation. These statements require modification in the light of more modern inquiries, but they merit attention here as being an important contribution to knowledge.

Schwann's conclusions were supported by many important experimental inquiries. Von Helmholtz showed that the oxygen produced by electrolysis in a sealed up fermentable fluid did not cause fermentation. He also performed a remarkable experiment in which he placed a bladder full of boiled grape juice in a vat of fermenting juice and found that the fluid in the bladder did not ferment. Thus the cause of fermentation could not pass through the wall of the bladder. Mitscherlich showed that when in a tube a layer of fermentable fluid was separated from a layer of yeast by a septum of filter paper no fermentation occurred. Hoffmann proved that a layer of cotton wool had the same effect, and Schrœder and Dusch made the important demonstration that, if a putrescible fluid be boiled in a flask and the neck of the flask be plugged with cotton wool at the moment the flame is removed from underneath the flask, putrefaction will not occur in the fluid. It might be objected that putrescible fluid will keep fresh in a hermetically sealed flask because no air has been admitted, but this objection will not hold good when applied to the experiment with the plug of cotton wool. Here evidently air, but filtered air, has been admitted, and still putrefaction does not occur. The cotton wool has sifted from the air the bodies that cause putrefaction, just as the wall of the bladder, the septum of filter paper,

or the layer of cotton wool, prevents the passage of the yeast cells from a fermenting to a fermentable fluid. Even if the neck of the flask be long drawn out and bent here and there in a zig-zag fashion without sealing up the tube, a putrescible fluid will remain free from putrefaction in the flask. In this case the organisms that cause putrefaction are caught at the bends of the neck of the flask. The conclusion then is irresistible that the living organisms in the air and in the yeast are the cause of putrefaction and of fermentation.

Such was the state of the question when, in 1857, Pasteur began those important researches which, continued in various forms up to the present time (1888), have revolutionized this department of science. Pasteur, in particular, showed that each kind of fermentation was connected with the growth and development of a special organism, and that as the yeast cell was specially the cause of the alcoholic fermentation, so there was another organism for the lactic fermentation by which the sugar of milk was changed into lactic acid, and a third which effected the transformation of lactic into butyric acid. Further, he introduced the method of cultivating certain organisms in fluids specially adapted to their wants and inimical or unsuitable to other organisms, and thus by repeated cultivations he demonstrated that it was possible to obtain a fluid containing one organism, the physiological properties of which might then be examined. Thus by sowing a mixture of organisms, *a*, *b*, *c*, and *d*, in a sterilized fluid suitable specially for the growth of *a*, and by repeating this process many times with the fluid of successive cultivations, a fluid is obtained in which only the organism *a* is found, the others having perished in the struggle for existence in exceptional conditions. This method has been of the greatest importance to science, and like many new methods has been the key to many problems.

It was in 1864 that Pasteur made the important observation that the ferment causing the butyric fermentation is capable of living and multiplying in a fluid containing certain mineral matters, along with sugar or lactate of lime, and that it does this in the absence of free oxygen or of free air. In this case the presence of oxygen or of air arrests the fermentation, for if the air be again excluded, the fermentation will be resumed. This led to the investigation of the behaviour of various fermentive organisms, with the result of showing that the same rule prevailed with many (as in the butyric fermentation), namely, that the fermentation is carried on most efficiently in the absence of free air or free oxygen. Further, it was ascertained that most fermentive organisms may assume two conditions, one aerobic, that is, living in the presence of oxygen or of air, and the other anaerobic, that is, living in the absence of the oxygen; and it is when it is in the latter condition, the anaerobic,

that an organism carries on the work of fermentation. If the organism receives a free supply of oxygen from the air, it consumes it, assimilates nutritious matters, and takes on a certain habit of growth, like the coloured moulds found on the surface of preserved fruits or on milk exposed to the air. On the other hand, if there is a limited supply of oxygen, or none at all, the organism removes oxygen from the fermentable matter and thus causes fermentation. The curious fact that these lowly organisms apparently may live without free oxygen is no exception to the general law that all living things require this gas, but they have a special method under certain circumstances of obtaining it, namely, by the decomposition of the fermentable matter. Thus Pasteur is justified in the aphorism, "*La fermentation est la vie sans air*," and this remarkable property points to the important rôle of fermentations in many of the phenomena of the living body. It is quite true that the actual removal of oxygen from sugar by the action of the yeast has not been demonstrated, and that the explanation is thus far theoretical; but without this theory, it is difficult to account for the fact that some organisms do live and multiply without the access of either oxygen or air. A few flourish best with less oxygen than in ordinary atmospheric air. Thus Engelmann showed that some species gather close to a bubble of air, whilst others keep away from it and only come near when the bubble has lost a part of its oxygen. On the other hand, some, such as *Bacterium aceti*, require oxygen, and are truly aerobiotic. It would appear that at the beginning of fermentation oxygen is required, but that after it has begun, free oxygen may or may not be requisite according to the nature of the organism carrying it on.

Thus, after many vicissitudes, the vitalistic theory of fermentation has been put on a secure footing. True ferments in the shape of living organisms have been found for the lactic fermentation, resulting in the production of butyric acid; for the viscous, by which sugar may be changed into mannite; for the acetous, by which sugar may pass through alcohol into acetic acid; for the conversion of urea into carbonate of ammonia in decomposing urine; and for the phenomena of putrefaction, in which, by the action of *bacterium*-forms, albuminous and other matters are changed into simpler bodies. It is to be noted, however, that while each fermentation is accompanied by the development of a special organism, the same substance, such as alcohol, may be one of the products of different fermentations.

It has been urged by some that the multiplication of organisms in a fermenting fluid or in a putrefactive fluid is the consequence and not the cause of the fermentation or putrefaction. In the case of the alcoholic fermentation, this is disproved by the experiments of Von

Helmholtz, Mitscherlich, and others, which showed that if the yeast cells were prevented from passing into a fermentable fluid by the interposition of an organic membrane, fermentation did not ensue. Further, as shown by Chauveau, the properties of vaccine lymph are due to the small organic particles in the fluid, and not to anything in the fluid itself; and it has been found possible to sterilize various fluids, that is, remove from them organisms capable of causing fermentation, by passing the fluids through thick septa of finely porous substances. A striking corroboration of the view that the organisms themselves are absolutely necessary we have in the fact that if the fluid containing a cultivation of a bacillus believed to be the cause of splenic fever be inoculated into an animal, it will die of the disease, but this result does not follow if the inoculated fluid has been previously elaborately filtered so as to remove the bacillus.

If then the organisms are the cause of the fermentations, how do they act? Do they live on the fermentable matter and oxygen, and are the substances we call the products of the fermentation to be regarded in the light of excreted or waste matters? Or, do the organisms, as held by Berthelot, Frèmy, Hoppe-Seyler, and others, produce a kind of ferment which in turn acts on the fermentable matter? There are reasons for holding that the latter view is not far from the truth. It is quite true that no substance has yet been separated from yeast capable of causing the alcoholic fermentation other than the yeast cell; but Berthelot has obtained from yeast a soluble ferment capable of changing starch and cane sugar into glucose. Further, as will presently be seen, there are many soluble ferments known to chemists, but these are all formed in the first instance in animal or vegetable cells that may be considered to be the analogues of the organized cells capable of causing fermentation.

Hoppe-Seyler has with great force pointed out that the action of the fermenting matter may not be a direct oxidation, but rather a reduction. Thus, in the lactic and alcoholic fermentations and in putrefaction there is the liberation of hydrogen, and this nascent hydrogen may seize hold of an atom of oxygen from ordinary oxygen, O_2, to form water, $H_2 + O_2 = H_2O + O$. The nascent oxygen thus liberated at once attacks any oxidizable matters present, or it may unite with more free hydrogen to form water, or it may unite with a molecule of ordinary oxygen, O_2, to form a molecule of ozone, $\frac{O_3}{2 \text{ vols.}}$. Thus the liberation of hydrogen may be the cause of active oxidations. On the other hand, suppose no free oxygen is present, then the hydrogen liberated attacks organic substances, reducing them. In this case the phenomena are not

those of oxidation, but of reduction, and we see how in fermentations and putrefactions there may be either oxidations or reductions according to the presence or absence of oxygen. Thus in putrefying fluids oxidations may be going on in the upper layers where there is abundance of oxygen, and reductions in the lower layers where the oxygen is deficient or absent.

This explanation throws some light on the phenomena of so-called oxidations occurring in the tissues. It may be supposed that some of the molecular changes concerned in the nutrition of living tissues are similar to those of fermentations in the absence of an abundant supply of oxygen. In these circumstances, the hydrogen set free in turn liberates nascent oxygen, which then attacks oxidizable matter. Thus, according to Hoppe-Seyler, supposing the oxidizable matter to be represented by n, the changes would be represented by the equation—

$$HH + O_2 + n = H_2O + On.$$

It will be observed also that this is equivalent to a dehydration.

Ferments may be classified under three divisions—(1) the soluble ferments; (2) the organized ferments; and (3) according to the nature of the chemical changes they are capable of exciting.

(1) The Soluble Ferments.

These are substances produced in the interior of animal or vegetable cells, and when obtained as free from extraneous organic matter as possible, they are solid, amorphous, colourless, tasteless substances, soluble in water, and precipitated from their aqueous solutions by alcohol, and the acetate of lead. Chemically, they resemble the derivatives of albuminates, but they contain no sulphur. Their chemical constitution is unknown. The following are a few examples of fermentative processes—

1. The conversion of starch into dextrin and glucose, produced by the action of the diastase of malt, of ptyalin in saliva, of a ferment in the pancreatic juice, and of all albuminous matters in a state of decomposition—

$$\underset{\text{Starch.}}{4(C_6H_{10}O_5)} + 3H_2O = \underset{\text{Dextrin.}}{C_6H_{10}O_5} + \underset{\text{Dextrose.}}{3C_6H_{12}O_6}.$$

or,

$$(a)\quad \underset{\text{Starch.}}{3(C_6H_{10}O_5)} + H_2O = \underset{\text{Maltose.}}{C_{12}H_{22}O_{11}} + \underset{\text{Dextrin.}}{C_6H_{10}O_5}.$$

$$(b)\quad \underset{\text{Dextrin.}}{2(C_6H_{10}O_5)} + H_2O = \underset{\text{Inverted sugar.}}{C_{12}H_{22}O_{11}}.$$

2. The transformation of cane sugar into inverted sugar, which is a

mixture of dextrose and lævulose, and into glucose, accomplished by a ferment in the intestinal juice, and by a ferment in yeast—

$$\underset{\text{Cane sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Lævulose.}}{C_6H_{12}O_6}.$$

3. The conversion of glucosides, such as amygdalin, into glucose and various compounds, accomplished by synaptase or emulsin—

$$\underset{\text{Amygdalin.}}{C_{20}H_{27}NO_{11}} + 2H_2O = \underset{\text{Glucose.}}{2C_6H_{12}O_6} + \underset{\text{Oil of bitter almonds.}}{C_7H_6O\,.\,NHC.}$$

$$\underset{\text{Salicin.}}{C_{13}H_{18}O_7} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Saligenin.}}{C_7H_8O_2}.$$

4. The conversion of glucose into glycerine and mannite—

$$\underset{\text{Glucose.}}{C_6H_{12}O_6} + H_2 = \underset{\text{Mannite.}}{C_6H_{14}O_6}.$$

5. The conversion of glycerine and of mannite into alcohol by the action of nitrogenous organic matter in a state of decomposition. (Berthelot.)

6. The splitting up of fats into fatty acids and glycerine by the action of a ferment in the pancreatic juice—

$$\underset{\text{Olein.}}{C_{57}H_{104}O_6} + 3H_2O = \underset{\text{Oleic acid.}}{3(C_{18}H_{34}O_2)} + \underset{\text{Glycerine.}}{C_3H_8O_3}.$$

7. The transformation of albuminates into peptones by the action of pepsin, the ferment of the gastric juice, and by a ferment in the pancreatic and intestinal juices.

The following are examples of the chemical changes effected in these processes of fermentation—

1. Simple isomeric transformations, as in the conversion of starch into dextrin and sugar.

2. Hydrations, as in the conversion of cane sugar into glucose.

3. Synthetic processes, as in the fermentation of glucosides.

4. The separation of water, and a change in the molecular condition of the remainder, as in the conversion of albuminates into peptones (*hydrolytic ferments*).

It is to be noted that, in many instances, chemical changes, similar to those produced by soluble ferments, may be effected by the action of heat and of the mineral acids. Thus, sulphuric and hydrochloric acids effect a hydration and change cane sugar into dextrose and lævulose, milk sugar into galactose, and starch into dextrin and dextrose according to the above equations.

Many of these ferments may be arranged under the following physiological classification, which is also practical—

I. *Proteolytic*—those changing albuminous matter into peptones (acid

digestion by pepsin of gastric juice) or into tryptones and then into leucin, tyrosin, etc. (alkaline digestion by tripsin of pancreatic juice).

II. *Amylolytic*—those changing starch into glucose with absorption of water, such as the ptyalin of saliva, a ferment in the pancreatic juice, and a ferment in the liver and in many tissues.

III. *Steatolytic*—those decomposing fats with water, as a ferment in the pancreatic juice.

IV. *Inversive*—converting cane sugar into inverted sugar. Such a ferment exists in the intestinal juice, possibly to a small extent in saliva and in hepatic cells.

V. *Blood ferment*—causing union of the elements that form the fibrin of blood clot.

Ferments of this class are the principal agents in the chemical transformation of food in the process of digestion, and in all probability processes of a fermentive character also occur in certain organs, as in the liver.

(2) The Organized Ferments.

These are living organisms, the type of which is the unicellular plant found in yeast, known as *Saccharomycetes cerevisiæ*, and including such organisms as many forms of fungi, vibrios, and bacteria.

1. Soluble ferments usually produce, as the result of their action, not more than one or two substances, but the organized ferments, as a rule, produce several substances. Thus glucose, in the presence of yeast not only may yield carbonic acid and alcohol but also glycerine, succinic acid, acetic acid, fatty matter, a nitrogenous matter, and other products.

2. Organized ferments, as already explained, do not absolutely require the presence of the oxygen of the air, as they have the power of obtaining oxygen from fermentescible matter itself. In certain cases it would even appear that excess of oxygen arrests fermentation. Pasteur has shown that vibrios are killed by a strong current of oxygen passed through the fluid containing them, and Paul Bert has found fermentation to go on much more slowly under a pressure of five atmospheres of pure oxygen.

3. As examples of the fermentations excited by such organisms, we may consider the following—

a. Alcoholic—

$$\underset{\text{Grape sugar.}}{C_6H_{12}O_6} + H_2O = 2CO_2 + \underset{\text{Alcohol.}}{2(C_2H_6O)} + H_2O.$$

b. Lactic acid—

$$\underset{\text{Milk sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Lactic acid.}}{4(C_3H_6O_3)}.$$

c. Butyric acid—

$$\underset{\text{Lactic acid.}}{2(C_3H_6O_3)} = \underset{\text{Butyric acid.}}{C_4H_8O_2} + 2CO_2 + 2H_2.$$

d. Acetous—

$$\underset{\text{Alcohol.}}{C_2H_6O} + O = \underset{\text{Aldehyde.}}{C_2H_4O} + H_2O; \text{ then } \underset{\text{Aldehyde.}}{C_2H_4O} + O = \underset{\text{Acetic acid.}}{C_2H_4O_2}.$$

e. Putrefaction—This cannot be represented by an equation but albuminous and fatty matters are changed into leucin, tyrosin, fatty acids, glycerine, ammonia, sulphuretted hydrogen, water, hydrogen, and possibly nitrogen.

(3) Classification of Ferments according to the Nature of the Chemical Changes they Produce.

Hoppe-Seyler[1] has given an important classification of ferments which is in the main chemical in its character, but it merits special attention because it illustrates the probable action of many fermentive substances whether soluble ferments or organized ferments.

I. The conversion of anhydrides into hydrates—

A. Ferments acting like weak mineral acids at the temperature of boiling water (100° C.).

1. Conversion of starch or glycogen into dextrin and glucose—

$$\underset{\text{Starch.}}{4(C_6H_{10}O_5)} + 3H_2O = \underset{\text{Dextrin.}}{C_6H_{10}O_5} + \underset{\text{Glucose.}}{3(C_6H_{12}O_6)}.$$

2. Conversion of cane sugar into glucose and lævulose (fruit sugar)—

$$\underset{\text{Cane sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Lævulose.}}{C_6H_{12}O_6}.$$

3. Conversion of a benzol-glucoside into sugar and a benzol derivative by the action of emulsin—

$$\underset{\text{Salicin.}}{C_{13}H_{18}O_7} + H_2O = \underset{\text{Sugar.}}{C_6H_{12}O_6} + \underset{\text{Saligenin.}}{C_6H_4\begin{cases}CH_2\,.\,OH\\OH\end{cases}}$$

$$\underset{\text{Helicin.}}{C_{13}H_{16}O_7} + H_2O = \underset{\text{Sugar.}}{C_6H_{12}O_6} + \underset{\text{Salicylaldehyde.}}{C_6H_4\begin{cases}COH\\OH\end{cases}}$$

$$\underset{\text{Glucoside of salicylic acid.}}{C_{13}H_{16}O_8} + H_2O = \underset{\text{Sugar.}}{C_6H_{12}O_6} + \underset{\text{Salicylic acid.}}{C_6H_4\begin{cases}CO\,.\,OH\\OH\end{cases}}$$

$$\underset{\text{Arbutin.}[2]}{C_{25}H_{34}O_{14}} + 2(H_2O) = \underset{\text{Sugar.}}{2(C_6H_{12}O_6)} + \underset{\text{Hydrochinon.}}{C_6H_4\begin{cases}OH\\OH\end{cases}} + \underset{\text{Methylhydrochinon.}}{C_6H_4\begin{cases}OCH_3\\OH\end{cases}}$$

$$\underset{\text{Amygdalin.}}{C_{20}H_{27}NO_{11}} + 2(H_2O) = \underset{\text{Sugar.}}{2(C_6H_{12}O_6)} + \underset{\text{Oil of bitter almonds.}}{C_6H_5\,.\,COH\,.\,CHN}$$

[1] *Physiologische Chemie*, 1877, p. 116 *et seq.*

[2] Arbutin, a crystalline substance in leaves of red bearberry (*Arctostaphylos uva ursi*).

$$\underset{\text{Coniferin.}^{1}}{C_{16}H_{22}O_8} + H_2O = \underset{\text{Sugar.}}{C_6H_{12}O_6} + \underset{\text{Conifer-alcohol.}}{C_{10}H_{11}O_2 . OH}$$

$$\underset{\text{Glucoside of vanillic acid.}^{2}}{C_{14}H_{18}O_9} + H_2O = \underset{\text{Sugar.}}{C_6H_{12}O_6} + \underset{\text{Vanillic acid.}}{C_8H_8O_4}$$

$$\underset{\text{Daphinin.}^{3}}{C_{31}H_{34}O_{19}} + 2(H_2O) = \underset{\text{Sugar.}}{2(C_6H_{12}O_6)} + C_{19}H_{14}O_9$$

$$C_{31}H_{50}O_{16}{}^{*} + 5H_2O = 3(C_6H_{12}O_6) + C_{13}H_{24}O_3$$

$$\underset{\text{Jalapin.}^{4}}{C_{34}H_{56}O_{16}} + 5H_2O = \underset{\text{Sugar.}}{3(C_6H_{12}O_6)} + C_{16}H_{30}O_3$$

4. Decomposition of a sulphur compound into sugar, sulphuric acid and oil of mustard through myrosin—

$$\underset{\text{Myronate of potassium.}^{5}}{C_{10}H_{18}NS_2O_{10}K} = \underset{\text{Sugar.}}{C_6H_{12}O_6} + \underset{\text{Sulphate of potassium.}}{KHSO_4} + \underset{\text{Oil of mustard.}}{C_4H_5NS}$$

$$\underset{\text{White mustard.}}{C_{30}H_{44}N_2S_2O_{11}} = \underset{\text{Sugar.}}{C_6H_{12}O_6} + C_{16}H_{24}N . SO_4H + C_8H_7ONS$$

B. Ferments acting like caustic alkalies at a higher temperature.

1. Decomposition of ethers and of the fats into an alcohol and fatty acid.

2. Decomposition of amides with absorption of water—

$$\underset{\text{Urea.}}{CH_4N_2O} + H_2O = \underset{\text{Carbonate of ammonia.}}{(NH_4)_2CO_3}$$

$$\underset{\text{Hippuric acid.}}{C_9H_9NO_3} + H_2O = \underset{\text{Glycocoll.}}{C_2H_5NO_2} + \underset{\text{Benzoic acid.}}{C_7H_6O_2}$$

$$\underset{\text{Tauro-cholic acid.}}{C_{26}H_{45}NSO_7} + H_2O = \underset{\text{Taurin.}}{C_2H_7NSO_3} + \underset{\text{Cholalic acid.}}{C_{24}H_{40}O_5}$$

II. Ferments causing the transference of the oxygen from the hydrogen atom to the carbon atom.

1. The lactic fermentation. Here one molecule of milk sugar, with the addition of a molecule of water, is changed into two molecules of sugar (dextrose and lævulose), which in turn become four molecules of lactic acid.

2. The alcoholic fermentation. The transference of O from H to C is thus shown—

$$\underset{\text{Glucose.}}{\begin{matrix} CH_2 . OH \\ CH . OH \\ CH . OH \\ CH . OH \\ CH . OH \\ COH \end{matrix}} + 2H_2O = \begin{matrix} CO . (OH)_2 \\ CH_2OH \\ CH_3 \\ CH_3 \\ CH_2OH \\ CO(OH)_2 \end{matrix} \left.\begin{matrix} \\ \\ \end{matrix}\right\} \text{Alcohol.} \quad \left.\begin{matrix} \\ \\ \end{matrix}\right\} \text{Alcohol.}$$

[1] Coniferin, a glucoside in the cambium of coniferous woods, *Abies excelsa*, *Larix Europæa*, etc.

[2] Vanillin, neutral odoriferous principle of vanilla, fruit of *Vanilla planifolia*.

[3] Daphinin, a crystallizable glucoside in *Daphne alpina* and other species of *Daphne*. * Convolvulin, a resin from *Convolvulus schiedeanus*.

[4] Jalapin, a resin obtained from scammony resin, yielded by *Ipomœa orizabensis*.

[5] A salt in the seed of black mustard.

3. Putrefaction. The following are examples—

$$\underset{\text{Formate of lime.}}{(CHO_2)_2Ca} + H_2O = \underset{\text{Carbonate of lime.}}{CaCO_3} + CO_2 + 2H_2$$

$$\underset{\text{Acetate of lime.}}{(C_2H_3O_2)_2Ca} + H_2O = \underset{\text{Carbonate of lime.}}{CaCO_3} + CO_2 + \underset{\text{Marsh gas.}}{2CH_4}$$

$$\underset{\text{Cellulose.}}{n(C_6H_{10}O_5)} + n(H_2O) = 3n(CO_2) + \underset{\text{Marsh gas.}}{3n(CH_4)}$$

$$\underset{\text{Lactate of lime.}}{(C_3H_5O_3)_4Ca_2} + H_2O = \underset{\text{Carbonate of lime.}}{CaCO_3} + 3CO_2 + 4H_2 + \underset{\text{Butyrate of lime.}}{Ca(C_4H_7O_2)_2}$$

(4) The Nature of the Organized Ferments.

These organisms belong to the group of bodies to which the name of *Schizomycetes* was given by Naegeli in 1857, and they include such as

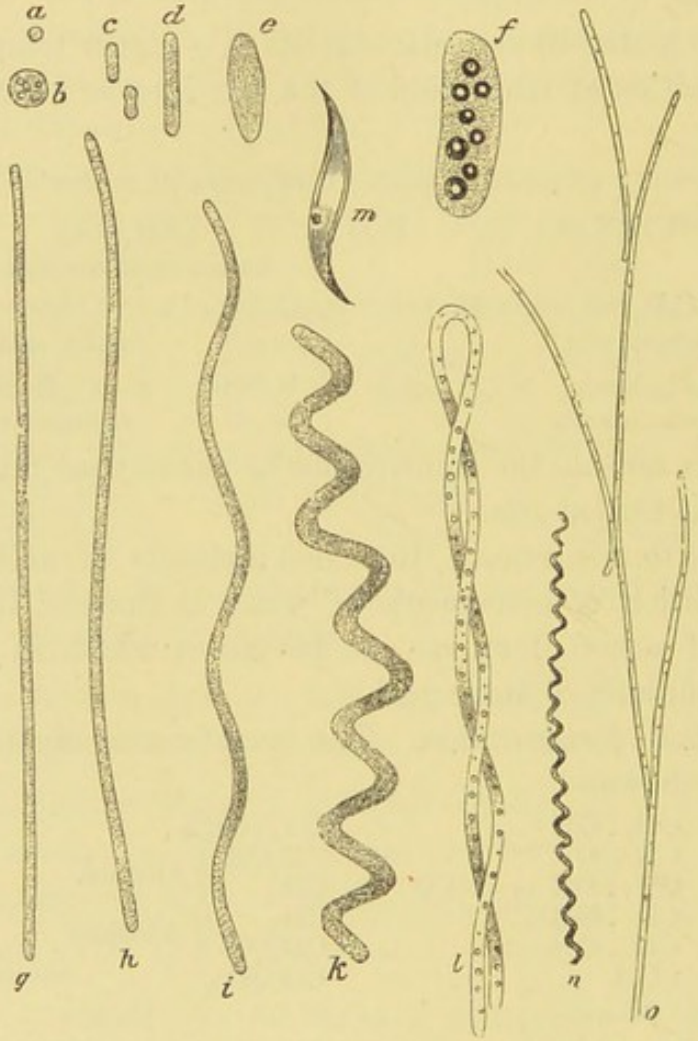


Fig. 66.—Typical forms of Schizomycetes (after Zopf). *a*, micrococcus; *b*, macrococcus, or "monas"; *c*, bacterium; *d*, bacillus; *e*, chlostridium; *f*, monas Okenii; *g*, leptothrix; *h*, *i*, vibrio; *k*, spirillum; *l*, spirulina (a form of Beggiatoa alba); *m*, spiromonas; *n*, spirochæte; *o*, cladothrix. The granules in *b*, *f*, and *e* are particles of sulphur.

are often termed bacteria, bacilli, micrococci, microphytes, microbes, etc. They are minute unicellular bodies of the nature of saprophytic

or parasitic schizophyta, devoid of chlorophyll, and multiplying usually by fission, and in some by a process of spore-formation. They vary in size from ·001 to ·005 mm. in length. The general appearance of the typical forms of these organisms is thus given by Professor Marshall Ward[1] (Fig. 66).

The form may be one of the following—

(1) *Globular* or *spheroidal*, usually termed *micrococcus*, or, if larger, *macrococcus* or *monas*.

(2) *Rod-like*, cylindrical cells, three or four times as long as broad, *bacterium*, when the rods are short, or with rounded ends, or *bacillus*, when the rods are longer and usually sharply cut off at the end.

(3) *Filaments*, consisting of single elongated filaments, or of several much elongated cells that have remained attached after division; such are seen in *leptothrix*.

(4) *Spiral forms*—If the sinuosity be slight, the body is a *vibrio*, and if more cork-screw like, a *spirillum*.

(5) *Plates* or *tablets*, irregular branching groups of cells, which, by successive divisions both longitudinal and transverse, give rise to little groups of square-shaped cells lying in groups of four, six, or eight, as in *sarcina*.

Some schizomycetes show pleomorphism, that is to say the same organism at one stage of its life, may appear in the form of a micrococcus, at a later, of a bacterium, and afterwards, as a filament or leptothrix. An example of such an organism is *Cladothrix dichotoma*,[2] and the special forms have been regarded erroneously as different species of schizomycetes. On the other hand, many of these organisms maintain the same form, generation after generation.

Schizomycetes are found practically almost everywhere, and if any putrescible fluid is exposed to the air for some hours, at ordinary temperatures, it soon swarms with micrococci and bacteria. They also abound in many animal fluids in the living body under certain conditions of disease, and some representatives may be found in small numbers in the tissues even in a state of health. In the alimentary canal, except where the secretions are highly acid, they are also plentiful.

Each schizomycete consists primarily of a minute cell, never nucleated, formed of a mass of "homogeneous or slightly granular protoplasm with a pearl-like lustre, and without vacuoles; this is enveloped by a membranous envelope which is so delicate as to be scarcely per-

[1] Marshall Ward, article "Schizomycetes," in *Encyclop. Britannica*, vol. xxi. p. 399.

[2] Figured in Marshall Ward's article. (Fig. 16, *op. cit.*)

ceptible."[1] This envelope in some cases consists of cellulose. Coloured pigments (red, yellow, green, or blue) have been found in some organisms. The protoplasmic matter consists of a colourless substance named *myco-protein*. Starch has been found in some bacteria, giving a blue colour with solutions of iodine. Minute crystals of sulphur have been found in filamentous schizomycetes (see Fig. 66, *f*), and oily and fatty matters have been detected. Sometimes, after these bodies have multiplied by transverse fission until a long chain-like structure has been formed, the chain may break into fragments and the particles thus set free—bacteria, bacilli, or micrococci—form dense swarms which "become fixed in a matrix of their own swollen contiguous cell walls, and pass into a resting state as a so-called *zoogloea*." They become entangled in this jelly-like matrix, and the zoogloea in itself may take on forms, spherical, ovoid, or filamentous, peculiar to the species. By-and-bye, the organisms in the zoogloea may become active, and, scattering in the surrounding medium, begin to grow and multiply.

The multiplication of schizomycetes by spores is now a well-ascertained fact, and the various types of this mode of development are shown in Fig. 67.

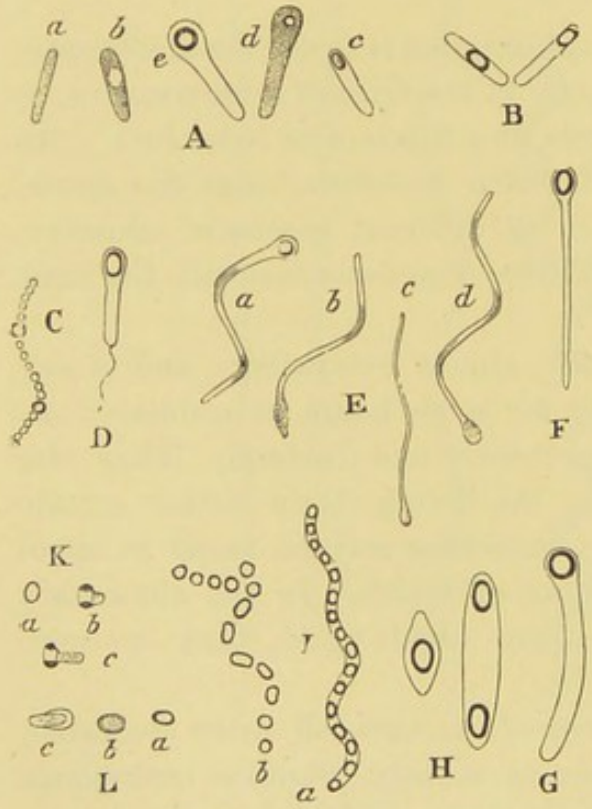


FIG. 67.—Types of spore-formation in schizomycetes. (After Zopf.) A, various stages in the development of the endogenous spores in a *Clostridium* (*bacillus*), the small letters indicating the order. B, endogenous spores of the hay bacillus. C, a chain of *cocci* of *leuconostoc mesenterioides*, with two resting spores, *i.e.* arthrospores. (After Van Tieghem.) D, a mobile rodlet with one cilium, and with a spore forming inside. E, spore-formation in *Vibro*-like (*c*), and *Spirillum*-like (*a*, *b*, *d*) schizomycetes. F, long rod-like form containing a spore (these are the so-called "Köpfchenbacterien" of German authors). G, vibrio-form with spore. (After Prazmowski.) H, *Clostridium*—one cell contains two spores. (Prazmowski.) I, *Spirillum* containing many spores—*a*, which are liberated at *b* by the breaking up of the parent cells. K, germination of the spore of the hay bacillus (*Bacillus subtilis*)—the axis of growth of the germinal rodlet is at right angles to the long axis of the spore. L, germination of the spore of *Clostridium butyricum*—the axis of growth coincides with the long axis of the spore.

It is now well known that schizomycetes are found in the blood, tissues, and organs of animals and of man suffering from certain specific diseases. One of the most striking instances of this occurs in

[1] Marshall Ward, *op. cit.*

splenic fever, where the blood abounds in *Bacillus anthracis*, shown in Fig. 68.

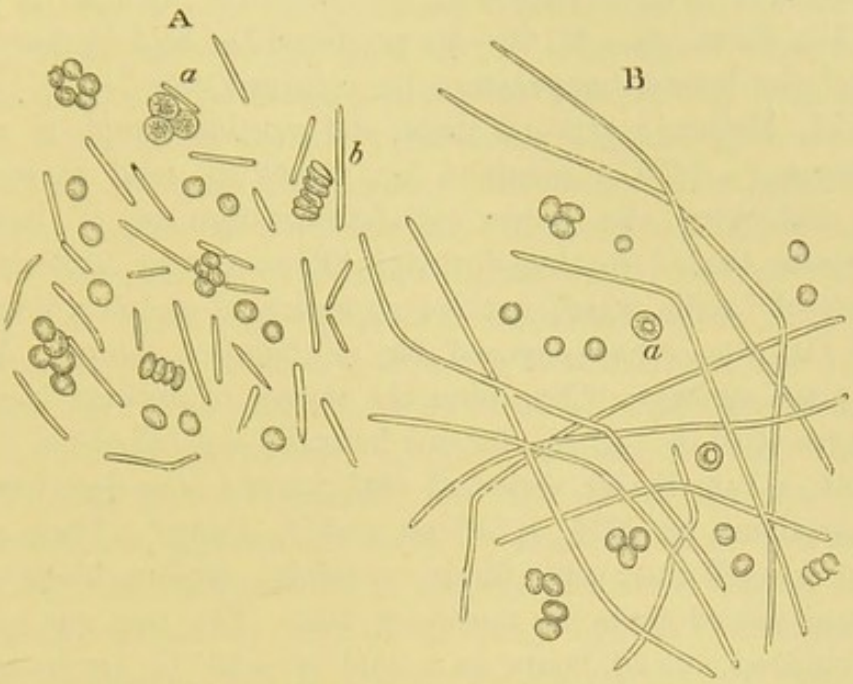


FIG. 68.—Bacillus anthracis. (After Koch.) A, *Bacilli* mingled with blood corpuscles from the blood of a guinea-pig, some of the *bacilli* dividing. B, the rodlets after three hours' culture in a drop of aqueous humour. They grow out into long *leptothrix*-like filaments, which become septate later, and spores are developed in the segments. (× 650.)

Much discussion has taken place as to whether the presence of such an organism is a casual, or even a concomitant phenomenon, or whether it is the real cause of the disease. It is beyond the province of this work to discuss a question that is chiefly pathological in its nature, and it is sufficient here to say that, from the purely physiological standpoint, the presence of such an organism in vast numbers cannot fail in being inimical to the well-being of its host. Even considering the affinity such organisms have for oxygen, it is clear that a struggle will ensue between them and the living tissues for this gas; and, if the organisms secure the predominance, the living tissues must suffer. In addition, it is not improbable (rather highly probable) that the organisms may secrete alkaloidal substances of a poisonous nature which, in their turn, may exert powerful physiological effects on the tissues and organs.

The growth and development of schizomycetes are influenced by various physical and chemical agents.

(1) *Temperature.*—A temperature of about 35° C. is the most favourable for the development of the majority of these organisms. Cohn[1] states that he has subjected bacteria to low temperatures without destroying their activity. He gives the temperatures as follows—

[1] Cohn, *Beiträge zur Biologie der Pflanzen*, 1870; *Zweites Heft*, s. 221.

Exposure for 12 hours 30 minutes to a temperature of 0° C.; for 1 h. 30 m., to −16° C.; for 1 h. 45 m., to −17° C.; for 3 h. 30 m., to −18° C.; for 4 h. 30 m., to −18° C.; for 5 h., to −17°·5 C.; for 6 h., to −14° C.; and for 7 h. 30 m., to −9° C. He produced the cold by freezing mixtures, and the lowest temperature he obtained was −18° C. = 0° F. In 1870-71, Melsens[1] exposed yeast and vaccine lymph to very low temperatures (−78° C.) obtained by means of solid carbonic acid, without destroying the power of fermentation or of inoculation. Klein[2] states that "freezing destroys likewise most bacteria, except the spores of bacilli, which survive exposure to as low a temperature as −15° C., even when exposed for an hour or more." Again, in another place, he says: "Exposing the spores of anthrax bacillus to a temperature of 0° to −15° C. for one hour did not kill them."

In 1884, a remarkable series of experiments was described to the French Academy by MM. R. Pictet and E. Yung.[3] These observers sealed up in small glass tubes fluids containing various kinds of microphytes, and placed them in a wooden box. The box was in the first place submitted for 20 hours to a cold of −70° C., produced by the evaporation of liquid sulphurous acid in vacuo. The box was then surrounded by solid carbonic acid for 89 hours, and a cold of from −70° to −76° C. was thus obtained. Finally the box was subjected for a third period of 20 hours to a cold produced by the evaporation of solid carbonic acid in vacuo—the temperature being estimated at from −76° to −130° C., that is, a minimum temperature of 202° below zero F. They sum up by stating that the organisms were acted on by a cold of −70° C. for 109 hours, followed by a temperature of −130° C. for 20 hours. The organisms tested were *Bacillus anthracis*, *Bacillus subtilis*, *Bacillus ulna*, *Micrococcus luteus*, and a micrococcus not determined. *Bacillus anthracis* retained its virulence when injected into a living animal. The vitality of the others was not affected. Experiment showed that whilst cold seemed to kill some of the micrococci, a great number resisted it. Yeast showed no alteration under the microscope, but it had lost its powers of fermentation. Vaccine lymph exposed to the low temperatures did not produce a pustule on the left arm of an infant, whilst another sample of the same lymph introduced into the right arm of the same child produced a pustule. Pictet and Yung conclude from their experiments that, in the conditions of cold indicated, many of the lower organisms were not destroyed.

[1] Melsens, *Comptes Rendus*, tome lxx. 1870, p. 629; also *Comptes Rendus*, tome xxi. p. 325.

[2] Klein, *Micro-organisms*, p. 35.

[3] Pictet and Yung, *Comptes Rendus*, tome xcviii. No. 12, p. 747.

In 1885, J. J. Coleman and the author[1] made numerous experiments in which very low temperatures were reached and maintained for many hours by the use of a special modification of the cold-air engines invented by Mr. Coleman, now largely used in the industry of importing meat into Great Britain. Although putrescible infusions and fresh meat contained in hermetically sealed bottles were exposed to a temperature of −83° C. for 100 consecutive hours, putrefaction soon began when the bottles were removed to a warm room. It was found impossible to produce sterilization, that is, to kill the schizomycetes or their spores, by cold.

On the other hand, high temperatures are more fatal. The spores of bacilli, however, may germinate after the fluid has been boiled (100° C.) for an hour, and it is said that they have withstood a temperature of even 110° C. Dry spores resist a higher temperature than spores in a moist state, and it would appear that ripe spores are more resistant than spores in the germinating state. Spores are killed also at a lower temperature in acid media than in alkaline media. Probably prolonged boiling would ultimately kill all spores. Tyndall[2] ascertained that discontinuous heating, that is repeatedly boiling the solutions containing spores for half an hour daily, sterilized the fluid in two or three days, although boiling the same kind of fluid (100° C.) for half an hour once did not produce this effect. The explanation offered is that spores not killed by the first boiling may germinate before the second boiling, and if caught in the germinating state they are killed, whilst some not germinating may again escape.

(2) *Water.*—Schizomycetes germinate only in a fluid, but spores may remain alive in a dormant dry state for months or years. Thus *Bacillus subtilis* has been kept alive in a dry state for years. More extended observations are required on this point with regard to many species, but it is obvious that they may live in the dry state and be wafted about by air currents.

(3) *Light.*—Brilliant sunshine appears to be inimical to the spores of certain species, and flasks may be partially, if not wholly, sterilized by being exposed to sunlight. On the other hand, it is not unlikely that light may encourage the development of other species, more especially in fluids and in the vicinity of chlorophyll-bearing-organisms, as shown by Engelmann's experiment described at p. 24. Aerobiotic forms collect where the oxygen is being evolved from chlorophyll, in the yellow-red part of the spectrum.

[1] Coleman and M'Kendrick, *Proceedings of Royal Institution of Great Britain*, Lecture on "The Mechanical Production of Cold and Effects of Cold on Microphytes," 29th May, 1885.

[2] Tyndall, *Floating Matter in the Air*, 1881.

(4) *Ozone.*—The author has attempted to sterilize organic fluids and solids by means of ozone, but without success, although it was evident that ozonization of the air or of an atmosphere of oxygen delayed considerably the advent of putrefaction.

(5) *Pressure.*—Paul Bert ascertained that putrefaction was prevented in flesh, moist bread, fruits, etc., by pure oxygen under a pressure of from 10 to 27 atmospheres, but that air under the same pressure had no effect in delaying putrefaction.

(6) *Chemical substances.*—Schizomycetes resist the action of certain substances that might be supposed capable of destroying them. Thus *Bacillus anthracis* survived several weeks' immersion in absolute alcohol. On the other hand, they are killed by carbolic acid, salicylic acid, quinine, hydroxylamine, sulphurous acid, corrosive sublimate, chlorine, and the mineral acids.

(5) Modes of Cultivating Schizomycetes.

Bacteriology, or the department of biology relating to the growth and development of the schizomycetes, has, during the past four or five years, become so important in connection with the germ theory of disease, as to have led to a remarkable development of experimental methods by which these organisms may be studied under scientific conditions. To describe these methods in detail is beyond the scope of this work, and all that is attempted is to give the student a short sketch of the general principles on which bacteriologists proceed.

Bacteria may be artificially cultivated in or upon media so prepared as to meet the requirements of microscopical investigation. As a rule, they grow well upon any organic substance containing nitrogen and carbon, of a slightly alkaline reaction, and at a suitable temperature. This substance may be either liquid or solid. *Pasteur's fluid, e.g.* consists of a solution of 1 part of tartrate of ammonia, 10 parts of candy sugar, and the ash of 1 part of yeast in 100 parts of water. *Cohn's liquid* consists of ·5 grm. phosphate of potash, ·5 grm. sulphate of magnesia, ·05 grm. tribasic phosphate of lime, and 1 grm. tartrate of ammonia, in 100 grms. of water. Infusions or decoctions of fruit, vegetables, or flesh are also employed. A very serviceable bouillon (or beef-tea) may be made by boiling 500 grms. of finely minced beef in a litre of water for three quarters of an hour. It is rendered alkaline by adding a few drops of a saturated solution of carbonate of soda, and filtered to obtain a perfectly clear fluid. This is placed in glasses with stoppers of cotton-wool and sterilized by exposure to the temperature of boiling water for about three quarters of an hour on three successive days—"discontinuous sterilization."

A great objection to the cultivation of bacteria in liquids is that it is exceedingly difficult to get a "*pure cultivation*," that is to say, a special variety of micro-organism growing without admixture of other kinds. Accordingly various solid or semi-solid substances are used, upon whose surface a single variety may be isolated and examined. Thus, the cut surface of a boiled potato affords a very suitable nidus for many varieties of bacteria. The potato is sterilized by being washed with a 1 to 1000 solution of corrosive sublimate. It is then boiled for about three quarters of an hour, cut into two pieces by a knife which has been sterilized by being heated in a flame, and placed under a glass cover for protection. Bread rubbed to a fine powder, moistened with distilled water, and sterilized by discontinuous heating is frequently employed for the study of moulds and fungi. An exceedingly useful, firm, clear, transparent *jelly* is made in the following way: 500 grms. of finely minced lean beef are soaked for 24 hours in a litre of water and strained through a muslin cloth so as to give a litre of meat-juice. To this are added 10 grms. of peptone powder, 5 grms. of common salt, and 100 grms. of the best (French) gelatin. The mixture is well shaken and heated till the gelatin is completely liquefied. It is then made slightly alkaline with a saturated solution of carbonate of soda, filtered while warm into glass vessels or test tubes, and sterilized by discontinuous heating. Sometimes 1 or 2 per cent. of grape sugar is added to the above preparation.

As this jelly melts at a temperature of 25° C., it is unsuitable for the cultivation of such organisms as grow only at temperatures more nearly approaching that of the human body (37°–38° C.). To meet this objection, from 10 to 20 grms. of *Agar-agar*, a gelatinous substance obtained from various kinds of seaweed, may be substituted for the gelatin, the other constituents remaining as above. The jelly so obtained is not quite so clear as when gelatin is employed, and the process of filtration is rendered very slow by the tendency of the agar-agar to gelatinize when the temperature falls below the boiling point. For the growth of certain micro-organisms, such as the *bacillus* of tuberculosis, blood-serum is employed, made firm by heating to 68° C. for about an hour. If heated to a higher temperature, it becomes grey, opaque, and unsuitable for microscopical research. It is sterilized by being heated to a temperature of from 56° C. to 58° C., two hours daily for about eight days, or if the blood of a healthy animal is carefully drawn off into cold sterilized vessels, the serum may be obtained at once free from micro-organisms and requires no further sterilization.

Before any of the above-mentioned substances can be used for the cultivation of bacteria, all instruments and utensils that may come in contact

with them must be completely sterilized. All metallic instruments required, such as knives, scissors, forceps, needles, and wires may be sterilized by being heated in the flame of a Bunsen burner for about half a minute. Glass utensils, such as covers, slides, and test-tubes are placed in an oven at a temperature of 160° C., and after the lapse of about half an hour are found to be sterilized. For the sterilization of nutrient media, a long cylindrical vessel with a jacket of felt and a perforated cover is used in which water is kept boiling and the substances to be sterilized are suspended in the rising steam for about three quarters of an hour for three successive days. But, if the substance contains much albumin, as, *e.g.* blood-serum, it is then warmed for about three or four hours daily to a temperature of from 56°–58° C. for a week, during which time all germs which have been present at the beginning of the process have developed into mature forms and, as such, been destroyed.

Suppose, now, we wish to make a pure cultivation of a special micro-organism, we may proceed by various methods. If the potato is to be used, a small quantity of the material supposed to contain the desired variety is taken upon the point of a sterilized knife and rubbed evenly over the cut surface of the potato, care being taken not to touch its circumference at any part. From the centre of this surface, a minute portion is now taken and rubbed over the surface of a second half; a little of the second is conveyed to a third, and so on, until six or seven halves have been successively inoculated one from the other. It will be readily understood that only an exceedingly minute portion of the original material can be present upon the surface of the sixth or seventh potato. All the pieces are placed under a glass cover, and they are kept from drying by placing moist blotting paper beside them. In the course of a few days, a vari-coloured film will probably be seen covering the surface of the potato first inoculated, composed of colonies of various species of micro-organisms. On the second potato, this layer will be slightly broken up as the germs which have given rise to the young colonies have been more separated from one another than in the first case. On the sixth or seventh there will probably be only one or two small colonies which have sprung from single germs, and in these we have a pure cultivation of a special variety ready for examination or further separate cultivation.

If the gelatin or agar-agar preparations are to be used, three test-tubes about half full of the jelly are placed in a water bath at a temperature high enough to liquefy the jelly. A small quantity of the material to be investigated is introduced into one of the test-tubes and stirred about in it by means of a sterilized platinum wire, the end of which has been bent round so as to form a

small loop. The wire is now sterilized in the Bunsen flame, cooled, and by its means a small drop of the liquid in the first tube is transferred to that of the second, and intimately mixed with it. Similarly, from the second a small drop is transferred to the third. As with the cultivation on the potato, so here there can be very little of the original material in the third test-tube. In order, now, to obtain a somewhat large surface upon which the micro-organisms may grow separate from one another, the mouths of the test-tubes are sterilized by gentle heating in the flame, and the liquid gelatin or agar-agar is poured on to cold sterilized glass plates, where it quickly forms a thin layer of firm jelly. The plates are covered with a glass shade, the air being kept moist as described above, and the growth of the cultivation observed. The special variety of micro-organism, if present, may be recognized either by the naked eye appearance of the colony, or by microscopical examination of a small portion of it, stained or unstained as may be desired. Let us suppose that the variety we wish to examine has been isolated and recognized, we may wish to cultivate it further by itself. In this case a minute portion of a colony is lifted on the point of a sterilized platinum wire, the cotton-wool stopper of a gelatin or agar-agar tube is removed, and the wire is thrust down through the jelly and quickly withdrawn, leaving, as it passes out, the micro-organisms adherent to the jelly, or, if the jelly has become firm in an inclined tube, so that it has a long sloping surface, a line may be drawn upon this surface with the end of the platinum wire, leaving the micro-organisms exposed to the air in the tube. Here the micro-organisms grow, and their appearance may be noted from time to time. Growth may go on at the ordinary atmospheric temperature, or it may require the temperature of the human body (37°–38° C.). In this case, tubes containing agar-agar or blood-serum are used, and are placed in an incubator, one form of which is represented in Fig. 69. In this the temperature is maintained constantly at the required height, any changes being prevented by the use of a thermo-regulator, one form of which is shown in Fig. 70. It consists of a three-way tube (*l*), the lower end of which passes down through a caoutchouc stopper to near the bottom of a test tube (*u*) containing mercury. In very sensitive instruments, a diaphragm through which the tube passes is placed at the centre of the test-tube, and immediately below this diaphragm is a small quantity of a mixture of alcohol and ether, which, when heated, expands much more rapidly than mercury. To the upper end of the tube (*t*) is fixed an india-rubber tube bringing gas. In this end of the tube, however, there is a caoutchouc stopper (not seen in the diagram), through which passes a smaller tube (*i*), the lower end of which is cut in a slanting direction

towards the mercury. Gas can only pass into the three-way tube through the smaller tube (*i*), and thence it is led by the horizontal arm

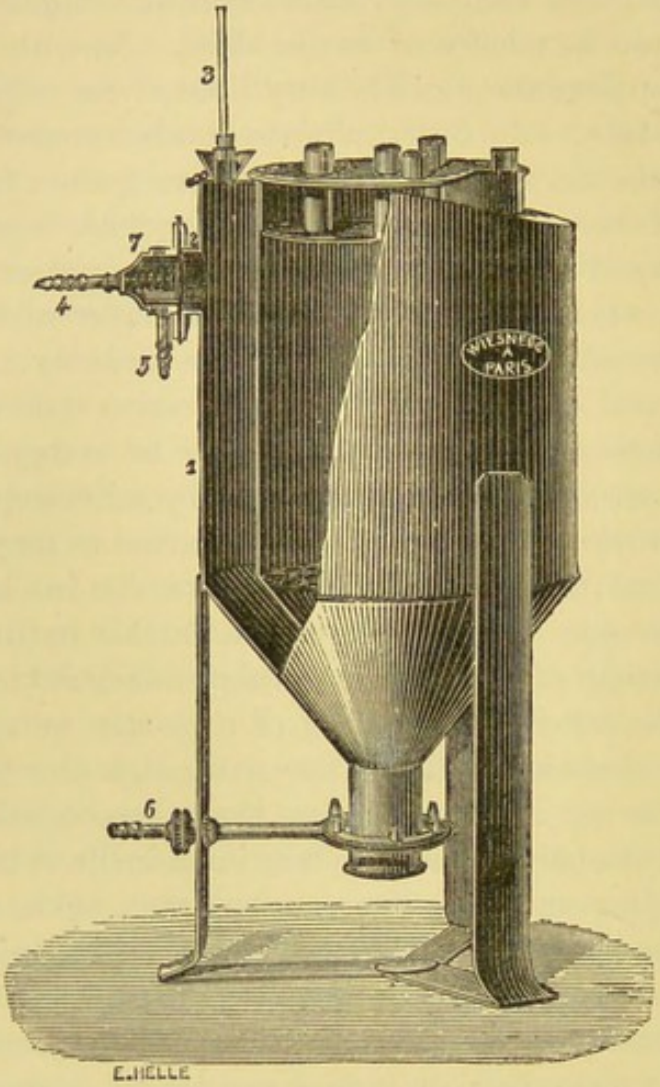


Fig. 69.—Incubator.

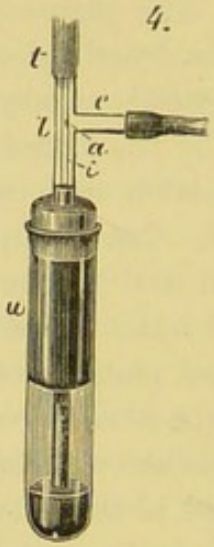


Fig. 70.—Thermo-regulator. *u*, test-tube with mercury; *l*, three-way tube; *i*, smaller tube through which gas passes into *l*; *a*, small aperture in *i*; *c*, tube to burner.

(*c*) and an india-rubber tube to a burner placed below the incubator. There is a minute opening in the tube (*i*) at (*a*), the object of which will soon become evident. When the instrument is to be used, the test-tube is passed into the incubator through an aperture in its cover, and the gas is lit. As the temperature inside the incubator rises, the mercury expands, and is forced up into the tube (*l*) until it reaches the tube (*i*). Continuing to rise, it gradually shuts off the gas, and the flame becomes smaller; but if the mercury still rose, the stream of gas would be cut off altogether, and, the flame being extinguished, the mercury would fall and the gas escape were it not for the minute aperture (*a*), which always permits a very small quantity of gas to find its way to the burner, and prevents the extinguishing of the flame. But the flame is now so small that the temperature of the incubator falls, the column of mercury descends, the tube (*i*) is opened, the gas streams

into (*l*), and the flame enlarges. The lower end of the tube (*i*) is placed at such a height above the surface of the mercury that the flame will be lowered when the temperature reaches any desired point. The flame is guarded by a screen, and when once heated, the incubator remains at the same temperature for any length of time.

By means of the incubator we can study the life history of many varieties of micro-organisms which would either perish or whose growth would be much retarded by exposure to lower temperatures. This is the more important because many such micro-organisms can, when introduced into the tissues of living animals, occasion serious disturbances in the vital processes of the part inoculated or even of the whole organism, and consequently the study of such forms is of the profoundest interest and importance alike to the physiologist and the physician. Much as has been done in this field of research, very much more still remains to be done. Much has still to be discovered about those varieties which have been isolated and cultivated artificially, and we know that there are still many forms which cannot be so cultivated partly because the different nutritive media in use are not suited to their requirements, and partly because we cannot supply the proper environment without which they must perish. Certain varieties can only, so far as we know, develop and be propagated in the bodies of living animals, some, indeed, seem to be limited to a single species of animal. Having once found a lodgment, however, they give rise to a series of symptoms, so definite in their order, so characteristic in their appearance, and so grave in their result, that we are frequently warranted in inferring their presence from the manifestation of the symptoms. We must not, however, regard any micro-organism as the specific cause of a definite disease unless it satisfies the following conditions—

Firstly, it must be present in the tissues of every animal suffering from the disease, and not in others.

Secondly, we must be able to isolate and cultivate it outside of the organism of the diseased animal, so that it is entirely free from contact with diseased tissue of any kind.

Thirdly, after inoculation with the pure cultivation, of the tissues of healthy animals, it must produce in them the same disease.

Consult on matters relating to physiological chemistry, in addition to the works referred to in the text, HOPPE-SEYLER—Physiologische Chemie, 1877-1881; ARTHUR GAMGEE—Physiological Chemistry of the Animal Body; BEAUNIS — Physiologie Humaine, vol. I.; the articles on the subject in HERMANN'S Handbuch der Physiologie; WITTHAUS—Medical Student's Manual of Chemistry, 1884. See also Prof. P. W. LATHAM'S Croonian Lecture, 1886.

In discussing the pathology of Gout and Diabetes, he has advanced a theory regarding the formation of uric acid, which may be briefly stated as follows—"The glycocin, instead of undergoing in the human subject the normal change into urea, combines in the liver with two molecules of urea, derived from the other amido-acids, leucin, etc., and forms the compound

$$\begin{matrix} CO\{NH_2 \\ \quad\{NH \end{matrix}$$
$$\begin{matrix} CO\{NH{-}{-}CH_2{-}COOH\,; \end{matrix}$$

that is a compound containing one more molecule of CONH than hydantoic acid, and allied to biuret and allophanic ether. This substance dehydrated forms

$$\begin{matrix} CO\{NH{-}CO \\ \quad\{NH \quad | \end{matrix}$$
$$\begin{matrix} CO\{NH{-}CH_2, \end{matrix}$$

which, like hydantoïn, is soluble in the blood, passes on to the kidneys, and is there conjugated with another molecule of urea, and forms ammonium urate—

$$\begin{matrix} CO\{NH{-}CO \\ \quad\{NH \quad | \\ CO\{NH{-}CH_2 \end{matrix} \quad + \quad \begin{matrix} CO\{NH_2 \\ \quad\{NH_2 \end{matrix} \quad = \quad H_2O + C_5H_3N_4O_3 . NH_4,$$

Urea. Ammonium urate.

which is excreted; but, a portion overflowing from the kidney into the general circulation and meeting with soda in the blood, is converted into sodium urate." (See *Lancet*, 1884, vol. I., p. 485; and *Lancet*, 1885, vol. I., p. 1120.)

SECTION III.

THE PHYSIOLOGY OF THE TISSUES.

Chap. I.—HISTORICAL INTRODUCTION.

The morphology and physiology of the tissues are included under the common term Histology. Before entering on the consideration of the individual tissues we shall take a historical survey of the steps by which our present knowledge has been attained. This survey will give an intelligent comprehension of the more modern views held regarding the structure of living matter and the origin of the various tissues.

It is evident that knowledge of the structure of the tissues must depend chiefly on the degree of excellence of the microscope, and on the skilful adaptation of methods of preparing these for examination by that instrument. No doubt simple lenses enabled Robert Hooke, Malpighi, Grew, and Leeuwenhoek, in the 17th century, to discover some of the cellular elements, and by such simple instruments Fontana, in 1780, saw and described the nucleus of the cell, adipose tissue, striated muscular fibres, and the elements of nervous tissue. Little progress, however, was made until early in the present century, when, by the application of the principle of achromatism by Fraunhofer, and by its practical development more recently by Lister and others, the compound microscope became a trustworthy instrument. Since then each improvement in the microscope, as to quality of lenses, modes of illumination, and mechanical adjustments, has been accompanied by a contemporaneous advance in histology. The application of the instrument has also been facilitated by the gradual development of methods of preparing the tissues for examination. Fontana, so long ago as before 1781, appears to have been the first to apply reagents to substances under the microscope, and he used alkalies and acids and even syrup of violets as a colouring matter.[1] Little, however, was done in this way until about the middle of the present century. Since then scarcely a year has elapsed without the invention of an improved method.

[1] Carnoy, *La Biologie Cellulaire.*

Thus Purkinje and Räuschel and Burdach used acetic acid as a reagent to clear up the tissues; Gerlach showed how to fill the minute blood-vessels with transparent injection; H. Müller devised the method of hardening tissues by steeping them for long periods in solutions of chromic acid or of its salts; the plan of staining certain elements was followed by Lord Sydney Godolphin Osborne, Gerlach, and Lionel Beale, with carmine, and by Waldeyer, with logwood or hæmatoxylin; Rainey showed how to render tissues transparent by glycerine; Lockhart Clarke made it possible to render nervous tissues transparent by passing them through alcohol, oil of turpentine, oil of cloves, and finally mounting them in Canada balsam; Rollet and Schwartz invented the method of double staining, so that one element of tissue might be tinted red by carmine, whilst other elements were tinted yellow by picric acid; Ranvier, Jürgens, Dreschfield, and others called to our aid the coal tar colours; and Krause, His, and Von Recklinghausen introduced the use of salts of silver, Max Schultze, the use of osmic acid, Cohnheim, the use of chloride of gold, and F. E. Schulze, the use of the salts of palladium—these observers having ascertained that certain elements of tissue reduce these salts, precipitating the metal in such a condition that by lines or marks the eye can follow details in structures that otherwise would show no optical indication of their presence.

By such operations of micro-chemistry, aided by improved methods of cutting thin sections, and by the use of microscopes that, considered merely as optical instruments, are probably very near the state of perfection, much progress has recently been made, more especially in the obscure department of histology dealing with the nature of protoplasm, the structure of cells, and the phenomena of fecundation.

As already stated, the earliest observers with the simple microscope undoubtedly saw some of the cellular elements of the tissues both of plants and of animals, but the first important step taken was by the botanist, Robert Brown, who, in 1831, made great progress in the knowledge of vegetable cells. In particular, he directed special attention to the nucleus, previously observed by Fontana, and established the fact that it was a normal element in the cell. In 1836, Valentin discovered the nucleolus, and he described it as a little round body, a kind of second nucleus, in the interior of the first. As to the physiological significance of cells, Turpin, so early as 1826, was the first to attribute to them distinct individualities, and to make the generalization that plants were formed by an agglomeration of cells. Then came the announcement of the cell theory in 1839, first applied to plants by Schleiden, and then to animals by Schwann. A careful study of the history of this subject has convinced me that even before 1830, the cell theory was held generally by botanists, and was discussed more especially in the writings of Dutrochet, Mayen, Schleiden, and Von Mohl; but it was not put on a sure basis until it was applied to animal tissues by Schwann. At this period then, 1839, the conception of a cell

was as follows—"*A vesicle closed by a solid membrane, containing a liquid in which floats a nucleus containing a nucleolus, and in which also one may find small granular bodies.*" Further, it was held by the founders of the cell theory that all cells might originate in a structureless substance or kytoblastema. The cell theory itself was contained in the statements that the bodies of all plants and animals are formed originally of cells, and that it is by the evolution of, and changes in, these cells that all the tissues are formed (Fig. 71).

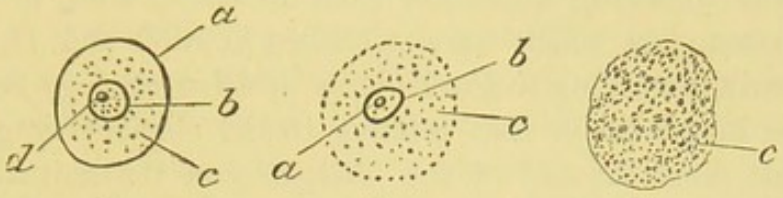


FIG. 71.—(1) Original conception of a cell. *a*, cell wall; *b*, nucleus; *c*, cell substance or contents; *d*, nucleolus. (2) Cell wall has disappeared. *b*, nucleus; *a*, nucleolus; *c*, cell substance or contents. (3) Modern view. Cell now consists of granular matter. Even the faint line, *c*, might be omitted; no cell wall exists.

The cell theory rapidly underwent modification under the influence of new facts and of philosophical speculation. Thus, in 1841, Henle showed that cells may multiply by budding, and in the same year Martin Barry observed that the reproduction of cells was accompanied by division of the nucleus. In 1845, Goodsir first promulgated the doctrine that cells never originate without pre-existing cells, a doctrine subsequently adopted by Remak and applied to pathological phenomena by Virchow. Then, in 1845, the botanist, Naegeli, showed that certain cells have no cell wall, and in 1857, Leydig defined a cell as a soft substance containing a nucleus. Lastly, in 1854, Max Schultze described a non-nucleated amœboid organism, *Amœba porrecta*, that is a cell in the physiological sense, but destitute of either cell wall, nucleus, or nucleolus, nothing in short, but a little mass of apparently structureless matter. Thus, we come back to a structureless substance, and in this connection it is interesting to read the following quotation from Schwann, because it indicates the comprehensive view taken by him of the nature of living matter—"In the fundamental phenomena attending the exertion of productive power in organic nature, a structureless substance is present in the first instance, either around, or in the interior of cells already existing; and cells are formed in it in accordance with certain laws, which cells become developed in various ways into the elementary parts of an organism."[1]

[1] For an interesting account of the earlier views regarding cells, see Dr. Drysdale's *Protoplasmic Theory of Life*, 1874. Dr. Drysdale in particular refers to

Now, we must retrace our steps to ascertain what were the earlier views held by naturalists regarding living matter. As early as 1839, Brisseau-Mirbel applied the term *cambium* to the living matter in plants, and a little later Schleiden called it *mucilage* or *schleim*. In 1835, the French naturalist Dujardin, in describing the Rhizopoda, first used the word *sarcode* to designate "a kind of mucus endowed with spontaneous movement and contractility." Then appeared the famous word, *protoplasm*. According to Carnoy, this term was first used by Purkinje, and he makes the following quotation from Reichert, who, in describing Purkinje's researches, which were published in 1839 and 1840, says—"There is only, according to Purkinje, a decisive analogy between the two organic kingdoms in that relating to the elementary granules of the vegetable cambium and of the *protoplasm* of the animal embryo." Six years later, in 1846, Hugo von Mohl, in describing the tissues of plants, used the word and defined the appearance of protoplasm in such a manner as to entitle him to the credit of the first scientific use of the term. He writes—"I am authorized to give the name of protoplasm to a semi-fluid nitrogenous substance, stained yellow by iodine, which is contained in the cavity of the cell, and which furnishes the materials for the formation of the primordial utricle, and of the nucleus." The word protoplasm, then, was first used to designate the living matter of plants, and although Dujardin, in 1835, described the properties of the living matter of animals under the name sarcode, it was a long time before naturalists recognized the practical identity of the two substances, the matter called sarcode being supposed to be peculiar to the lower orders of the invertebrates. It was not until 1861 that Max Schultze maintained the identity of the sarcode or protoplasm of the lower beings with the living matter in the tissues of the higher beings, and it is worthy of notice that this identity was grounded not on structural but on physiological properties. Thus it was found that the living matter in vegetable cells, as shown by the well-known phenomenon of the streaming of the granules, and by the movements of the sexual elements of fungi, had properties identical with those of the sarcode of the lower animals, and with those of the living matter in the higher

the early speculations of Dr. John Fletcher in his work, published in 1835, entitled *Rudiments of Physiology*. The following quotation from that book shows how advanced Dr. Fletcher's speculative opinions were before 1835: "Admitting that irritability or vitality, general and specific, is a property of the organized solids alone, it becomes a question of the highest interest whether it be directly inherent in each of the organized tissues, either of plants or animals, or *whether it merely appears to be possessed by them all in virtue of some one which is universally distributed over the organized being, and inextricably interwoven with every other.*" (ii. p. 55.)

animals. The living stuff, wherever found, manifested *irritability* and *contractility*, and thus it was at last held that the living matter, or *protoplasm*, was the same in kind in both kingdoms of organized beings, from the lowest to the highest. Then it was necessary, about 1865, to define protoplasm as follows—"*A diaphanous semi-liquid, viscous mass, extensible but not elastic, homogeneous—that is, without structure—without visible organization, having in it numerous granules, and endowed with irritability and contractility.*"

Protoplasm having been established as a living stuff, for a time it was regarded as a structureless material, presenting only a few granules, and with or without a nucleus. When a bit of protoplasm was very small, even although it had no nucleus, it was considered physiologically to be a cell. But again the restive onward movement began, which led to new conquest. As early as 1844, Von Mohl, in his elaborate investigations into the structure of the cell, pointed out the differentiation of the outermost layer of the protoplasm next the cell wall to form the *primordial utricle*, the vacuolation of the protoplasm, and the nature of the nucleus, and in 1851, he limited his definition of protoplasm so as to exclude the primordial utricle and the nucleus, in the following words—"The remaining part of the cell is more or less filled with an opaline, viscous, white fluid, having granules in it, and I call this protoplasm." Thus, as remarked by Carnoy, Von Mohl limited the name protoplasm to the hyaline viscous portion of the cell matter. These efforts of Von Mohl were the first attempts to differentiate the structure of protoplasm; they are the starting point of a vast number of researches in this direction, in which the investigator into vegetable tissues goes hand in hand with the animal morphologist. In 1853, Professor Huxley wrote on the subject, and distinguished between the endoplast, or the matter within the cell wall, and the periplast, or surrounding matter. He attached special importance to the endoplast, homologous with the primordial utricle of Von Mohl, and held that all differentiations of tissue were from changes in the periplast. Nor can we forget the impetus given to physiological speculation in this country by his famous lecture on protoplasm.

The first step in the direction of differentiation of the elements of protoplasm was made by Stilling, when, in 1859, he discovered a fibrillar structure in the interior of the ganglionic nerve cell. Five years later, in 1864, Leydig described a similar appearance in the cells of the intestines of certain isopod crustaceans (*Cloporte, etc.*). Between the years 1865 and 1867, Frommann showed that a fibrillar network existed in many cells, and in 1873, Heitzmann demonstrated the same fact. The two latter observers may be regarded as the founders of the modern

view that almost all cells, and nearly all kinds of protoplasm, show a delicate network of fine fibres, and they established this detail of structure as a general property of protoplasm. Thus protoplasm is not structureless, it is not a homogeneous mass having a few granules interspersed in its substance, but it has details of structure so fine as to require the highest microscopic powers, the finest optical definition, and the most refined micro-chemical operations to make them visible (Fig. 72).

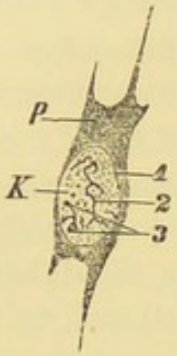


FIG. 72.—Connective tissue corpuscle or cell from the skin of *Triton tæniatus* × 560. *P*, protoplasm; *K*, nucleus; 1, membrane of nucleus; 2, intranuclear network; 3, small bodies in the nucleus. The coarser threadlets of the intranuclear network are easily seen; the finer threadlets appear with this power as mere points. (Method No. 1, Appendix.)

The next important generalization is as to the chemical nature of protoplasm. Loose expressions were much in vogue eight or ten years ago, regarding this aspect of the subject. Life was said to be identified with a little albuminous matter, containing phosphorus—protoplasm was merely a drop of living albumin—and the conception one was offered of albumin was, that it was a substance like a little white of egg. But micro-chemistry, and a study of the chemical reactions of living matter have effected a revolution in scientific opinion. It is evident that living protoplasm cannot be analyzed. The attempt at analysis by the application of any reagents, or by the use of physical agencies, such as heat or electricity, causes it to fall to pieces; only the fragments remain, whilst the peculiar properties which we associate with the living state have vanished. We can, however, gather up these fragments and determine their chemical nature. They are found to consist of water, of substances having the character of the albumins, of nitrogenous matters rich in phosphorus, of fats, of carbo-hydrates, such as glycogen (animal starch) and glucose, of bodies that behave like ferments, and of mineral matters, such as chlorides, sulphates, and phosphates of the alkalies, and of the alkaline earths. Thus protoplasmic stuff is seen to have a highly complex chemical constitution. (See p. 26.)

Let us return to the cell. During the last ten or twelve years, and especially during the last three or four years, important advances have been made, more especially as to the structure of the nucleus and the functions which it performs in the reproductive processes of the cell. It is at this stage that, if one wishes to appreciate the true position of

the cell doctrine at the present time, he must study the mode of the genesis of cells, starting with the changes in the ovum before and after fecundation. Before, however, entering on this difficult, though profoundly interesting, subject, let us turn our attention to the remarkable observations that have recently been made on the structure of the nucleus, beginning with the appearance of a work by Professor W. Flemming, of Kiel, in 1882. In this work Flemming gives a historical account of previous observations, and he applies a new nomenclature to the different parts of the nucleus that can be revealed by high powers. He says that three substances exist in a fully formed nucleus—first, a *reticular network* of fine fibres; second, *nucleoli*; and third, a *nuclear fluid* or intermediate substance, the whole being surrounded by a *membrane*—the membrane of the nucleus. The network he calls *karyomiton* (κάρυον, a kernel, μίτος, a thread), as opposed to the *kytomiton* (κύτος, a cell), or network in the body of the cell. The meshes of the network vary in size—they are sometimes uniform, sometimes irregular, and they often present nodosities or swellings at the points where the fibres interlace. The fibres forming the network are fixed by acids, chloride of gold and alcohol, so as to become more apparent. The substance forming the fibres has a strong affinity for colouring matter, and on account of this property Flemming has termed it *chromatin*; but it is important to note that this word has more of a morphological than of a chemical significance, and does not involve any theory as to its chemical character. Flemming has also corroborated an observation first made by Balbiani that the fibres of chromatin in the nuclei of the salivary cells of the larva of *Chironomus* (a *Tipula*, an insect resembling a gnat) show a transverse striation, indicating the existence of segments of different constitution (Fig. 73).

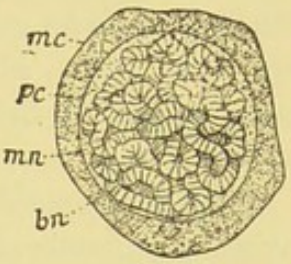


FIG. 73.—Cell from a young embryo of *Hydrophilus piceus*—*mc*, cellular membrane; *pc*, protoplasm: *mn*, membrane of nucleus: *bn*, nuclear thread or filament striated, immersed in a finely granular plasma.

In the meshes of the reticulum lie one or more *nucleoli*, small portions of nuclear substance of different constitution from that of the reticulum or of the nuclear fluid. They are not to be regarded, however, as variable fragments of nuclear matter, but as specific products, the result of those molecular actions of which the nucleus is the seat. Flemming holds that these minute structures have probably a determinate physiological purpose. Further, he states that there are differences between the nucleoli of the same nucleus, classifying the larger and more regular in appearance as principal nucleoli, and the others as accessory nucleoli. There are also differences between the nucleoli of

different cells, more especially as regards the existence in them of minute vacuoles, or round spaces. He has also noticed extremely slow changes of form in the nucleoli of certain living cells. In the changes attending the division of the nucleus in cell division, the nucleoli disappear, and after cell division they reappear. Flemming is of opinion that their disappearance arises from a distribution of their substance through the nucleus, and that their reappearance or genesis is a function of the reticular network. He has also observed that in general, but not always, the absolute volume of the nucleoli is proportional to the volume of the nucleus.

Flemming has observed in the great majority of nuclei a limiting *membrane*, or envelope, and he distinguishes in some cases an achromatic and a chromatic membrane. The first—the achromatic—so termed because it does not stain readily, is found in the germinal vesicle or nucleus of many ova. The chromatic membrane is sometimes perforated by small holes, and, according to Frommann, Klein, and others, an anastomosis of the intranuclear and intracellular networks may thus exist.[1]

Lastly, the *nuclear fluid* is probably a solution of saline matters, along with albuminous and other organic constituents.

As I have detailed these characters according to the descriptions of Flemming, it will have been evident that the nucleus is not so simple a structure as it has been hitherto supposed. These observations have been repeated by many of the most careful workers, by men such as Strasburger, Leydig, Pfitzner, E. van Beneden, Carnoy, Frommann, Klein, Brass, furnished with the best microscopic appliances, and thoroughly familiar with the interpretation of microscopical appearances. It is not surprising, however, that whilst all admit that a new world of real phenomena has been opened up, there are considerable differences of opinion, and a good deal of confusion, from the unfortunate tendency to the introduction by each new observer of his own nomenclature. Recently, Professor van Bambeke, of Gand, in Belgium, has attempted to introduce order into this chaos by a systematic study and classification of the observations and views of different authors.

Thus with reference to the nature of the fibrous appearance seen in nuclei, observers may be arranged in two classes: 1st, those who follow Flemming in regarding it as a reticulum or network, namely Pfitzner, Retzius, Leydig, Van Beneden, etc.; and 2nd, those who hold the reticulated appearance to be due to the presence in the nucleus of a coiled-

[1] It is unfortunate that the terms *chromatic* and *achromatic* have been introduced in the terminology of nuclear structures, as these words have a definite meaning in physical science, employed in describing optical phenomena.

up filament, namely, Strasburger, Balbiani, Carnoy, etc. Most of those in the first category agree in the general description of a reticulum, but they differ as to terminology, and as to certain minute details of structure which each observer believes he has demonstrated. Thus, E. van Beneden, in his researches on the fecundation of an intestinal worm found in the horse, *Ascaris megalocephala*, describes the nucleus as consisting of a membrane, within which there is a network of chromatin, containing in its meshes a fluid substance. The whole of this mass forming the membrane and reticulum he calls *nucleoplasm*, and he describes it as consisting of two portions: 1st, an *achromatic substance* arranged in fine moniliform filaments; and, 2nd, *a chromatic substance* which is imbibed by the membrane and by the reticulum, and which also fills the meshes. Further, he supposes that each fibre or filament consists of numerous minute bodies, *nucleomicrosomata* (readily stained), between which the chromatic substance exists, and the more elongated fibrils he terms *nuclear-threads*.

Strasburger also regards a nucleus as consisting of *nucleoplasm* (or *karyoplasma*), in which, as in kytoplasma, there are two matters: the *nucleomicrosomata*, taking up pigment, and the *nucleohyaloplasma*, which is not stained. The nucleoplasma is an elongated filament, of variable thickness, crossing in loops in all directions, and thus giving rise to the reticulated appearance.

Arnold Brass, of Marburg, takes a somewhat different view of the constitution of the chromatin filament. He says it is the seat of metamorphoses by which it may vary much in consistence, so that sometimes it may be squeezed out of nuclei into long filaments. Further, he holds that the chromatin filament is not one substance, but a mixture of various substances, as he does not find the reagents he employs act upon it uniformly. If the substance or substances be in a state of solution, then there may be no appearance of structure in the nucleus, and it may be called a *homogeneous nucleus* (a condition rarely met with); or they may appear in the form of granules or filaments which may either anastomose or form one long coiled-up filament.

Carnoy of Louvain describes a nucleus as a kind of little cell containing a cord or coiled filament of *nuclein*. Each nucleus has then (1) a protoplasmic body, and (2) a portion formed of nuclein, which is the chromatin of Flemming. He also terms the protoplasmic portion *karyoplasma*, a hyaline mass, homogeneous in appearance, and having granules interspersed. Carnoy sums up his conclusions as to the second constituent, the nuclein (or chromatin), in the following propositions—

1. In typical nuclei the element nuclein presents a filamentous form; it is composed of a continuous or coiled filament.

2. The chromatic reticulum of Flemming is only an appearance due to the regular crossing of its convolutions, rarely to their temporary union (Fig. 74).

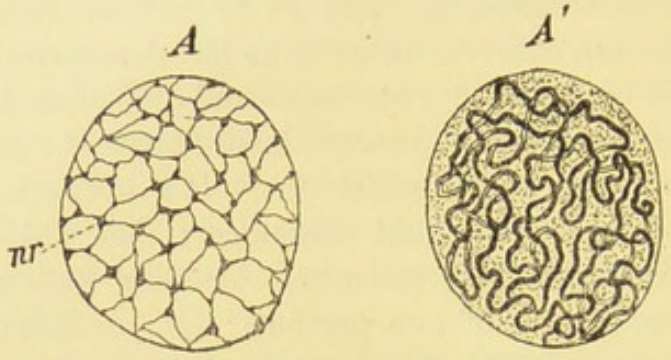


FIG. 74.—Two nuclei of the epidermis of *Salamandra maculata*. *A*, nucleus at rest: the nuclear filament forms a network by the convolutions being fused so as to present thickenings, *nr*. *A*, nucleus before division, or karyokinetic process. The filament seen in *A'* is now swollen, and its convolutions are separated so that one long coiled filament is seen.

3. The nuclear filament is subject to profound changes. Thus it may be broken into fragments, or even into spherules, or it may dissolve and totally disappear.

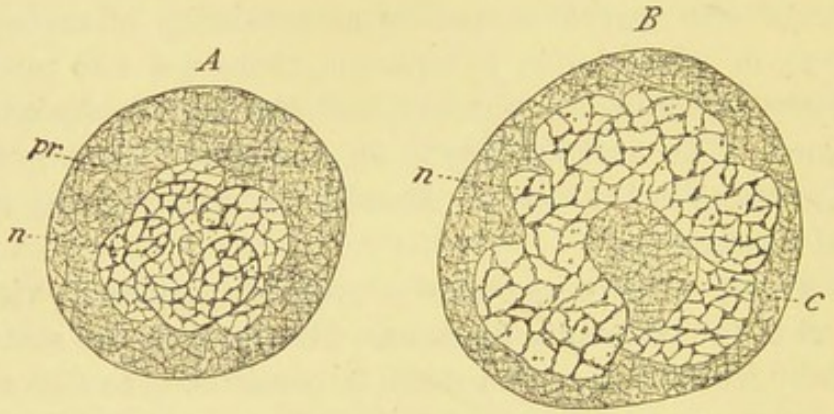


FIG. 75.—Giant cells of the marrow of a rabbit. *pr*, delicately reticulated protoplasma, with a rich enchylema or juice; *n*, irregularly formed nucleus containing a filament of nuclein, coiled irregularly and in a complex manner, so as to form a network by the points at which the filaments cross being fused together.

4. The nuclear filament is structural. One may distinguish a wall, a tube, and contents. Even the contents may be organized, appearing in the form of discs, etc.

5. Usually the nuclear filament does not occupy a determinate position, but it may be localized towards the centre of the nucleus forming a nucleolus.

The karyoplasma, or protoplasmic portion, of Carnoy, like the kytoplasma (cellular plasma), is thus formed of a *reticulum* and an *enchylema* (χυλός, juice or moisture of plants, afterwards applied to animal fluids, such as chyle), a substance in the meshes, or the intermediary substance, or nuclear fluid of other authors (Figs. 75 and 76).

One other research it is important to notice as it relates to the chemical nature of nuclear matter. In 1871, Miescher found in the nuclei of cells a substance to which he gave the name *nuclein*. (See p. 78.) In 1882 and 1883, Zacharias repeated Miescher's observations

on nuclein, only using the methods of micro-, instead of macro-chemistry, and he arrived at the important conclusion that two chemical matters

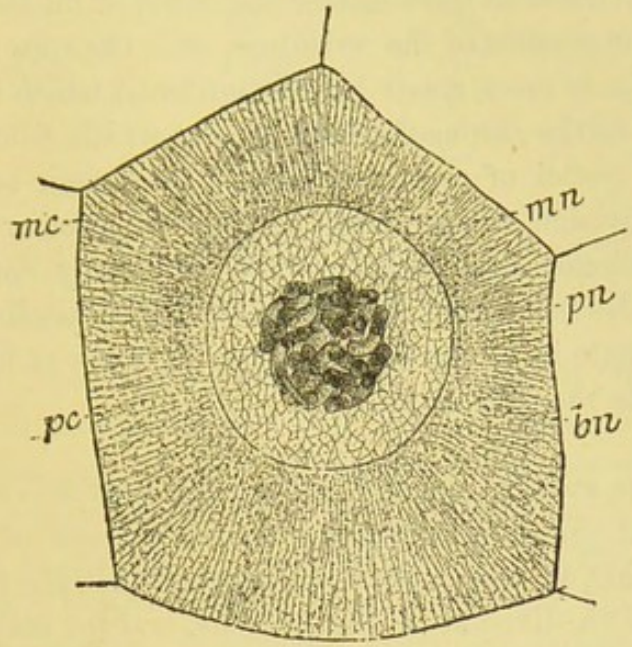


FIG. 76.—Cell and typical nucleus of the intestinal epithelium of a maggot. *mc*, cellular membrane; *pc*, cellular protoplasm; observe the radiating reticulum with the enchylema or juice enclosed in the meshes; *mn*, membrane of the nucleus; *pn*, plasma of the nucleus; here observe also a reticulum and plasma, distinct from those of the protoplasm; *bn*, continuous nuclear thread or filament, contracted in the centre of the nucleus and showing numerous loops.

exist—(1) *nuclein*, which gives the same reactions as the soluble nuclein of Miescher; and (2) *plastin*, a matter which gives different reactions. Further, he states that nuclei contain a *fundamental substance* nearly related to protoplasm, which is chiefly, if not wholly, plastin, whilst chromatin is the same substance as nuclein. He supposes also that the matters termed chromatin by Flemming are probably mixtures of plastin and nuclein.

If we attempt to make a critical estimate of the result of these numerous investigations, we may reach the following conception of a nucleus: (1) It is a small round or oval body, having an envelope or membrane, and containing a reticulum or net-work, in the meshes of which there is a semi-fluid matter; (2) in some cases, possibly in all, at a definite period in the life of the nucleus, instead of a reticulum there may be a coiled filament or a series of filaments, not anastomosing, floating in a nuclear fluid; (3) to the matter forming the reticulum, or forming the filament or filaments, the name chromatin may be given, inasmuch as this readily takes up colouring matters; but as staining fluids do not stain the whole of the chromatin filament uniformly, it is highly probable that another substance exists in the chromatin filament,

and in the membrane of the nucleus, which may be provisionally termed achromatin, and which possibly forms the organic basis of the whole structure; (4) to the semi-fluid matter the name of nuclear fluid may be given; (5) in the meshes of the reticulum, or in the coils of the filament, there may be one or more small bodies (nucleoli) which appear to be of the same nature as the chromatin filament, but which, from their uniform presence in the nuclei of certain cells, and from their behaviour in the process of cell division, are probably portions of nuclear matter having a special physiological significance, at present only conjectural; and (6) there is no absolute proof that there is any connection between the chromatin filaments in the nucleus and the net-work of fibres in the cell substance outside the nuclear wall.

Such being the structure of the nucleus, the next and most important question is, what part does it perform in the process of cell division? It was in 1841 that Martin Barry first observed that the division of cells was associated with division of the nucleus, and for many years it was supposed that this was simply a fission of the nucleus, followed by a fission of the whole cell, so that the daughter nucleus was contained in the new cell. It was also observed that in many tissues nuclei might apparently divide without fission or division of the matter in which they existed, so that certain cells or tissues contained several nuclei. The simple division of the nucleus, without any changes occurring in that structure, and followed by division of the protoplasm of the cell, is called akinetic, or direct division (κἱνέω, to move, to set agoing). A better term, I think, is that suggested by Carnoy, *karyostenosis* (κάρυον, a kernel; στένωσις, a being straitened; στένω, to make narrow). This mode of division has been observed by Ranvier and Lavdowski in the formation of leucocytes, epithelial cells, certain fat cells, and it appears to be the mode of division of the parenchymatous cells of plants, and of some algæ and infusoria (Fig. 77).

The other mode of division, sometimes called indirect division, or *karyokinesis*, a term first used by Schleicher, was first described by Professor Bütschli, of Heidelberg, in 1876, as occurring in the cells of the testis of *Blatta germanica*, in two blastodermic cells of *Musca vomitoria*, and finally in the parthenogenetic egg of *Aphis*. Since then, this mode of division has been found by others in many organisms, both plant and animal. It appears to be common in the vertebrates, and may be readily demonstrated in the epidermal cells of amphibians. The process may be described as follows (Fig. 78), using the terms suggested by Flemming—The chromatin of the nucleus is increased, and is developed into a complexly coiled thread, called the *spirem* (σπείρημα, a wreath, a

coil or spire), while the nucleoli and the membrane of the nucleus disappear. The thread is next divided into portions which assume the

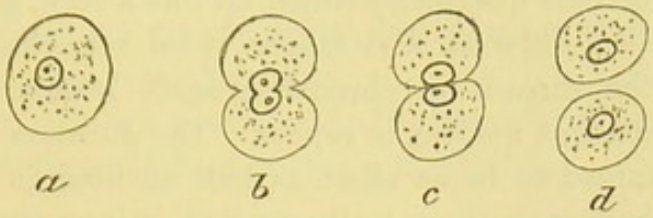


FIG. 77.—Direct cell division, or karyostenosis. *a*, fully formed cell; *b*, beginning of division of nucleus and also of cell substance; *c*, division of nucleus and of cell substance complete; *d*, formation of two new cells.

form of *loops*. These are at first placed irregularly, but they are soon arranged in such a way that the tops of the loops are turned towards the clear middle part of the nucleus, while the free ends of the loops are directed towards the circumference. This form is termed the *aster* (ἀστήρ, a star), or *garland*. The loops are next divided in the direction of their length, whereby their number is doubled, but the loops themselves become thinner. Then the loops are collected towards the middle or equator of the nucleus, so as to form a flat body around it, called the *equatorial plate*. Shortly afterwards the loops separate into two groups, each group retreating towards the pole of the nucleus, giving rise to the *barrel-form* or *pithode* (πιθώδης, like a cask). The

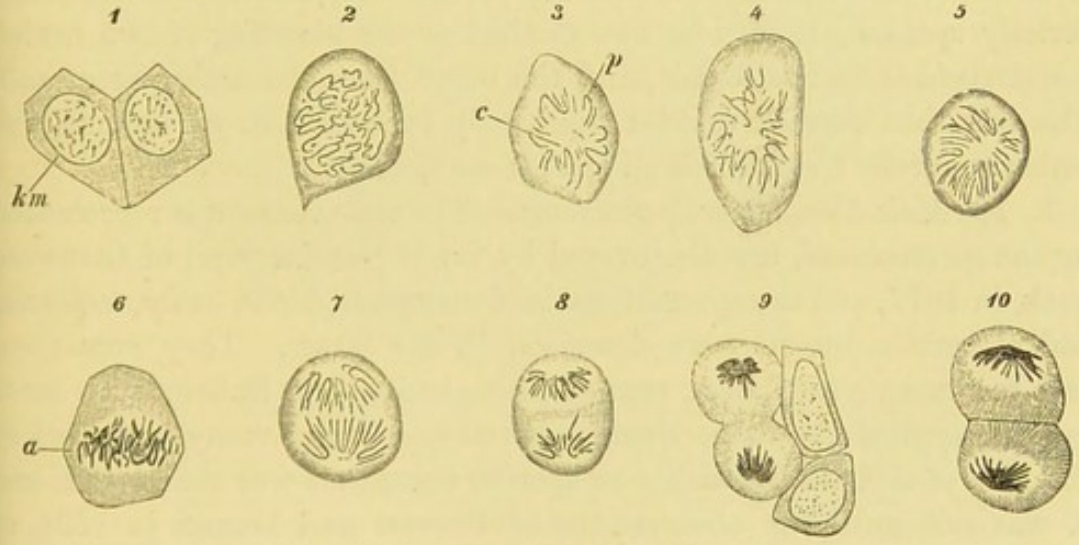


FIG. 78.—Views of the various stages of karyokinesis, or division of the nucleus from a section of the epidermis of *Triton tæniatus*, ×560 d. 1. Two epithelial cells, in which are seen the nucleus, the membrane of the nucleus *km*, and the dark-coloured net-work. 2. Disappearance of the nuclear membrane—*Spirem*. 3. Nuclear threads in groups and having the loops dividing at the centre *c*, or at the periphery *p*. 4. *Monaster*, in which the loops are directed to the centre. 5. The loops are thin and very numerous, in consequence of division. 6. *Equatorial Plate*. 7. *Barrel-form*. 8. *Dyaster*. 9. Contraction of the protoplasm of the cell; both the neighbouring cells show nuclei, the net-work in which is not distinguishable. 10. Complete division of the cell, the nuclei in which have not yet returned to the original resting condition seen in 1. (Method No. 2, Appendix.)

groups of loops now separate still further, and having the closed ends of the loops towards the poles, give rise to a double star, or *dyaster*.

Then there begins at the equator of the cell a division of the protoplasm, which leads to its complete separation into two equal parts. The groups of loops again become irregularly coiled up like a clew, giving rise to an appearance, called *dispirem*, and finally these remarkable movements subside, and the threads of chromatin again assume the form and arrangement seen in a nucleus in repose. The duration of this process has been ascertained to be as short as half an hour in man, whilst in amphibians, where it is most easily studied, it may occupy five hours. According to Carnoy, several of these changes may be omitted, and he states that there are many gradations between the karyostenotic and the karyokinetic modes of nuclear division.

Chap. II.—THE ORIGIN OF THE TISSUES.

Having thus endeavoured to give a concise account of the modern views held as to the nature of protoplasm, cells, and nuclei, we now proceed to a description of the changes that have been observed during the process of fecundation by which the primitive cells of the body are formed, because, as previously remarked, the phenomena of fecundation and those of cell division ought to be studied together if one desires a comprehensive view of the subject. It is of course well known that fecundation consists in the blending of the male and female elements. Strictly speaking it may be now defined as the blending of two nuclei, one derived from the female, and the other from the male parent cell. The cell thus formed divides repeatedly by fission so as to form the embryonic cells from which all tissues are derived.

1. *The Male Elements or Spermatozoa.*—The male element is represented by the *spermatozoid*, first discovered by Louis Ham, a pupil of Leeuwenhoek, in 1677, and the spermatozoids of mammals, birds, fishes, molluscs, and of certain insects were described by the latter. They were then, and for many years after, termed animalcules, but Buffon, with more insight, applied the name living molecules. It was many years before the idea of their individuality as specific organisms was abandoned, and it was not until the observations of Prevost and Dumas in 1824, of Von Kölliker in 1841, and of Newport in the year 1850, that their true position as constituent elements of the body was recognized. Their development was first traced to some extent by Von Siebold and Leuckart in 1836; in 1846, Von Kölliker asserted that they were essentially nuclei developed in certain cells of the testes. At first, Von Kölliker held that they were developed in the interior of nuclei, but as Henle, in 1854, showed that not only the nucleus but part of the protoplasm of the cell (forming the tail) took part in their evolution,

Von Kölliker, in 1851, modified his opinion to the extent of stating that the whole nucleus entered into the formation of the spermatozoid. In 1865, Schweigger-Seidel went a step further, and asserted that the spermatozoids were not merely nuclei, but were, on the contrary, entire cells, each carrying a vibratile cilium.

The spermatozoids are developed in the two glands termed the *testes*, the general structure of which is shown in Fig. 79.

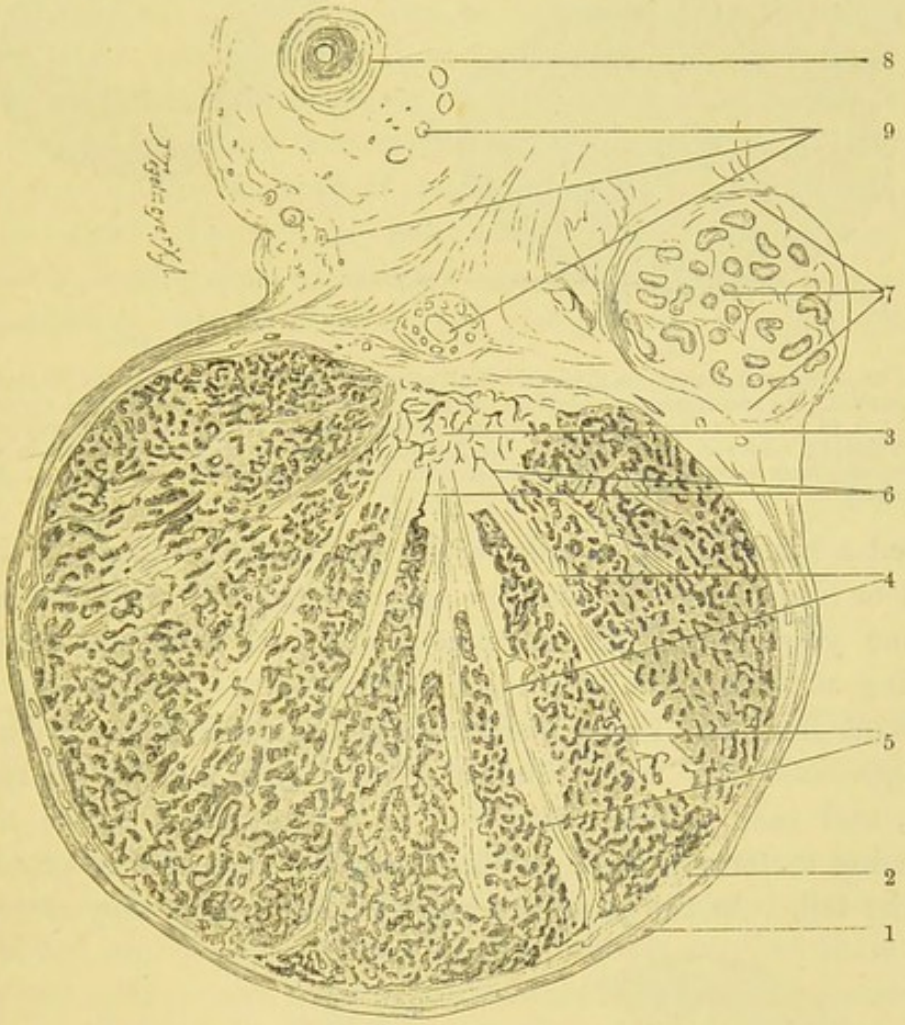


FIG. 79.—Transverse section of the testis of a newly-born boy, 10 times magnified—1, tunica albuginea ; 2, tunica vasculosa ; 3, corpus Highmorianum or mediastinum testis, containing the rete testis ; 4, septa of the testis ; 5, lobules, consisting of convoluted seminiferous tubules ; 6, straight tubes or vasa recta, passing into the rete testis ; 7, globus major of epididymis ; 8, vas deferens, or excretory duct of testis ; 9, blood-vessels. (Method No. 3, Appendix.)

The essential structures in the testis are the *tubuli seminiferi*, long convoluted tubes lined with epithelial cells in which the spermatozoids are developed. Sections of these tubules are seen in Fig. 80.

It has been found difficult to trace the origin of the spermatozoids in the cells in the tubes of the testicle, and the question cannot even now be regarded as settled. Von Kölliker, in 1856, described two kinds of cells, an outer layer with large nuclei and nucleoli, and an inner layer of

smaller cells, which were the true sperm cells. The sperm cells became cysts in which the spermatozoids were developed. In 1871, Merkel

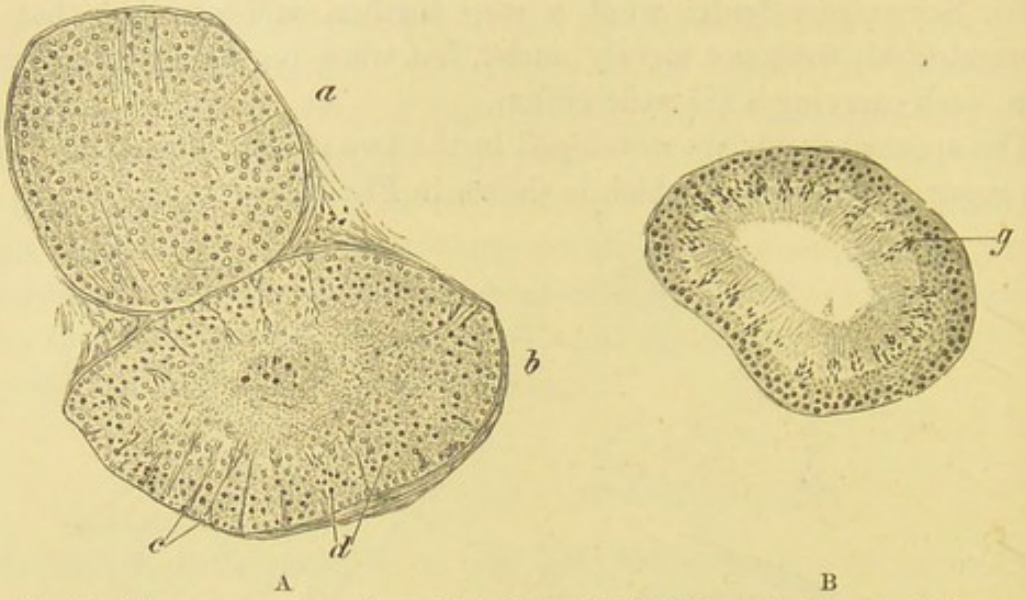


FIG. 80.—Transverse sections through tubuli seminiferi of the testis of an ox, 80 times magnified. A, two transverse sections of the smaller tubules—*a*, tubule in a state of rest; *b*, in a state of activity; *c*, spermatoblasts; *d*, nuclei of indifferent seminal cells deeply coloured. B, transverse section of a tubule in an active condition—*g*, group of young spermatozoids, connected with the spermatoblast. (Method 4, Appendix.)

described a ramified set of cells in the tubules which formed a kind of support or framework on which the spermatozoids were formed, and in the same year, von Ebner developed this idea and figured peculiar branching structures on which the spermatozoids originated. These outgrowths from the living membrane of the seminiferous tubules he called *spermatoblasts*. Each spermatoblast has a number of rounded lobules, and each lobule gives rise to a spermatozoon, the nucleus lengthening to form part of the head, while a thin film of protoplasm forms the tail. As the young spermatozoids develop, they are at first

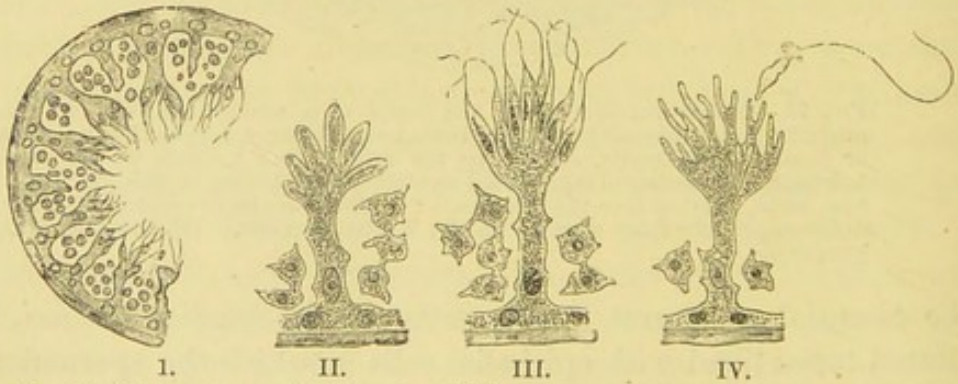


FIG. 81.—Spermatogenesis (after Landois). I. cross section through a seminal tubule; II. unripe spermatoblast, with blunt rounded laplets, the young sperms; III. spermatoblast with ripe ciliated heads; IV. spermatoblast after separation of spermatozoids.

closely connected with the spermatoblast, but when set free they roll off into the tubule (Fig. 81). In 1875, Sertoli described the development

of spermatozoids in the Elasmobranchs (sharks, rays, etc.). He traced the invagination of the primitive germinal epithelium into the underlying stroma, so as to form a follicle, and in certain of the cells of this follicle—"mother cells"—the nucleus divided so as to form a number of minute bodies, each of which became a spermatozoid. Von la Valette St. George examined spermatogenesis in the vertebrates and published his results in 1878. According to him, two sets of cells exist in the tubules; one set, like young ova, he calls *spermatogonia;* these divide into numerous smaller cells, *spermatocytes*, each of which may form a spermatozoid, as in mammals, or they may take part in the formation of supporting structures or envelopes for the spermatozoids, as in fishes and amphibia; the other set of cells does not take any part in spermatogenesis. Lastly, Renson, in 1882, describes the larger cells of Von la Valette as forming cysts called *spermatogemmæ*, each containing a number of *nematoblasts*, which become the young spermatozoids (Fig. 82).

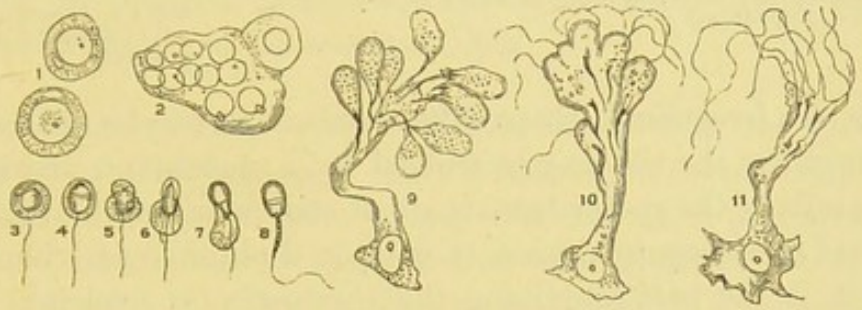


FIG. 82.—Spermatogenesis (after Renson). 1, follicular cells; 2, spermatogemmæ; 3, 4, 5, 6, 7, 8, separate nematoblasts, developing into spermatozoa; 9, nematoblasts grouped on supporting cell; 10 and 11, successive stages of the penetration of the young spermatozoa.

These nematoblasts, when liberated from the cyst, cluster round certain elongated epithelial or supporting cells, similar to the spermatoblasts of von Ebner, and become buried in the protoplasm until their full development. They may even penetrate to the bottom of the spermatoblast (supporting cell), but this structure then grows outwards and carries the spermatozoids to the centre of the seminiferous tubule, where they are finally discharged.

Many other views have been offered, but those which I have detailed may be regarded as typical expressions of opinion on this difficult question. It is evident that the spermatozoon is a highly differentiated structure, requiring specific arrangements for its maturation and development (Fig. 83).

Spermatozoids vary much in form. The familiar type is that showing an oval head and a long filament or tail (Fig. 84). It is remarkable that this comparatively simple form is found both in the higher and lower groups. Thus it is met with in the sponge, in medusæ, in tape-

worms, and in mammals generally, including man. On the other hand, the spermatozoa of various intestinal worms (*ascaris*), and of

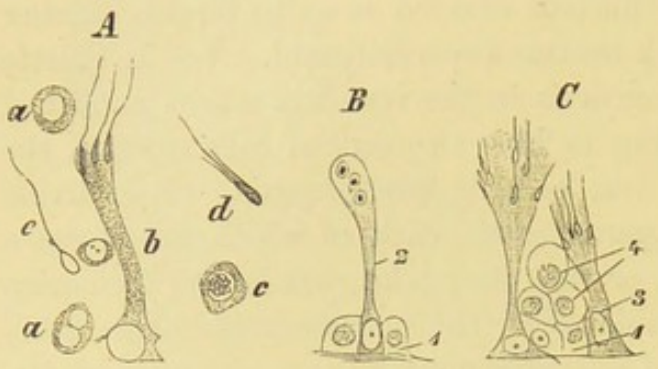


FIG. 83.—*A*, Isolated elements of the testis of an ox. *a*, seminal germ cells; *b*, spermatoblast; *c*, indifferent seminal cell; *d*, torn off (?) process of a spermatoblast; *e*, almost finished thread with a remainder of protoplasm near the middle. *B* and *C*, from a section of testis of ox. 1, Seminal germ cell; 2, spermatogemmæ; 3, spermatoblast; 4, indifferent seminal cells; × 240. (Method No. 5, Appendix.)

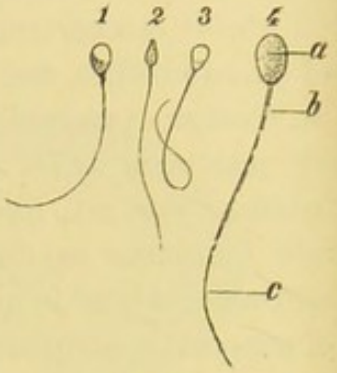


FIG. 84.—Spermatozoids of man, 560 times magnified. 1, seen from surface; 2, from the edge; 3, with fine filamentous tail; 4, spermatozoid from semen of ox. *a*, head; *b*, middle portion; *c*, tapering end of tail. (Method No. 6, Appendix.)

arachnids, are irregular in shape, like amœboid corpuscles; in the newts and salamanders the tail may be fringed by a membrane, and in many birds the tails of the spermatozoids are of enormous length (Fig. 85.)

The *head* of the spermatozoon is of special physiological importance because it is the part containing the chromatin (or nuclein), derived from the nucleus, consequently it is readily stained with colouring matters. The *tail* is formed of protoplasm. Some have described certain small nuclei in the spermatoblasts that do not enter into the formation of the spermatozoids, and in accordance with the theory to be afterwards mentioned, that all cells may be regarded as hermaphrodite, that is to say, as containing both male and female elements these have been termed *female nuclei*. This is a very doubtful interpretation.

In man the head of the spermatozoon is from 3 to 5 μ long, and 2 to 5 μ broad; the middle portion, that is to say, immediately behind the head, is about 6 μ in length, and 1 μ broad, and the tail is from 40 to 50 μ long, and runs out to a fine point (Fig. 84).

Spermatozoa have remarkable powers of resistance. The sinuous movements belong to the tail, which pushes the head before it. While the spermatozoids are in the testicle these movements scarcely exist, and it is only when they reach the fluid secreted by the epididymis, by the *vesiculæ seminales*, by the prostate gland and by Cowper's glands, that the movements become active, probably owing to a stimulating action of these fluids. The movements may go on for from 24 to 48 hours

after removal of the spermatozoids from the body, under favourable circumstances. Water arrests the movements, but they may recom-

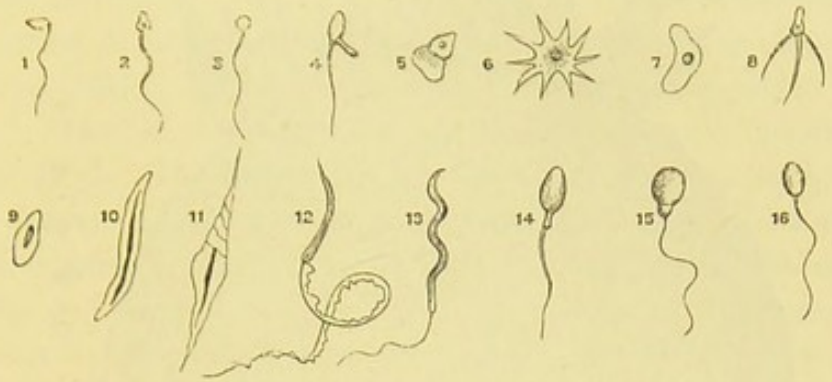


FIG. 85.—Forms of spermatozoa—1, sponge; 2, medusa; 3, *bothriocephalus* (tape worm); 4, *cleta* (chætopod); 5, *ascaris* (thread worm); 6, *moina* (daphnid); 7, crab; 8, lobster; 9, 10, 11, *plagiostoma* forms with elongated nucleus; 12, salamander; 13, ray; 14, man; 15, *cobitis* (fish); 16, mole.

mence on the addition of very weak alkaline fluids. Even extremely weak acids at once kill the spermatozoids.

2. *The Female Elements or Ova.*—We must now turn our attention to the nature of the *ovum* or egg. This is found in a special organ termed the ovary, an organ for many years, from the time of Galen to the middle of the 17th century, described as *testis muliebris*. De Graaf, in 1672, was the first to describe the ovary carefully. He described the vesicles that bear his name, the *Graafian vesicles*, which, however, he regarded as ova, and he stated that ova were structures to be found throughout the whole range of the animal kingdom. He also observed the ovum itself in the rabbit. Little progress, however, was made till 1825, when Purkinje discovered the *germinal vesicle* in the ovum of the common fowl. Then in 1827, Von Baer traced the changes in the ovum from the uterus back to the Graafian vesicle. Soon afterwards, Coste showed that Purkinje's vesicle occurred in the mammalian ovum, and in 1836, Wagner discovered the *germinal spot*, sometimes, therefore, called Wagner's spot. From this date until the last few years little progress was made in the study of the minute structure of the ovum, and it was described as a typical cell, having a cell wall, the *zona pellucida*, or *vitelline membrane*, surrounding the contents, or *yolk*, in which lay embedded the *germinal vesicle*, or nucleus, which had a nucleolus—the *germinal spot*. The ovum has shared, however, in the recent progress made in the study of the structure of the cell and the structure of nuclei, and now we know that the nucleus shows the threads of chromatin, or a reticulum with wide and irregularly formed meshes (Fig. 86).

The *ovaries* consist of connective tissue and of a glandular substance.

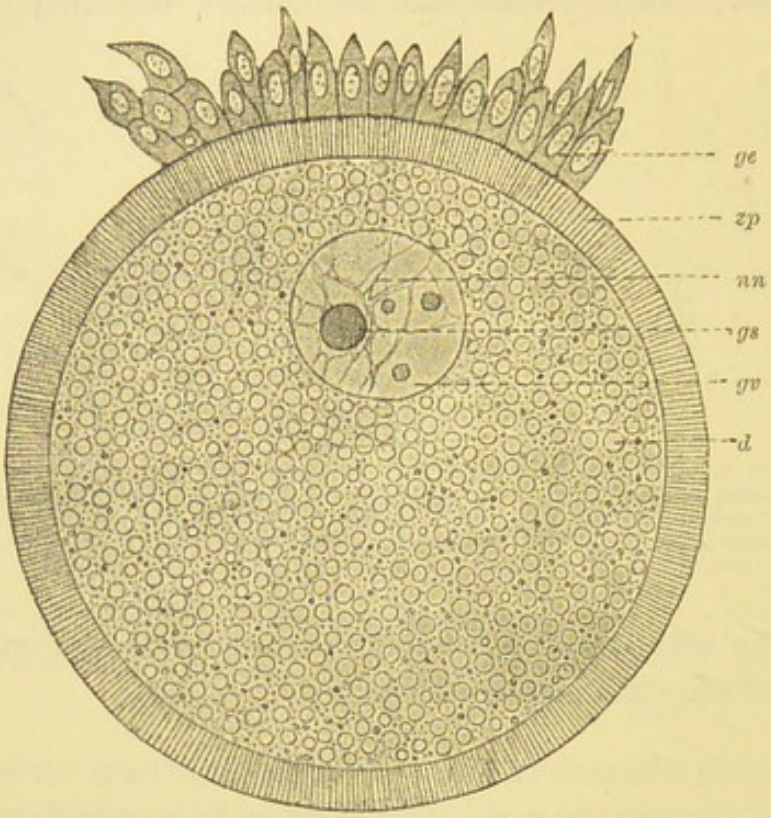


FIG 86.—Ovum of rabbit from an egg follicle 2 mm. in diameter. *zp*, zona pellucida, having at the top, cells of the germinal epithelium *ge*. The yolk shows corpuscles of deutoplasm *d*. In the germinal vesicle *gv* is the nuclear network *nn*, with a large nucleolus the germinal spot, *gs*.

The connective tissue, forming the *stroma* of the ovary, is arranged in various strata, and contains numerous vessels (Fig. 87).

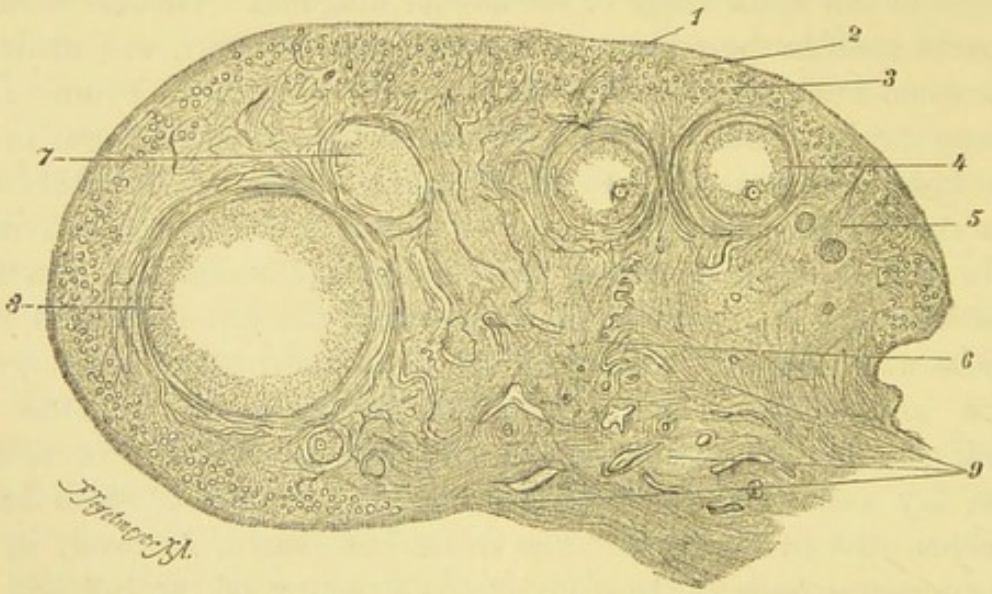


FIG. 87.—Transverse section of the ovary of a girl 8 years old, 10 times magnified. 1, germ epithelium ; 2, tunica albuginea, still weakly developed ; 3, outermost zone of the cortical substance, containing numerous small follicles ; 4, larger follicle ; 5, inner portion of the cortical substance ; 6, central substance, or stroma, with numerous arteries ; 7, follicle, with the outer wall cut off ; 8, larger follicle, the discus proligerus of which has not been exposed in the section ; 9, hilum of the ovary, containing wide veins. (Method No. 7, Appendix.)

In the outer part of the stroma is the so-called glandular substance, which contains numerous small follicles, about 36,000 in the human being, each having in it a small ovum or egg. It is remarkable that at an early period of intra-uterine life vast numbers of ova are found in the ovary, even during its development. Many of these never come to maturity. Most of these follicles are extremely small, measuring not more than 40 μ. The larger follicles in which the ova reach maturity lie much deeper. The upper surface of the ovary is covered with *germ epithelium*, consisting of small, short, cylindrical cells. In the embryonic period, the ova pass through only the first stage of development, and their further development takes place in the Graafian vesicles. In the fœtal period, and even after birth, we find between the cylindrical cells of the germ epithelium large roundish cells, provided with a nucleus and nucleolus. These are the *primordial ova*, which have originated from single cells of the germ epithelium (Figs. 88, 90). It is important to note

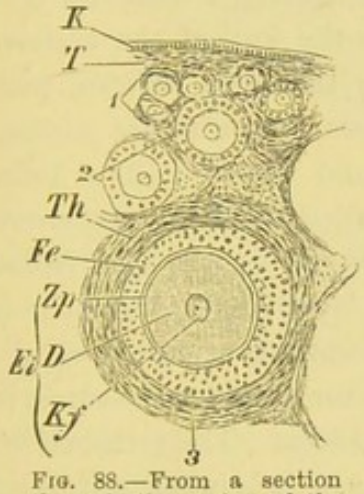


FIG. 88.—From a section through the cortex of the ovary of a rabbit, 90 times magnified. 1, primary follicle; 2, follicle lined with a layer of cylindrical epithelial cells; 3, follicle with stratified epithelium. *K*, germ epithelium; *T*, tunica albuginea (slightly developed); *Th*, theca folliculi; *Fe*, follicle epithelium; *Zp*, zona pellucida. *D*, yolk; *Kf*, germinal vesicle with punctiform germ spots. (Method No. 8, Appendix.)

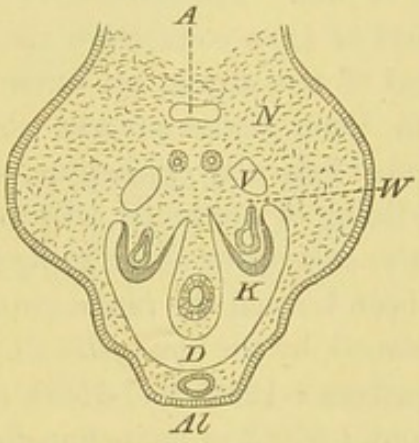


FIG. 89.—Diagrammatic section through the posterior end of a young warm-blooded embryo, the spinal cord not shown. *A*, Aorta; *V*, veins; *Al*, canal of allantois; *D*, intestinal canal. Round this is the peritoneal cavity, and at *K* the germinal epithelium. *W*, Canal of the primitive kidney.

in passing that this germ epithelium is the same as gives rise in the male to the cells that have to do with the production of spermatozoids (Fig. 89). In the course of development, groups of cylindrical cells grow up from the stroma of the ovary so as to enclose the primordial ova. These may even form tubular-like structures, called by some German writers the *ova tubes*. By-and-bye, each ovum is surrounded by cells,

forming a little round body called the primary follicle, which consists of the ovum and of epithelial cells surrounding it. The formation of this follicle around each ovum is for the purpose, in due time, of ejecting the ovum from the ovary. The size of the follicle increases by the multiplication of epithelium cells, and, by-and-bye, a space exists round the ovum which is filled with a fluid, the *liquor folliculi*. This liquid may be formed by transudation from the surrounding blood-vessels, and, as some have suggested, by the disintegration and melting away of some of the epithelial cells. We have now a vesicle filled with fluid, the Graafian vesicle, having a diameter of from ·5 to 5 mm. The connective tissue forms the wall of the vesicle. It consists of (1) a connective tissue covering the *theca folliculi*, which is formed of two strata, an outer (*a*) of fibrous tissue, *tunica fibrosa*, and (*b*) an inner *tunica propria*, rich in cells and vessels; (2) a lining of stratified follicular epithelium, sometimes called the *membrana granulosa*. This lining of epithelial cells forms a prominence at one side, called the *cumulus ovigerus* or *discus proligerus*, and the layer surrounding the ovum has been termed the *tunica granulosa*. The space is occupied, as already mentioned, by the *liquor folliculi*. A mature human ovum measures in diameter from 1-125th to 1-150th of an inch = 180 μ. The germinal vesicle is about 1-500th of an inch in diameter = 50 μ. It is important to note in this connection that the head of the spermatozoid is the 1-6250th of an inch in length, and about 1-8000th of an inch broad, so that the size of the male nuclear element is only about 1-12th part of that of the female nuclear element (Fig. 91).

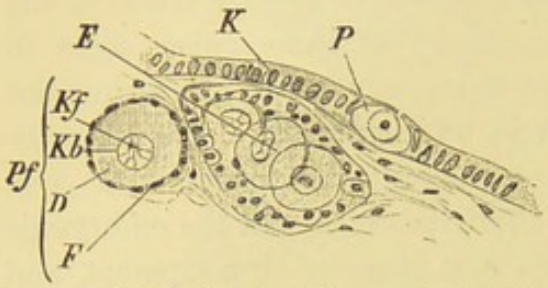


FIG. 90.—Vertical section of ovary of a girl four weeks old, 240 times magnified. *K*, germ epithelium; *P*, primordial ovum, with large nucleus and nucleolus; *E*, group of three ova surrounded by cylindrical cells; *Pf*, primary follicle; *F*, epithelium of follicle; *D*, yolk; *Kb*, germinal vesicle; *Kf*, germinal spot. (Method No. 9, Appendix.)

When the ovum reaches maturity, the Graafian vesicle is full of fluid, and, as already mentioned, bulges out from the surface of the ovary. The vessels in the neighbourhood of the vesicle become much congested, and at a given time the vesicle bursts and extrudes its contents. This is assisted by a rupture of some of the finer vessels, causing a hæmorrhage into the cavity of the vesicle. Coincident with these changes, by a reflex mechanism, the exciting cause of which is not known, the end of the Fallopian tube is applied to the ovary, and the ovum escapes into it and passes to the uterus. During its passage down the Fallopian tube it meets with the spermatozoids, and then fecundation occurs.

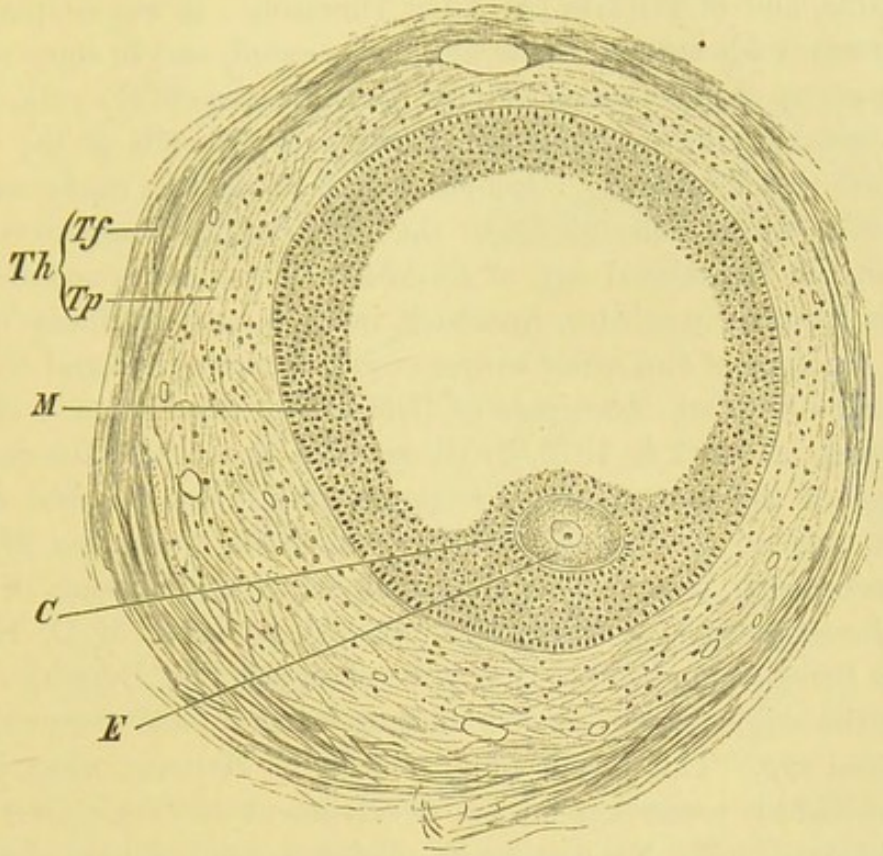


FIG. 91.—Section of the Graafian follicle of a girl eight years old, 90 times magnified. *Th*, theca folliculi ; *Tf*, tunica fibrosa ; *Tp*, tunica propria ; *M*, membrana granulosa—follicular epithelium ; *C*, discus or cumulus ovigerus ; *E*, ovum, with zona pellucida, germinal vesicle, and germinal spot. The clear space in the middle contains the liquor folliculi. (Method No. 10, Appendix.)

3. *Influence of Spermatozoid on Ovum.*—The question now arises, what is the influence of the spermatozoid upon the ovum? It is interesting for a moment to notice the historical development of knowledge upon this question. The first step in this direction was made by Kölreuter in 1761, when he succeeded in artificially producing hybrids by the cross fertilization of plants. Soon after this Jacobi artificially fertilized the eggs of the trout and salmon, and in 1780, Spallanzani operated in the same way on the frog, the tortoise, and the dog. Curiously, however, he thought that the virtue lay in the fluid and not in the living particles. In 1824, Prevost and Dumas proved that the seminal fluid lost its effect after filtration Martin Barry observed spermatozoids in the ovum of the rabbit in 1843 ; Leuckart made the same observation in the frog in 1849 ; Nelson, in 1852, observed the spermatozoids in the egg of *Ascaris mystax ;* and in 1853, Keber observed the actual entrance of the spermatozoon into the egg of the common mussel. The subject also attracted the attention of Bischoff, whose researches into the development of the dog were of

great value, and of the late Dr. Allen Thomson. It was supposed that the spermatozoids entered the ovum, disappeared, and in some mysterious way set up changes which resulted in the cleavage of the yolk, or more strictly speaking, in the formation of the first two cells of the embyro —the first two *blastomeres*. Opinion remained in this vague condition until 1872, when Bütschli made the remarkable observation of *two* nuclei in the fecundated egg of *Rhabditis dolichura*, a nematode worm. As often happens in science, Auerbach, in 1874, made a similar observation on the eggs of two other worms—*Ascaris nigrovenosa* and *Strongylus auricularis*—without knowing of Bütschli's description. Bütschli followed up his work in 1875, by discovering the same phenomenon in the eggs of the gastropods—*Lymnæa stagnalis* and *Succinea Pfeifferi*. He also noticed in the eggs of these molluscs a peculiar fibrillated appearance, such as occurs in connection with the division of nuclei. These investigations were quickly followed by those of O. Hertwig, Ed. van Beneden, and Fol. It is to be observed that Bütschli did not explain the origin of the second nucleus which he had observed in the fecundated egg. This was accomplished by O. Hertwig, when, in 1875, he published his researches on the development of *Toxopneustes lividus*, an echinoderm. He has the merit of demonstrating that the second nucleus is the head of the spermatozoon. Further, O. Hertwig at first supposed that the other nucleus was the germinal spot of Wagner set free by the destruction of the germinal vesicle. This error was pointed out by Van Beneden, and O. Hertwig accepted the correction, with the result that the other nucleus was soon shown to be, as Bütschli had previously conjectured, the germinal vesicle. The distinguished Belgian naturalist, E. van Beneden, early recognized the importance of these observations. In 1875, he described the fusion of the nuclei, and expressed the opinion that in the fusion of the two nuclei there was a phenomenon comparable to the conjugation of the Protozoa and Protophyta; and in his magnificent monograph on the fecundation of *Ascaris megalocephala* (1883) he has largely advanced our knowledge of the subject. Although the entrance of the spermatozoon had been previously observed, no specific changes were immediately noticed. This was reserved for Fol, a Swiss naturalist, who, in 1877, observed the entrance of the spermatozoid into the eggs of *Asterias glacialis* (a starfish), and of various species of *Echinus* (sea-urchins); and he studied and figured the phenomena that ensued. These observations have been confirmed by many naturalists (Fig. 92).

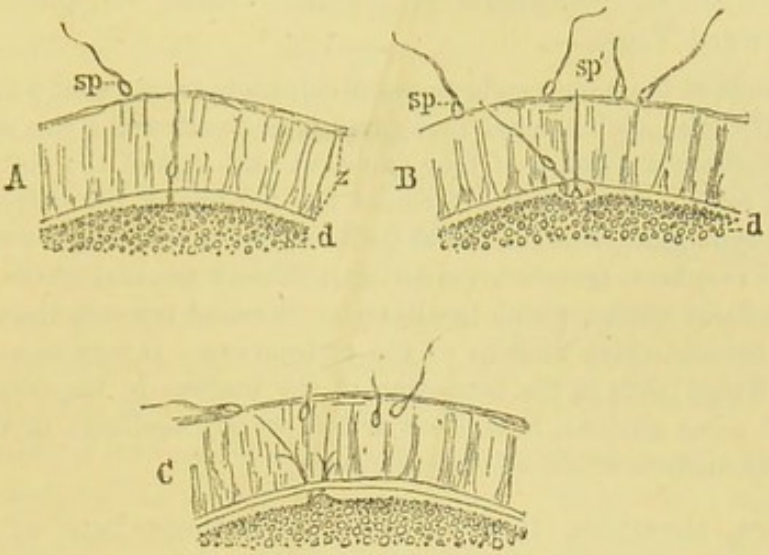


FIG. 92.—Fecundation of the eggs of *Asterias glacialis*. *sp*, Spermatozoids. The spermatozoids are in the mucus covering the egg. At *A*, a spermatozoid has reached the periphery of the yolk. At *B*, several are moving onwards towards a prominence in the surface of the yolk. At *C*, the heads of one or two spermatozoids have united with and penetrated into this prominence.

4. *The Formation and Expression of Polar Bodies.*—Another series of phenomena has been brought to light. It is well known from the researches of Strasburger and others that, in the development of the reproductive organs of plants, certain portions of the matter that is devoted to the formation of spores, antherozoids, and oospheres (or ova) are not used for that purpose, but are extruded or otherwise laid aside from taking part in the reproductive process. Thus, in the sporangium of many fungi, a part of the protoplasm, called the *epiplasm*, remains over after the formation of the spores. In the development of the zoospores of algæ a part of the protoplasm, not used up, is extruded in the form of a vesicle from the sporangium, and Strasburger states that in some cases, just before the division of the spore-mother-cell, a mass of stuff is extruded from the nucleus, called the *paranucleolus*. Similar phenomena have been observed in sexually-reproductive cells. The male element of mosses, the antherozoid, has an appendage attached to its posterior end. This appendage or vesicle has been found to be the "unused protoplasm of the mother-cell," along with actually a portion of the nucleus, which is thus excluded. In the angiosperms, during the development of the nucleus and the protoplasm of the pollen grain (the male portion) cell-like bodies are formed which are separated from the generative cell. Similar phenomena are noticed in the development of the female structures or *gametes*, that is to say, portions of protoplasm are extruded from the oogonium. Even in angiosperms nuclear divisions take place in connection with the

development of the oosphere or ovum. Thus, to quote from Dr. Sydney Howard Vines—

"The nucleus of the young embryo-sac divides into two, one of which travels to each end of the sac; each nucleus then divides, and each of the two nuclei divides again, so that there is a group of four nuclei at each end of the embryo-sac. Of those at the micropylar end, one becomes the nucleus of the oosphere, two the nuclei of the synergidæ, and the fourth (polar nucleus), which is the sister nucleus of that of the oosphere, travels towards the middle of the sac, where it fuses with one of the chalazal nuclei, which has likewise travelled towards the middle of the sac, to form the definitive nucleus of the embryo-sac. It may be suggested that the division which leads to the formation of the nucleus of the oosphere, and of the so-called polar nucleus, is the one which we are seeking; in that case the so-called polar nucleus would be the polar body."

It appears, therefore, that in the development both of non-sexual spores and of male and female elements in plants a portion of the original nuclear substance is extruded or thrown aside and takes no share in the reproductive process. The portions thus laid aside or extruded have been called *polar bodies*. It is remarkable that analogous phenomena occur in the development of the ova and of the sperm cells of animals.

The extrusion of minute particles of matter, or polar bodies, from the egg was first observed by Dumortier in the egg of *Lymnæa stagnalis* in 1837, and the phenomenon was investigated by F. Müller in 1848. No relation between these bodies and the germinal vesicle was noticed until the researches of Bütschli in 1875, to which I have previously referred. He observed a curious spindle-shaped structure passing between the germinal vesicle and the surface of the ovum. In 1876, Ed. van Beneden detected the extrusion of two polar bodies from the egg of *Asteracanthion rubens*. The researches of Fol, Selenka, and O. Hertwig not only corroborated these observations, but many details of the phenomena connected with their formation and extrusion were observed. Thus Calberla noticed that changes occurred in the germinal vesicle along with the extrusion of the polar bodies, and that these bodies might be eliminated even before the egg reached its maturity. In the great majority of cases the formation of the polar bodies preceded the union of the sexual elements, and researches by Fol and others have shown that the genesis of these bodies is independent of fecundation.

Let us now examine what actually occurs. When the ovum is ripe it consists, as already described, of a cell wall enclosing contents, in which is embedded the nucleus or germinal vesicle, in which again is the nucleolus or germinal spot. Previous, then, to actual fecundation (although the spermatozoid may have penetrated the ovum), a fusiform

body is seen (the "direction-spindle" of Bütschli, and the "amphiaster of rejection" of Fol), as was first described by Bütschli, stretching from the germinal vesicle (Fig. 93) towards the surface of the ovum, so that one end of the spindle touches the surface whilst the other is in contact with the germinal vesicle. At the same time, or rather immediately before the appearance of the spindle, the wall of the germinal vesicle becomes less and less distinct, and the protoplasm in its immediate vicinity assumes a radiated appearance. There is every reason to believe that a portion of the substance of the germinal vesicle forms part of the spindle. By-and-bye a small globule protruding from the surface of the ovum appears at the external end of the spindle. This is the first polar body. In most cases a second spindle is formed, and there is the extrusion of a second polar body. Bütschli thought that the spindle was entirely extruded in the polar body, but Fol and Hertwig have

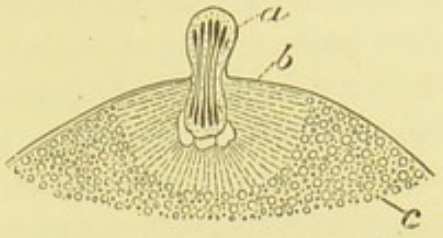


FIG. 93.—Extrusion of a polar body from the egg of a snail, *Succinea Pfeifferi*. *a*, polar body showing elongated filaments of nuclear matter; *b*, radiated appearance around the polar body; *c*, yolk.

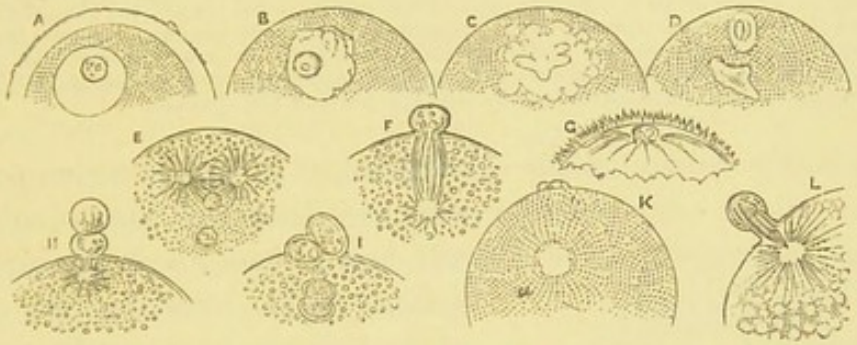


FIG. 94.—A, ripe ovum of *Asterias glacialis* with excentric germinal vesicle and spot. *B*, *C*, *D*, *E*, gradual metamorphosis of germinal vesicle and spot; *F*, detachment of first polar body and withdrawal of remaining part of nuclear spindle within the ovum; *G*, portion of living ovum with first polar body; *H*, formation of second polar body; *I*, after formation of the same, showing the remaining internal half of the spindle in the form of two clear vesicles; *K*, ovum with two polar bodies and radial striæ around female pronucleus; *L*, expulsion of polar body (*A*—*K*, after Fol; *L*, after Hertwig).

shown that this is not the case. As to the genesis of the spindle itself there is still considerable difference of opinion. Bütschli conceived that the fibres of the spindle were formed by the coalescence of granules. Fol described them as due to a swollen condition of the "filaments bipolair," meaning certain filaments passing from the germinal vesicle towards each pole of the egg. O. Hertwig supposes that they arise from the fragments of the wall of the germinal vesicle and of the germinal spot; and these views of Hertwig have been supported by Trinchese and Blochmann.

Considerable discussion has taken place as to whether these pheno-

mena belong to the class of changes occurring in karyokinesis. Flemming, in 1882, after a careful study of the egg by the methods followed in the observation of karyokinesis, came to the opinion that the phenomena are the same, with this difference, that in the egg the chromatin element does not bulk so largely as the achromatin constituent, the result being that in the karyokinesis of cells the chromatin figures are the more apparent, whereas in the egg the achromatin figures arrest the attention. This view is well illustrated by the figures of ordinary karyokinesis given by Rabl (Fig. 95), in which the achromatin spindle-like figures are

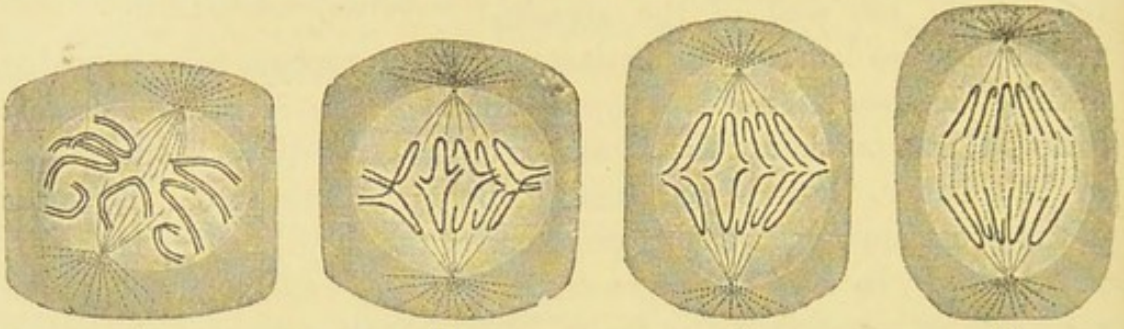


FIG. 95.—Diagram showing the mode of nuclear division. *A* shows strongly marked chromatin filaments with the spindle figure. At the ends or points of the spindle observe the star-shaped or radiating arrangement of the protoplasm, and in the middle of the spindle the chromatin filaments. In the latter a longitudinal division of the filaments has already begun. *B* shows the number of chromatin filaments multiplied by division and beginning to be arranged with the loops in the same direction as the spindle. *C* shows the loops still more regularly arranged. *D*, both groups of the filaments or loops have retreated towards the poles of the spindle.

very distinctly shown; on the other hand, as E. van Beneden points out, Hofmann is the only observer who has described the spindle in the mammalian egg, whilst he himself has looked for it in vain, and he inclines to the view that the extrusion of the polar body is not an example of ordinary karyokinesis, in which the ovum may be regarded as simply dividing, so that one half is the minute polar body, whilst the other half is the rest of the ovum, but that it is really a physiological process peculiar to the ovum. In the development of the egg of *Ascaris megalocephala*, E. van Beneden describes a peculiar figure termed by him the ypsiliform figure from its resemblance to the Greek letter Υ (Fig. 96). It may be compared to the toy known as the "cup and ball," the stalk of the cup being the vertical portion of the Υ, and supposed by Van Beneden to be like a thread or cord, whilst the two diverging

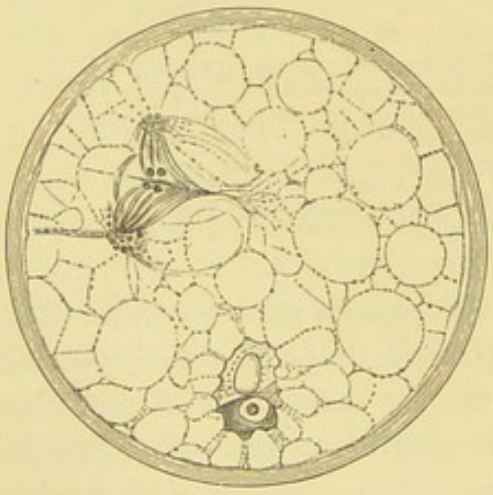


FIG. 96.—Egg of *Ascaris megalocephala* showing the Y-shaped figure, having delicate protoplasmic filaments connected with it The altered spermatozoid is seen in the lower left hand portion of the egg.

limbs are really sections of the sides of the cup. In the hollow of the cup there is a mass of clear homogeneous matter, containing chromatin globules, corresponding to the ball in the well-known toy. The fibrils of the Y-shaped body originate in fine granules, which are the remains of the membrane of the germinal vesicle, along with some of the surrounding protoplasm. At the end of each limb of the Y there is also a little mass of granules, and around the stem of the Y, and running up so as to assist in forming the cup, there is a clear transparent mass to which he gives the name of *prothyalosome*. The axial fibres, those forming the stem of the Y, appear first. Thus, in the first instance, a fusiform figure appears, the pointed ends corresponding to the ends of the stem of the Y. Next two other centres of attraction appear in the prothyalosome, like two stars or radiant points, the fibrils from each of which coalesce at the upper end of the stem of the Y, thus giving rise to the Y-shaped figure. At first the Y-shaped figure is near the centre of the ovum, but it gradually approaches the periphery, and the point of each limb of the Y touches the vitelline surface, although not at the same time (Figs. 97, 98, and 99). The form of the Y then changes in consequence of fibres in the two limbs uniting transversely, so that it becomes more homogeneous in appearance; but at each point corresponding to the free end of the limb of the Y there is a little star-shaped cluster of chromatin granules. As the Y-shaped body approaches the surface, the stem of the Y lengthens, and the fibres become less and less distinct, and at the lower end the matter forming the head of the spermatozoid is apparent. The first polar body is formed of a portion of the chromatin stars at the ends of the limbs of the Y, along with a portion of the clear matter surrounding the limbs and forming the cup—the prothyalosome; and E. van Beneden attaches great importance to the fact that division does not occur so as to cut the pole (that is to say, the limb of the Y, with surrounding matter) transversely, but the division takes place equa-

FIG. 97.—Shows Y-shaped figure near the surface, with the upper limbs of the Y flattened out so as to make the figure T-shaped. Observe also the transverse striæ. Egg of *Ascaris megalocephala*.

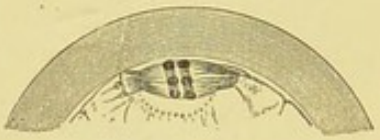


FIG. 98.—The upper part of the Y-shaped figure has now reached the surface of the egg, or stage prior to the genesis of the first polar body, which is formed by transverse cleavage. Egg of *Ascaris megalocephala*.

FIG. 99.—Formation of the first polar body in egg of *Ascaris megalocephala*, indicating one of the last modifications of the Y-shaped body.

torially, just as if the limbs of the Y had opened out, and each limb had been cut in the direction of its length. In all cases of ordinary karyokinesis the cleavage always takes place transversely to the direction of the filaments of the spindle, and if we recognize the Y-shaped figure of E. van Beneden as being formed of two spindles, the importance of his observation will be appreciated. The formation of the polar globules cannot therefore be deemed an example of karyokinesis: at all events, it is not of the usual type. After the removal of both the polar bodies, the portions of the chromatic stars or discs, along with the neighbouring portions of the prothyalosome, unite to form a body, which is in all respects similar to the polar bodies extruded. This he terms the *deuthyalosome*.

This body, the deuthyalosome, may be regarded, then, as formed of the remains of the germinal vesicle. The vesicle, by this elaborate process—which occurs *before* fecundation, although it may be going on while the spermatozoon is in the egg—has thrown off a portion of itself, and the body which remains at the end of the process cannot be regarded, morphologically or physiologically, as an ordinary cell nucleus.

Now we approach true fecundation. It has been ascertained that one single spermatozoon is sufficient for the process. Entering the ovum, either by a distinct aperture (in the ovum), termed the *micropyle* (Fig. 100), or, where no special aperture has been observed, through the cell wall, it quickly undergoes chemical and physical changes. It becomes more susceptible to the action of colouring matters, and instead of being granular it assumes a homogeneous appearance. The tail disappears, and the head becomes globular in form. E. van Beneden has observed in many eggs a refringent globule, similar in appearance to a body seen in the spermatozoid, and he has no doubt that this body has been thrown off by the spermatozoid, and finally ejected from the egg itself, probably with the second polar body. This important observation is of profound

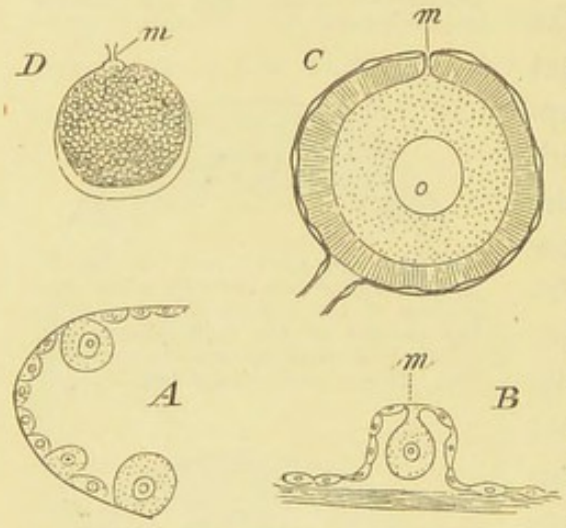


FIG. 100.—*A*, blind end or *cul-de-sac* of the ovary of a star fish. *B* and *C*, development of the egg of *Holothuria Bohadschia*, after Semper. *B*, young egg with the surrounding epithelial cells detached; at *m*, the opening of the micropyle. *C*, ripe egg, showing a thickened zona pellucida, and the epithelial cells much elongated; *m*, micropyle, very narrow. *D*, egg of Unio, showing micropyle at *m*.

significance, as showing that the spermatozoid (as well as the germinal vesicle) gets rid of a part of its substance (*seminal granule*) before fecundation occurs.[1]

The body left in the egg after the formation of the two polar bodies is termed the *female pronucleus*, while that formed by the head of the spermatozoid is the *male pronucleus*. Until the second polar body has been extruded, the spermatozoid remains inactive, but when this has occurred a series of changes ensues, which ends in the division of the first cell of the embryo into two cells (Figs. 101 and 102).

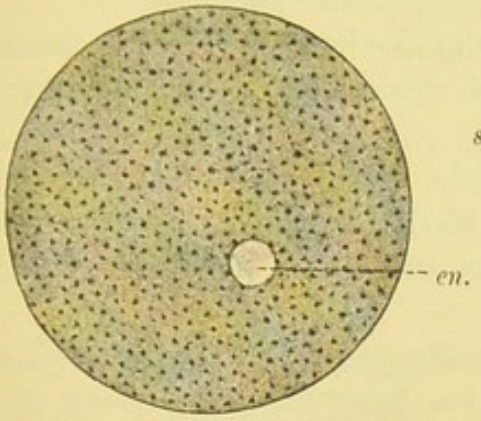


FIG. 101.—Ripe egg of a sea urchin (*Echinus*). *en.*, egg nucleus, or germinal vesicle.

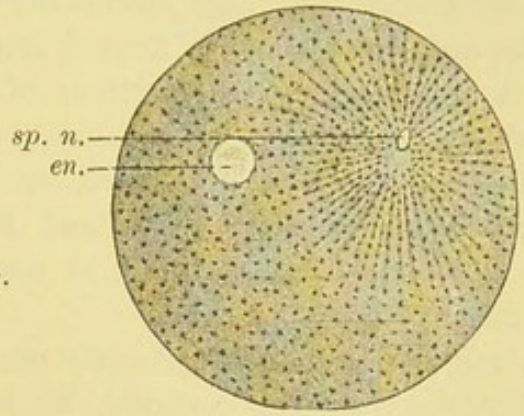


FIG. 102.—Fecundated egg of a sea urchin. The head of the spermatozoid, constituting the spermatozoid nucleus, *sp. n.* or male pronucleus, is surrounded by radiated protoplasm; *en.*, egg nucleus, or female pronucleus.

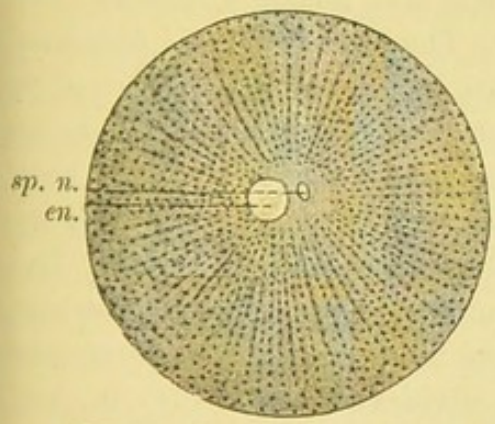


FIG. 103.—Fecundated egg of a sea urchin, *sp. n.* Spermatozoid nucleus, or male pronucleus; *en.*, egg nucleus, or female pronucleus. The two pronuclei are close together, and are both surrounded by a radiated mass of protoplasm.

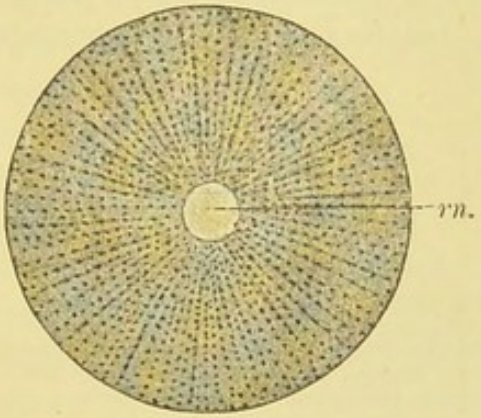


FIG. 104.—Egg of a sea urchin immediately after fecundation. *rn.*, fecundation nucleus. Both the male and female pronuclei have disappeared, and around the fecundation nucleus the protoplasm has a radiated appearance.

[1] Further, E. van Beneden and Ch. Julin have discovered the expulsion by the spermatomere of a minute globule, which does not therefore enter into the formation of the spermatozoid. La spermatogenèse ches l'ascaride megalocéphale. *Bull. de l'Académie Royale de Belgique*, 1884. The separation of a globule has also been described by J. E. Blomfield on The Development of the Spermatozoa, part 1, Lumbricus (Earth worm), *Quart. Jour. of Micros. Science*, 1880. See also on

The female pronucleus is now a nucleus containing two chromatin filaments, or loops, originally derived from the germinal vesicle of the egg. It is very curious to find that the number of loops seen in the various stages of these processes is very constant. Thus, in the germinal vesicle there are two chromatin plates, each composed of four chromatin globules; the same in the Y-shaped figure; in the first polar body two chromatin bodies, each formed of two smaller ones; in the deuthyalosome, two chromatin filaments, each dividing into two; the same in the second polar body; and now in the female pronucleus again two chromatin loops, each formed of two smaller portions.

The male pronucleus is formed from the head of the spermatozoid, and it also contains two portions of chromatin. It is found near the lower pole of the egg.

The two pronuclei approach each other, and ultimately unite. According to O. Hertwig, Fol, and Mark, there is a complete fusion. This is fecundation, the union at last of the two elements (Figs. 103 and 104).

E. van Beneden, in the *Ascaris megalocephala* has not observed complete fusion; on the contrary, he has been able to observe in the karyokinetic process which immediately follows, that the chromatin filaments supplied by the male and female elements remain distinct, so that in the two cells formed by the division of the first cell each new nucleus receives an equal portion of male and female elements. He divides the process into four stages. 1st. The four chromatin filaments increase in size and become coiled to a greater or less extent. It is to be remembered that two have been supplied by the male and two by the female pronucleus. 2nd. The contours of the pronuclei become less and less distinct; each chromatin loop divides transversely in the middle so that there are now eight loops, and the eight loops of chromatin move so that the shut end of each loop is directed towards the equator of the fecundation nucleus. Imagine an orange with eight loops of cord placed equatorially in it, the ends directed towards the axis passing from pole to pole. 3rd. Each loop is then divided longitudinally, thus giving rise to sixteen loops, and the two groups of eight are separated by a thin layer of

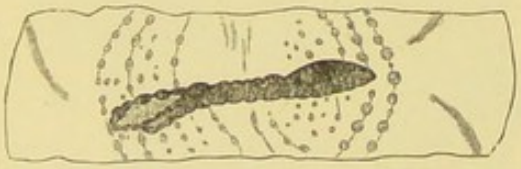


FIG. 105.—Arrangement of chromatin filaments in the equator of the egg of *Ascaris megalocephala*. The filaments form the dark irregularly-shaped bar. Only the middle portion of the egg is shown.

this question Herbert H. Brown's paper on Spermatogenesis in the rat, *Quart. Jour. of Micros. Science*, 1885, in which Prof. Ray Lankester's opinion is given as regards the globule in case of Earth worm, p. 20.

matter. 4th. Each of these groups of eight is now situated in what E. van Beneden terms sub-equatorial planes, parallel of course with the equator (Figs. 105, 106, and 107).

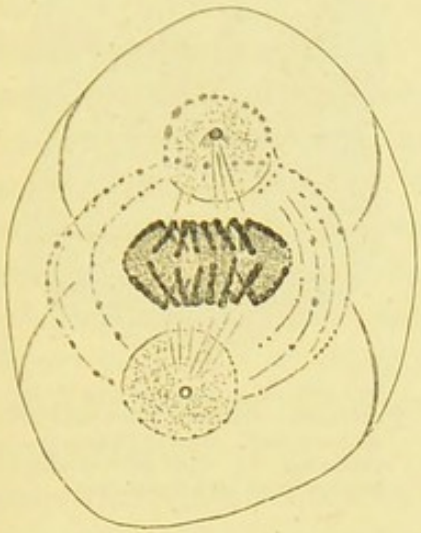


FIG. 106.—Stage in the division of the egg of *Ascaris megalocephala*, showing the chromatin loops separating into two sets and retreating from each other.

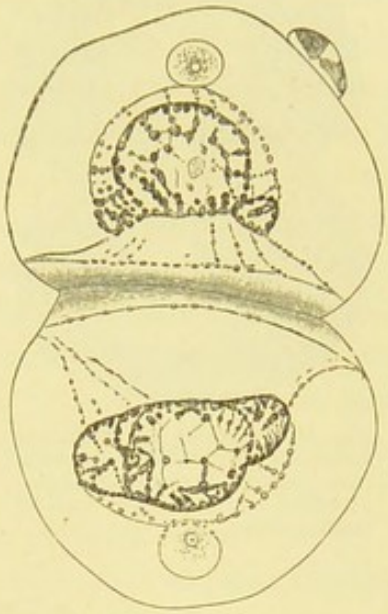


FIG. 107.—Stage of division of the egg of *Ascaris megalocephala*, showing the breaking up of the chromatin threads into minute globules, and in some places a reticulated appearance from the crossing of short filaments.

Thus it is evident that each loop has given a half of its substance to each of the daughter nuclei, and as there was an equal number of loops from the male and female portions to start with, it follows that each daughter nucleus must have male and female elements. The two sets of loops recede towards the poles of the nucleus, and then there is division of the body of the yolk, according to the ordinary method of karyokinesis already described. Further, the two cells thus formed, nourished by the fluids in which they exist, increase in size, and again divide karyokinetically into four. The process is repeated over and over again so as to form the primitive cells from which all the tissues and organs of the body are derived; and if the karyokinetic division is repeated so that portions of the chromatin derived from the male and female elements (multiplied and increased in quantity by nutrition, but the same in kind) are divided equally, it follows that all cells contain equal quantities of matter related to the male and to the female parent; and, to crown all, according

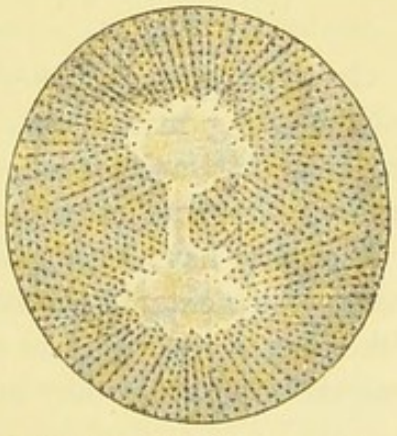


FIG. 108.—Egg of a sea urchin preparatory to cleavage.

to this view, each living cell is a hermaphrodite being[1] (Figs. 108, 109, 110, and 111).

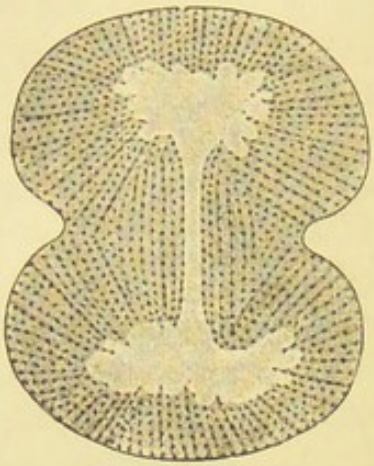


FIG. 109.—Egg of sea urchin at moment of division, showing constriction of the protoplasm at right angles to the axis of the nucleus.

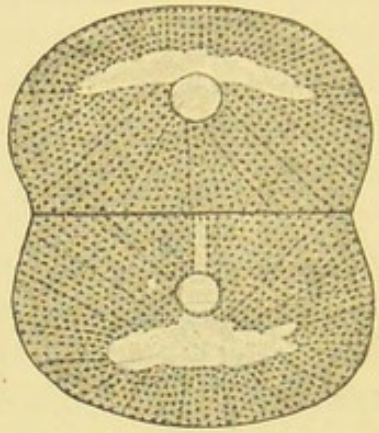


FIG. 110.—Egg of sea urchin after division. Observe the nucleus in each half. The streaked appearance of the protoplasm begins to be less distinct.

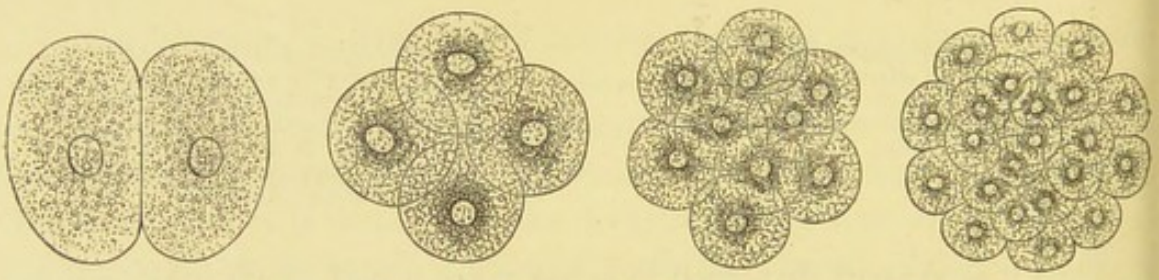


FIG. 111.—Various stages of the cleavage of the egg to form embryonal cells.

CHAP. III.—THEORIES AS TO THE PHYSIOLOGICAL BASIS OF HEREDITY.

In the previous chapter, we have discussed the origin of the cells and of the living matter that form our bodies. One is naturally inclined to ask, does a knowledge of these phenomena throw any light upon the well-known facts of hereditary transmission?[2] Not a few theories of heredity have been advanced. The oldest of all is that the soul of an ancestor entered the body of a newly born being and made the body like that in which it had at one time existed. The theory of the transmigration of souls may be traced to very remote times, and it exists

[1] This view is associated with the name of Professor Sedgewick Minot of Harvard. *Science*, vol. iv. 1884. Also *Proceed. Boston Society of Natural History*, 1877.

[2] Ch. van Bambeke, "Pourquoi nous ressemblons à nos parents." *Bull. de l'Académie Royale de Belgique.* 3me. serie, tom. x. 1885. This is an admirable esumé of recent speculations.

almost unmodified in the opinions of some of the so-called savage races of the present day, races probably representing, however, not the primitive type of thought and practice, but opinions modified in the course of ages, like those of the higher and civilized races. Stahl, in accordance with his mystical views, held that the soul formed the fœtus and shaped it according to the paternal or maternal type. Then, in our own day, came the doctrine of Pangenesis of Charles Darwin,[1] which assumed the existence in all cells of small corpuscles which circulate free in the body. These gemmules are transmitted by the parents, and they are usually developed in the next generation, but may lie dormant for generations, and their evolution depends on their union with gemmules of a kindred nature, supplied from another organism. This theory is capable of explaining many of the facts, but it labours under the disadvantage that there is no vestige of proof of the existence of such gemmules.

Haeckel[2] has endeavoured to solve the problem by supposing that what is transmitted is not matter but a certain kind of molecular movement which the tissues acquire by constant repetition, and retain by a kind of unconscious memory. Heredity, according to this view, would depend on the more or less accurate transmission of special kinds of molecular movement, while variability would be caused by the action of external agencies changing more or less the character of the movement so transmitted. The obvious criticism on this theory is, that we as yet know far too little of the character of molecular movements, to make our knowledge the basis of any such theory.

Naegeli rejects both the theory of Pangenesis of Darwin and the theory of Perigenesis of Haeckel, because the one calls in the aid of hypothetical germs and the other of hypothetical movements, and he endeavours to establish a theory on the basis of fact. Protoplasm, according to him, consists of two portions, one, fluid, called *hygroplasm*, and the other solid or insoluble, *stereoplasm*. The stereoplasm, however, is partly nutritive, but the other portion called *idioplasm* is the seat of all active changes. This idioplasm ramifies through the body, is in every cell and tissue; and each tissue has its own kind of idioplasm, so that there is a

[1] Charles Darwin, on the *Variation of Plants and Animals under Domestication*, 1867, chaps. xxxvii. and xxxviii. See also in this relation, Herbert Spencer's *Principles of Biology*, vol. i. chaps. iv. and viii. Also, Sir Richard Owen's work on *Parthenogenesis*, 1849. It is interesting also to read Professor Owen's criticism of Pangenesis in his *Comparative Anatomy and Physiology of Vertebrates*, vol. iii. p. 813.

[2] Haeckel, *Die Perigenesis der Plastidule oder die Wellenzeugung der Lebens theilchen*, Berlin, 1876.

vast variety of idioplasms, differing, however, more dynamically than in any detail of structure. Reproductive cells contain idioplasm that has returned from the condition of somatic idioplasm to the state of the idioplasm of the germ from which the organism sprang. Further, he supposes that the specific properties of the idioplasm depend on the grouping of more minute particles, the *micellæ*, and that this grouping is more complex in the idioplasm of the higher beings than it is in the lower. Suppose, then, that each parent transmits an equal portion of idioplasm, there will be an equal transmission of the peculiarities of each parent; and if one idioplasm predominates, the balance may lean to the side of the father or of the mother. This mass of idioplasm, as already said, extends through the body, having subsidiary characters peculiar to each kind of cell, but always carrying with it the more special peculiarities which it had at first. During growth it retains all its specific characters, and the germ cell is simply a cell containing idioplasm, having chiefly the primitive character. As, however, the somatic idioplasm is influenced by external conditions, to some extent, and as the return of the somatic idioplasm to the state of germinal idioplasm is not always exact, the offspring never exactly resemble their parents. Hence arises variability.

But while this theory is founded on the fact that there is an all-prevalent matter—the protoplasm,—it is liable to the same objection as was advanced to the other two theories—it is too conjectural. The question is, where is this idioplasm? If *it* is the reproductive matter, it must exist in many cells, more especially in those which yield the male and female elements. That it does exist in certain cells of plants is evident from the fact that cuttings produce roots, and when a stem is cut across new shoots appear from the lateral buds. Almost any part of the leaf of a *Begonia*, if suitably planted, will give origin to a fully-formed plant. Similar phenomena may be observed in certain animals—more especially in amphibians. If the limb of a salamander be removed, the limb may be renewed, that is to say, the cells forming the various tissues of the stump possess the power of producing cells of the same kind, and of rebuilding the limb. This has been aptly compared by Pflüger,[1] to the growth of a crystal. A crystal of alum has a certain molecular structure, and if a small fragment of such a crystal is placed in a saturated solution of the same salt, the broken fragment is repaired, so as to form a perfect crystal. This is more than an analogy. The molecular processes of the crystal are the cause of the deposition of the particles of alum in such an order as to renovate the crystal; and, in like manner, the molecular processes in the cells of the outermost layer

[1] Pflüger, *Ueber den Einfluss der Schwerkraft auf die Theilung der Zellen und auf die Entwicklung des Embryo. Archiv f. Physiol.* vol. xxxii.

of the stump lead to the formation of new cells of the same kind out of the material furnished by the blood plasma in their vicinity.

Seizing hold so far of Naegeli's idea, Strasburger[1] has modified it, and made the position more intelligible. Like him, he considers protoplasm as consisting of two substances, a nutritive hyaloplasm, and a formative hyaloplasm; but he identifies the formative hyaloplasm with the chromatin filament of the nucleus, which we have already studied. This substance he called nucleoplasm or nucleohyaloplasm. It is the active substance in the nucleus and even in the protoplasm around the nucleus, as Goodsir long ago stated. All the metabolic changes in the cell substance are controlled and directed by the nucleoplasm. Strasburger states that the reproductive power of a cell depends on its being in the embryonic state, and in plants reproductive cells in this sense are not uncommon, seeing that many parts of plants are capable of vegetative reproduction.

This view throws light upon the phenomena of the expulsion of polar bodies from the ovum, and from the spermatozoid substance. These phenomena express the return of the cells to their embryonic conditions. The late F. M. Balfour held that the extrusion of the polar bodies from the ovum, or rather the extrusion of a part of the germinal vesicle, was "requisite for its functions as a complete and independent nucleus," . . . "to make room for the supply of the necessary parts to it again by the spermatic nucleus. My view," he says, "amounts to the following, viz.: that after the formation of the polar cells, the remainder of the germinal vesicle, with the ovum (the male pronucleus), is incapable of further development without the addition of the nuclear part of the male element (spermatozoon), and that if polar cells were not formed, parthenogenesis might normally occur. A strong support for this hypothesis would be afforded were it to be definitely established that a polar body is not formed in Arthropoda and Rotifera, since the normal occurrence of parthenogenesis is confined to these groups. It is certainly a remarkable coincidence that they are the only two groups in which polar bodies have not so far been satisfactorily observed." Further, he says, "The function of forming polar cells has been acquired by the ovum for the express purpose of preventing parthenogenesis."[2]

[1] Strasburger, *Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen als Grundlage für eine Theorie der Zeugung.* Jena, 1884.

[2] F. M. Balfour, *Comparative Embryology*, vol. i. p. 72. It is only right to note that this theory is strongly opposed by Carnoy, and that he especially disputes the correctness of the interpretation of the extrusion of the polar bodies, *La Cytodiérèse de l'œuf, La Cellule.* Tome iii. fasc. i. p. 60. It is also asserted that

This notion, which has been somewhat modified by various authors, and is refused by Carnoy as an explanation, is that the extrusion of the polar bodies from the nucleus of the ovum is the removal of the male idioplasm, so that what is left is truly female, and the extrusion of the particle from the spermatozoid is the removal of the female idioplasm, so that what remains is truly male. The female idioplasm would thus transmit peculiarities of the mother, whilst the male idioplasm would send on those of the father. But, as Strasburger and Von Kölliker acutely remark, the mother transmits not only her own peculiarities, but also those possibly of the maternal grandfather and of the maternal great-grandfather; and, on the other hand, the father may send onward traits of the paternal grandmother and of the paternal great-grandmother. The maternal idioplasm cannot, therefore, be regarded as entirely female, nor the paternal as entirely male. Strasburger holds that the nucleoidioplasm filament may be regarded as made up of a number of segments derived from previous generations, and that in certain circumstances one portion may influence the kytoplasm of the ovum more than the other, thus giving rise to a manifestation of some of the hereditary qualities of the particular generation to which the portion of nucleohyaloplasm corresponded. It appears to me that here Strasburger passes entirely into the region of theory. The one statement of importance in his theory is the identification of the idioplasm of Naegeli, or, as he terms it, the nucleohyaloplasm with the chromatin filament. It is at this point that the important observations of E. van Beneden come in with great effect. If it be the case that the chromatin filaments from the male and those from the female remain distinct, and are communicated in equal amounts to the nuclei formed by the division of the fecundation nucleus, and if this process be multiplied indefinitely in the formation of the cells of the body, we see that each cell is representative of both father and mother, to a greater or less extent, according to the amount of maternal and paternal idioplasm present (Fig. 112). Further, it is necessary to assume that this idioplasm is capable by nutrition of being increased in quantity, but by the action of surrounding conditions it may be acquiring properties peculiar to itself, giving rise to the individuality of

Weismann has seen polar bodies extruded from the eggs of certain crustaceans that are parthenogenetic (Weismann, *Richtungskörper bei parthenogenetischen Eiern. Zool. Anz.* 27th Septr. 1886). Even if this be the case it does not seem to me to destroy the validity of Balfour's theory, but only to show that it is less extensive in its application. The extrusion of polar bodies by parthenogenetic ova may be the survival of an ancestral habit, and possibly after removal of the polar body there may still be enough of the male matter left to allow of development going on, without the entrance of a fresh spermatozoid.

the person in whose body these changes are supposed to take place. A time comes, however, when cells lose the power of thus multiplying

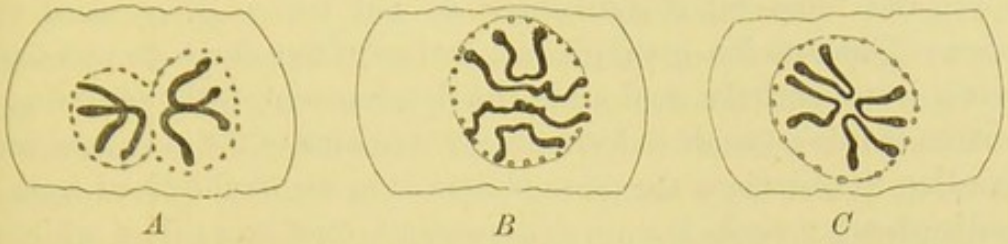


FIG. 112.—Different arrangements of the primary chromatin loops in the equatorial plane of the egg of *Ascaris megalocephala*.

indefinitely by fission, the growth of the body is arrested, and the general characters of the individual are fixed. One can suppose, then, that if any cell in the body could get rid of its male portion of idioplasm, and if another got rid of its female portion, the two remaining parts might conjugate, and the process of cell division might start afresh. The stimulus would still be greater if the two idioplasms were more different than is likely to be the case if obtained from the cells of the same individual. The best effect would be produced if a certain amount of difference existed, and this is attained by the idioplasm of two individuals being brought into contact. May not this be the key to the advantage gained by sexual difference, and may it not explain the infertility that results if two idioplasms from two individuals too closely related be brought into mutual relationship? This rendering and expansion of Strasburger's theory I have reached after a careful consideration of the question in the light of the facts of modern investigation.

The peculiarity of Strasburger's view is that the object of the extrusion of the polar bodies is simply the attainment of the embryonic condition, and that the reproductive matter—call it idioplasm or any name you choose—is formed by the conversion of somatic into reproductive idioplasm. In the latter statement Strasburger follows Naegeli. The essential distinction between Strasburger and Naegeli is that while the conceptions of the former are structural and material, those of the latter are dynamical. This supposed conversion of somatic into reproductive idioplasm is a difficulty, and a consideration of it has led Weismann[1] to promulgate the theory of what is termed the *continuity* of the germ-plasma. This view is that heredity consists in the transmission of a nuclear substance of special molecular structure, not the idioplasm of Naegeli, nor the nucleohyaloplasma of Strasburger (identical with chromatin), but a substance special and peculiar to the germinative cells.

[1] August Weismann, *Die continuität des Keimplasma's als Grundlage einer Theorie der Vererbung*. Jena, 1885. See also an account of this memoir by Professor H. N. Moseley in *Nature*, vol. xxxiii. p. 154.

This nuclear matter which he calls *keimplasma*, germ-plasma, is passed on from generation to generation without alteration; it is therefore continuous and immortal if individuals do not fail in propagating their species. This specific germ-plasma, derived from the male and female parents, is not entirely used up in the development of the offspring, but a portion of it is set aside to form the germ-plasma of the next generation. According to this view, the germ-plasma is in no way derived from the somatic-plasma; each has an independent existence; but while the somatic-plasma cannot influence the germ-plasma, the portion of germ-plasma not laid aside to form the germ-plasma of the next generation exercises a potent influence over the development of the somatic-plasma, transmitting to it the characters of the parents. Professor Weismann supports this view with many striking illustrations, and he adapts the theory to the explanation both of the extrusion of polar bodies and of parthenogenesis. Thus the ovum has two kinds of plasma in it, histogenetic or somatic-plasma and true germ-plasma. In its early stages, the histogenetic plasma is engaged in forming the yolk and membranes, and as it is of no further use in the further development of the embryo, it is got rid of in the form of one or more polar bodies to make room for fresh histogenetic plasma, derived in turn from the germ-plasma of the spermatozoon. The polar bodies are thus ovogenous nucleo-plasma, got rid of to allow new nucleo-plasma to have free play. A similar explanation applies to the separation of particles from the male sperm cell.

As to parthenogenesis, Weismann supposes, in the first place, that the male and female elements in fecundation, that is to say, the germ-cell and the sperm-cell, are practically identical, and that after the extrusion of the polar bodies, development is started by the sudden addition to the so-called female element of the so-called male element. This sudden addition necessitates cleavage, and as nutrition goes on actively, for the same reason, cleavage goes on again and again to form the embryonic cells. In some ova, however (parthenogenetic ova), even after the extrusion of polar bodies, nutrition goes on so actively as to start the developmental process without the stimulus caused by the sudden addition of the so-called male element. Parthenogenesis, according to this view, is thus merely a modification of the power of growth. The theory is not open to the objection urged against that of F. M. Balfour, namely, the undoubted extrusion of polar bodies from, at all events, some parthenogenetic ova.

It appears to me, however, that this theory, so ably maintained by Weismann, while it undoubtedly removes some of the difficulties, leaves the facts of hereditary transmission unexplained. If the portion of germ-

plasma not laid aside influences the development of the body, conferring on each tissue and organ at least some of the proclivities of the parent, how does it do so? The molecular mechanism by which this is accomplished is inexplicable. On the other hand, if this germ-plasma does not so influence development, how are the facts of hereditary transmission to be explained? It is, I humbly think, quite possible that Weismann may be right in the conjecture that a portion of the original germ-plasma may be laid aside at the earliest period of development to constitute the germ-plasma of the next generation; but surely this germ-plasma has received a molecular imprint from the parent in whose body it at one time existed so that it transmits certain of the characteristics of that parent. If the germ-plasma cannot be altered, how are we to account for the transmission of special characters of any kind? Weismann has admitted this difficulty by denying the alleged transmission of acquired characters by sexual reproduction; but all characters must have been acquired at one time or another, and any organism is what it is at the present moment, in consequence of this transmission of acquired peculiarities through countless generations. The germ-plasma cannot then remain the same, and therefore the only way in which we can suppose it to be modified is through the agency of the idioplasm throughout the body. This view will explain to some extent at least the transmission of acquired characters.[1]

In all these discussions, one aspect of the subject, as it relates to the higher organisms, and especially to man, is often omitted, namely, the influence which the female parent exerts on the development of the offspring, while in the uterus, in consequence of the intimate union that exists for a considerable period of time. It is a familiar observation that animals reared outside the mother's body, as in most invertebrates, in fishes, amphibians, and birds, are remarkably similar to each other. The individuals in a shoal of fish or a flock of sea gulls or sparrows can scarcely be distinguished. It is when we reach the stage of develop-

[1] I have to express my admiration of Weismann's theory as a guide to an experimental investigation into this most difficult field of inquiry. I would further observe that it can hardly be expected that acquired characters in their full intensity will be transmitted to the immediately succeeding generation, seeing that the ova destined to become the individuals of that generation are found in the ovary of the mother during embryonic life. The influence of an acquired character might have a certain effect on the ova during the life of the mother, but not enough to reproduce the acquired character with any degree of strength in the offspring. If the influences producing the acquired character acted also on the offspring, its effect on the offspring of the third generation would be intensified, and thus a process of accretion during several generations may be required to stamp acquired characters on offspring.

ment by placental connection that we begin to observe diversity of appearance, and if along with this we suppose that in consequence of a great development of the nervous system, the female becomes more and more susceptible to external impressions which react on her body and through her body on her offspring *in utero*, an explanation, so far, of individual peculiarities of the human race becomes apparent. There can be no doubt that pregnancy makes a remarkable change on the mother, to such an extent that the characters of subsequent offspring may be influenced. Thus it is well known to breeders of animals that a pregnancy induced by a certain kind of male, may deteriorate the characters of offspring that owe their origin to subsequent impregnations by other males. It is possible, therefore, that not a few maternal characters may arise in this way, and that if we could hatch human beings as we can hatch eggs, human beings might be so similar in appearance as to be practically indistinguishable.[1]

There is still one other view of the matter which I shall briefly notice. If the germinal vesicle be only the 1-500th of an inch, and the head of the spermatozoid be only the 1-6250th of an inch in diameter, dare we suppose, on purely physical grounds, that so small a particle of matter as is formed by the union of the two can contain a sufficient number of organic molecules to account in any way for the transmission of hereditary peculiarities? The late Professor Clerk Maxwell, in his profoundly interesting article on the Atom in the *Encyclopædia Britannica*, thus states the case—

"The first numerical estimate of a diameter of a molecule was that made by Loschmidt, in 1865, from the mean path and the molecular volume. Independently of him, and of each other, Mr. Stoney, in 1868, and Sir W. Thomson, in 1870, published results of a similar kind—those of Thomson being deduced not only in this way, but from considerations derived from the thickness of soap bubbles, and from the electric action between zinc and copper.

"The diameter and the mass of a molecule, as estimated by these methods, are of course very small, but by no means infinitely so. About two millions of molecules of hydrogen in a row would occupy a millimetre, and about two hundred million million million of them would weigh a milligramme. These numbers must be considered as exceedingly rough guesses; they must be corrected by more extensive and accurate experiments as science advances; but the main result, which appears to be well established, is that the determination of the mass of a molecule is a legitimate object of scientific research, and that this mass is by no means immeasurably small.

"Loschmidt illustrates these molecular measurements by a comparison with the smallest magnitudes visible by means of a microscope. Nobert, he tells us,

[1] A. Harvey. 1, Relative influence of male and female parents, *Monthly Journal of Medical Science*, 1854. 2, On the fœtus *in utero*, Edinburgh, 1850. 3, Fœtus *in utero*, Glasgow, 1859.

can draw 4,000 lines in the breadth of a millimetre. The interval between these lines can be observed with a good microscope. A cube, whose side is the 4,000th of a millimetre, may be taken as the *minimum visible* for observers of the present day. Such a cube would contain from 60 to 100 million molecules of oxygen or of nitrogen ; but, since the molecules of organized substances contain on an average about 50 of the more elementary atoms, we may assume that the smallest organized particle visible under the microscope contains about two million molecules of organic matter. At least half of every living organism consists of water, so that the smallest living being visible under the microscope does not contain more than about a million organic molecules. Some exceedingly simple organism may be supposed built up of not more than a million similar molecules. It is impossible, however, to conceive so small a number sufficient to form a being furnished with a whole system of specialized organs. Thus molecular science sets us face to face with physiological theories. It forbids the physiologist from imagining that structural details of infinitely small dimensions can furnish an explanation of the infinite variety which exists in the properties and functions of the most minute organism.

"A microscopic germ is, we know, capable of development into a highly organized animal. Another germ, equally microscopic, becomes, when developed, an animal of a totally different kind. Do all the differences, infinite in number, which distinguish the one animal from the other, arise each from some difference in the structure of the respective germs ? Even if we admit this as possible, we shall be called upon by the advocates of Pangenesis to admit still greater marvels. For the microscopic germ, according to this theory, is no mere individual, but a representative body, containing members collected from every rank of the long-drawn ramification of the ancestral tree, the number of these members being amply sufficient not only to furnish the hereditary characteristics of every organ of the body, and every habit of the animal from birth to death, but also to afford a stock of latent gemmules to be passed on in an inactive state from germ to germ, till at last the ancestral peculiarity which it represents is revived in some remote descendant.

"Some of the exponents of this theory of heredity have attempted to elude the difficulty of placing a whole world of wonders within a body so small and so devoid of visible structure as a germ, by using the phrase structureless germs. (See F. Galton on "Blood Relationship," *Proc. Roy. Soc.*, June 13, 1872.) Now, one material system can differ from another only in the configuration and motion which it has at a given instant. To explain differences of function and development of a germ without assuming differences of structure is, therefore, to admit that the properties of a germ are not those of a purely material system."

It is since that article was published in 1875, that most of the results detailed in Chap. II. (p. 214), have been reached. The opinions expressed by the eminent physicist require modification in the light of more recent research. Neither ovum nor spermatozoid is now to be regarded as destitute of structure, and the tendency of research is to show that the peculiarities of different ova depend on differences of molecular structure. Further, small as the reproductive body formed by the fusion of the male and female elements is, it is still large enough to contain *millions* of organic molecules having a complexity of structure as great

as that of a molecule of albumin. The physiologist also lays stress on the fact that all the tendency of his work on these minute structures is in the direction of detecting *differences*, not necessarily physical in the sense of being discernible by any possible microscope, but differences of a chemical nature—in other words, molecular. Nor are the tissues so various, nor the individual peculiarities so numerous, as the physicist is apt to suppose. Much depends on vegetative repetition, so that, given the peculiarities of any individual, it is not improbable that a few thousand special types of cell formation, as regards molecular structure, would be sufficient to account for the special peculiarities. There is room enough for many such molecules in a cube of idioplasm having a side of 1-500th of an inch.

For bibliographical references to the subjects discussed in the three preceding chapters, see a paper by the author, on "The Modern Cell Theory," in the *Proceedings* of the Philosophical Society of Glasgow, Vol. XX.

Chap. IV.—FORMATION OF THE BLASTODERMIC LAYERS.

Having considered the phenomena that immediately follow the union of the ovum and spermatozoid, we shall next direct our attention to the earlier differentiation of the embryonal cells into the layers from which the tissues and organs are developed. This can be done without discussing difficult embryological questions, and without entering much into detail; and it is important to follow this course, because it will enable us to classify the tissues genetically and to observe relations existing between tissues in the embryonal condition that would not be suspected when we contrast their characters as seen in fully formed tissue.[1]

As already explained in Chap. II. of this section, p. 214, the phenomena of fecundation are followed by segmentation in which the ovum divides into two, then into four, eight, sixteen, etc., cells (Fig. 111). At the end of the segmentation process, the ovum consists of a sphere formed of primitive cells, or *blastomeres*, of uniform size, and these cells are arranged in a single layer to form a wall around a central cavity. The wall of the ovum is now called the *blastoderm*, or *germinal membrane*, and the cavity in the centre is the *segmentation cavity* of Von Baer. This is the arrangement in the early development of Amphioxus, the most primitive vertebrate, in which the stages are simple and easily understood. One side or wall of the blastosphere then becomes thickened and invaginated, so as to give rise to a layer of cells forming a lining to the outermost layer. The embryo has now the form of a

[1] Details as to the formation of organs and as to the formation of the embryonal vesicles will be given in discussing the reproductive process. It is not necessary to allude to these at present.

sphere, the wall of which is formed of two layers, an outer, termed the *epiblast* or *ectoderm*, and an inner, termed the *hypoblast* or *endoderm*, and the cavity or space in the sphere, called the *archenteric cavity* or *archenteron*, communicates with the exterior by a small aperture, the *blastopore*. At this stage the embryo is termed a *gastrula*. The embryo soon becomes much elongated, so that the blastopore is at first at the posterior end, and a little later on is seen on the dorsal surface. A groove now forms on the dorsal surface of the elongated embryo by the development of two folds of the epiblast. The lips of this groove coalesce so as to form a canal, the *neural canal*, and the blastopore opens into this canal by a foramen or passage, *canalis neurentericus*, seen in Fig. 113. This canal is closed at a later period.

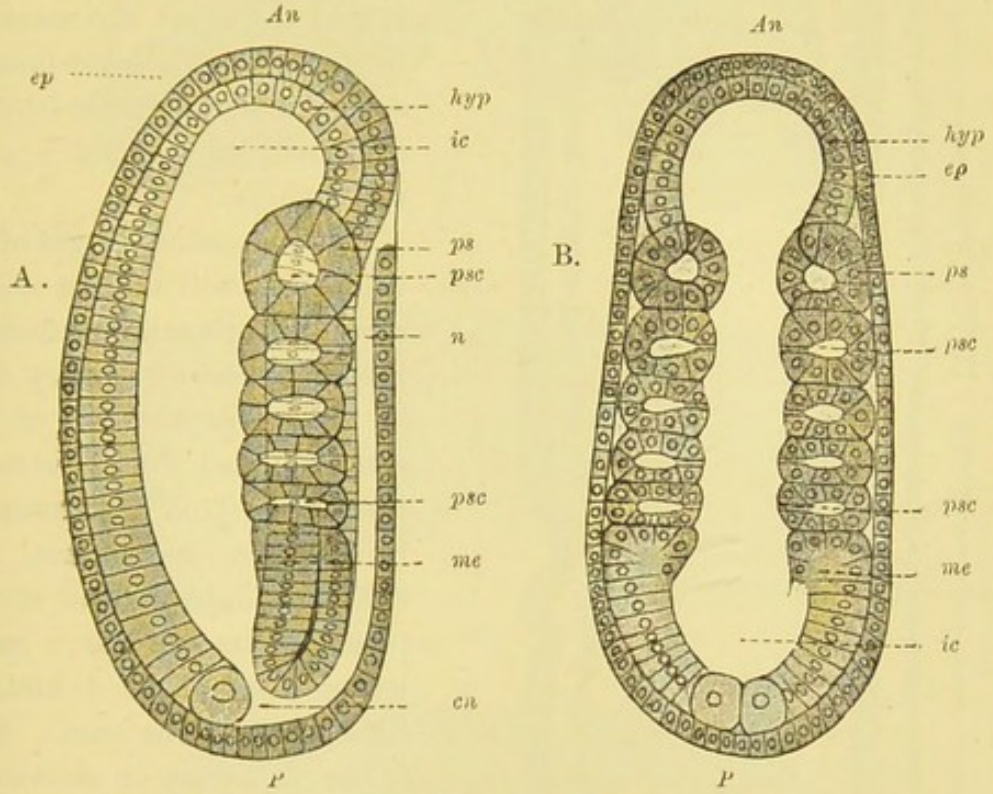


FIG. 113.—A. Longitudinal section of Amphioxus. B. Transverse section of Amphioxus. *ep*, epiblast; *hyp*, hypoblast; *me*, mesoblast; *ps*, first primitive segment; *psc*, cavity in primitive segment; *ic*, archenteric cavity; *n*, primitive neural canal; *cn*, *canalis neurentericus*. *An*, anterior; *P*, posterior end of embryo.

According to E. van Beneden, segmentation occurs in the ovum of the rabbit within one or two hours after the union of the male and female pronuclei, and the whole process is accomplished within seventy to seventy-five hours thereafter. In the segmentation process an early differentiation of cells has been noticed. Even in the cleavage of the first cell the result is the formation of two cells, one, the upper, somewhat larger than the other, the lower, and when eight cells have been formed, the four lower cells occupy a more central position than the four upper cells. Further, the upper or outer cells increase more

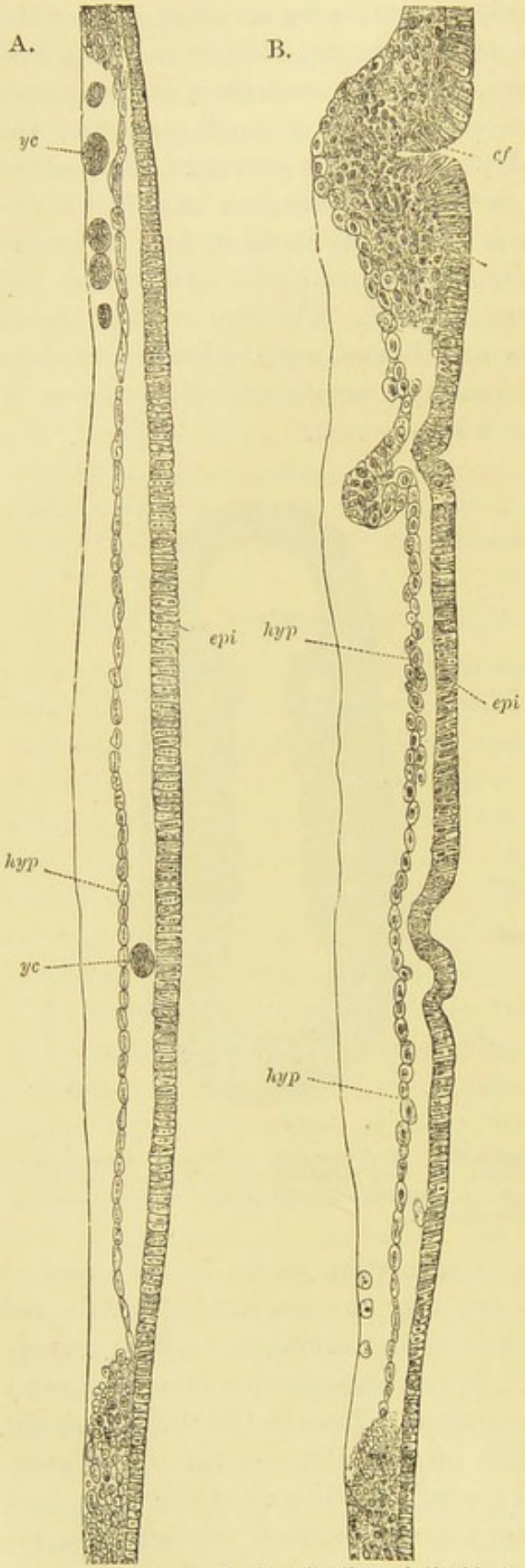


FIG. 114.—A. Longitudinal section through the blastoderm of hen's egg at end of first hour. B. Transverse section through the same. *epi*, epiblast, outer layer; *hyp*, hypoblast, inner layer; *yc*, yolk cells; *cf*, crescentic furrow. To see these layers in their natural position, turn the page so that the epiblast is uppermost.

rapidly than the lower or inner cells, so that the outer cells ultimately entirely enclose the inner cells. The invagination process producing a gastrula has not been seen in the mammalian ovum, but there exists an aperture which Van Beneden regards as the homologue of the blastopore. This aperture is soon closed by the growth of cells around it, and the ovum of a mammal now consists of a dense mass of cells. The outermost layer is composed of cells of a prismatic shape.

The succeeding stages of development will be best understood if we examine them in the first instance as they may be traced in the egg of the common fowl during incubation. The yolk is surrounded by a thin membrane, the *vitelline membrane*, and at one point of its surface a small white disc, about 4 mm. in diameter, may be seen. This is the *blastoderm* or *cicatricula*, corresponding to the layer of cells which entirely surrounds the ovum of the mammal. In the centre of the blastoderm there is a clear transparent portion, the *area pellucida*, in which the embryo is formed. A vertical section through the *area pellucida* of a fecundated egg, in which segmentation has taken place, shows that the blastoderm is formed of two layers. The upper layer consists of somewhat elongated

cells, having their long axes vertical, and adhering by their sides. These cells are of uniform size, about 9 μ, and each shows usually an oval nucleus. This layer constitutes the *epiblast*. The deeper layer of the blastoderm, termed the *hypoblast*, consists of somewhat larger cells, so highly granular as to conceal the nucleus. Between the blastoderm and the underlying yolk there is a space, the homologue of the segmentation cavity to which reference has already been made. See Figs. 114 and 115.

After a few hours of incubation, the *area pellucida* loses its circular

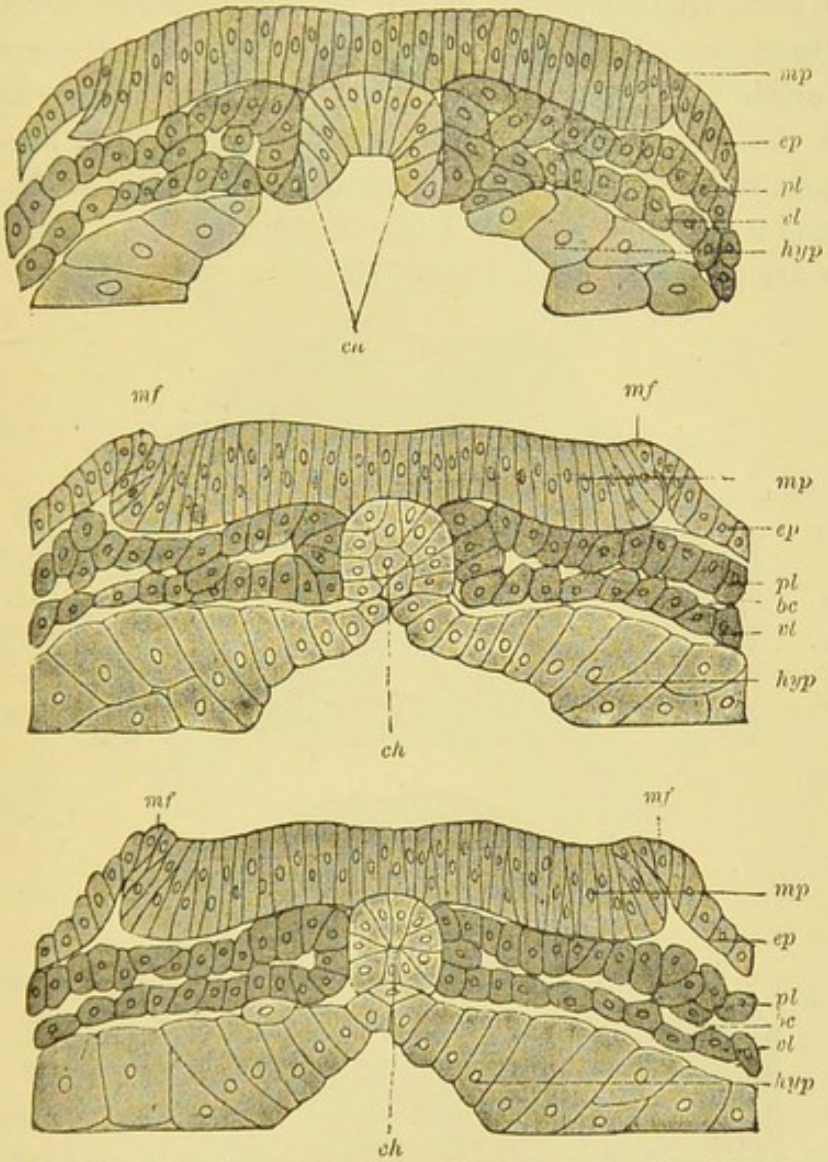


FIG. 115.—Three transverse sections through an egg in which the medullary ridge is beginning to form. The sections show the stages in the development of the *chorda dorsalis*, and the formation of the two layers of the mesoblast. *ep*, epiblast; *hyp*, hypoblast; *pl*, parietal layer of mesoblast; *vl*, visceral layer of mesoblast; *mp*, medullary plates; *mf*, medullary folds; *ch*, chorda; *bc*, body cavity or pleuro-peritoneal cavity.

outline and becomes oval and then pear-shaped, and towards the narrow end of the pear-shaped area an opaque thickened portion makes its

appearance. This is the primitive *streak* or *trace*. A groove—the *primitive groove*—then appears on the upper surface of the streak. About this period, that is to say, between the eighth and twelfth hour of incubation, a middle layer of the blastoderm—the *mesoblast* or *mesoderm*—begins to be formed. The primitive streak is a mass of cells arising from proliferation of cells derived from the epiblast, and may be regarded as the commencement of the middle layer in connection with the epiblast. About the sixteenth hour, two ridges or folds of the epiblast rise on each side of the primitive streak. These ridges, termed the *dorsal ridges* or *medullary folds*, enclose plates known as the *medullary plates*, and the ridges ultimately coalesce so as to form a closed tube—the *neural canal*—giving origin to the primitive cerebro-spinal axis. Below the medullary tube thus formed, the *chorda dorsalis* or notochord, an elongated cartilaginous rod, appears, and this becomes the centres of the bodies of the permanent vertebræ and the basis of the cranium. Some of these structures are seen in Figs. 115 and 116.

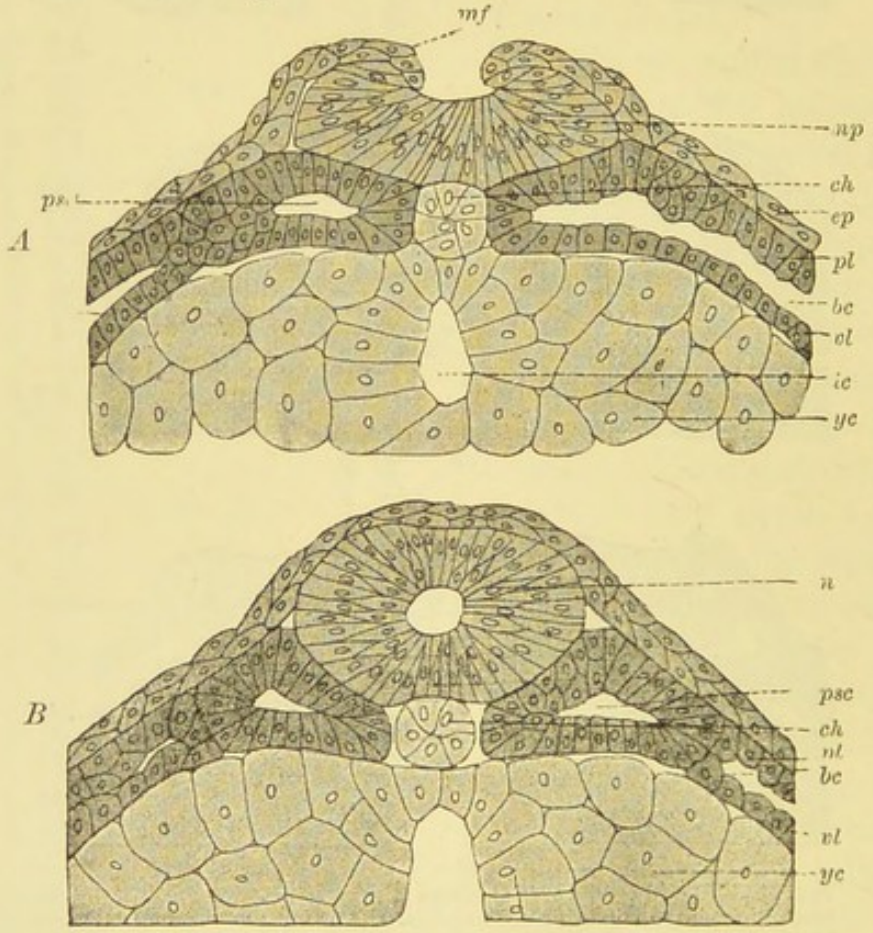


FIG. 116.—Two transverse sections through the embryo of a Newt. *A*, Transverse section through the body, in which the neural canal is not yet closed and the primitive segments are beginning to separate from the ventral laminæ. *B*, Transverse section in which the neural canal is closed and the primitive segments are fully formed. *mf*, medullary folds; *mp*, medullary plate; *n*, nervous tube; *ch*, *chorda dorsalis*; *ep*, epiblast; *pl*, parietal layer of mesoblast; *vl*, visceral layer of mesoblast; *ic*, intestinal canal; *bc*, body cavity; *psc*, cavity in primitive segment; *yc*, yolk cells.

As already pointed out, the mesoblast arises chiefly from the deeper layer of cells of the epiblast, but according to F. M. Balfour and His, it proceeds also in part from the hypoblast and from nuclei in the germinal wall of the yolk at the outer surface of the latter. The mesoblast, in turn, divides into two layers, an outer, termed the *somatic* or *parietal mesoblast* or *somatopleure*, which uniting with the epiblast gives origin to the osseous, fibrous, muscular, and tegumentary substance of the body-

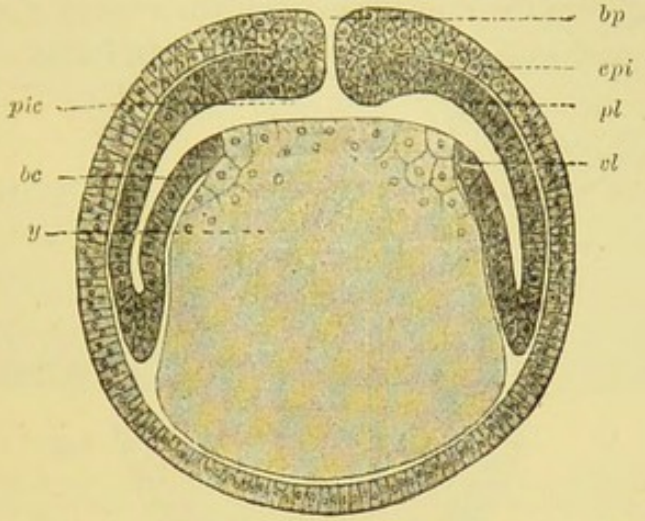


FIG. 117.—Diagram showing the development of the mesoblast and of the body cavity in the vertebrata. Transverse section through the blastopore of an embryo. *bp*, blastopore; *pic*, primitive intestinal cavity; *bc*, body cavity; *y*, yolk; *epi*, epiblast; *pl*, parietal layer of mesoblast; *vl*, visceral layer of mesoblast.

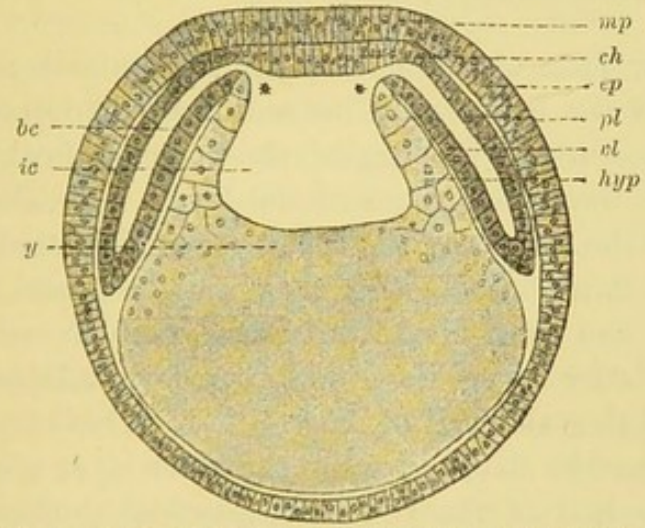


FIG. 118.—Diagram of the development of the mesoblast and of the body cavity of a vertebrate. Transverse section through an embryo in front of the blastopore. *mp*, medullary plate; *ch*, beginning of the *chorda dorsalis*; *ep*, outer germinal layer or epiblast; *hyp*, inner germinal layer or hypoblast; *pl*, parietal layer of middle germinal layer or mesoblast; *vl*, visceral layer of middle germinal layer or mesoblast; *y*, yolk; *ic*, intestinal canal; *bc*, body cavity.

wall and limbs; and an inner, the *visceral mesoblast* or *splanchnopleure*, which, uniting with the hypoblast, forms the fibrous and muscular wall

of the alimentary canal, the lymph and blood-vascular system, and the urinary and generative organs. Between the two layers of the mesoblast we have the general pleuro-peritoneal space or the body-cavity. The formation of these layers is well shown in Figs. 117 and 118.

The mesoblast in amphioxus, instead of arising from the epiblast, originates in two diverticula or recesses constricted off from the archenteric cavity, and the notochord is also developed from a third diverticulum springing from the dorsal aspect of the archenteric cavity, a type of development met with in various invertebrate forms. This is shown in Fig. 119.

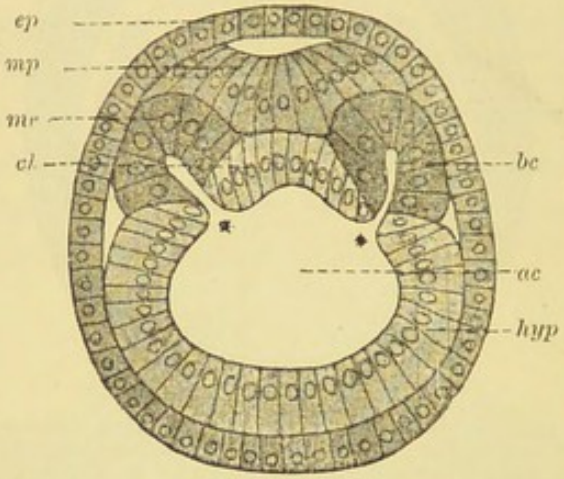
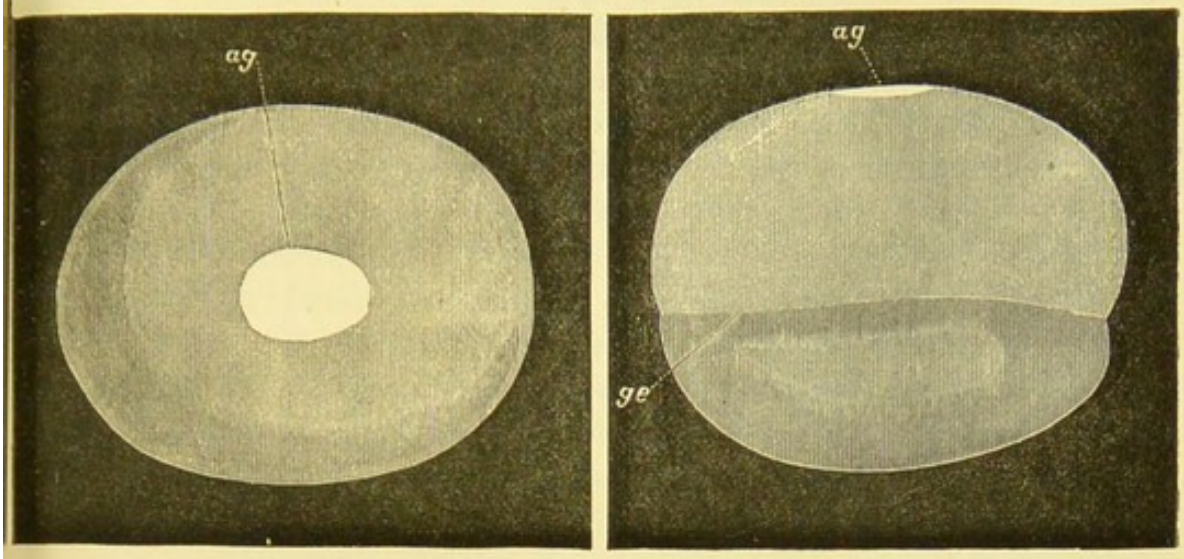


FIG. 119.—Transverse section through embryo of Amphioxus. *ep*, epiblast; *me*, mesoblast; *hyp*, hypoblast; *mp*, medullary or neural plate; *ch*, *chorda dorsalis*; *ac*, archenteric cavity; *bc*, body cavity.

The vesicular form of the blastoderm of mammals, arising from the segmentation of the entire yolk instead of segmentation of only a portion of it, as occurs in the egg of the common fowl, causes certain modifications in the development of the layers. As already described, the covering of the ovum consists of a layer of elongated nucleated cells (Fig. 115), and in the interior there is a semi-fluid mass of broken down yolk substance and some of the primitive granular cells, or segmental spheres. The latter by proliferation form a layer adapted to the outermost layer, and thus the wall of the vesicle soon has two layers. In the rabbit, about the fifth day, and when the inner layer of cells has spread over about one half of the vesicle, a discoidal thickening or opacity appears in the middle of the blastoderm. This is the embryonal area seen in Fig. 120, and in it the primitive streak and primitive groove appear as in birds, and then follows the development, in a similar manner, of the nervous system and of the chorda dorsalis.

The formation of the three layers of the embryo having thus been traced, it remains to refer to these layers the genesis of the tissues and of the more important organs of the body.

1. The *Epiblast* gives rise to (*a*) the central nervous system; from the infolding of the medullary plates to form the neural tube (see Fig. 116), the white and grey matter of the brain and spinal cord is formed;



A. B

FIG. 120.—Views of the Blastodermic Vesicle of a rabbit on the seventh day, without the *zona pellucida*, or outer layer of the egg. A, From above; B, from side. *ag*, area in which embryo appears; *ge*, boundary of the hypoblast.

and (*b*) the sympathetic nervous system and the peripheral nerves, both cranial and spinal, and ganglia, are also derived from the epiblast. (*c*) The ciliated epithelium in the central canal of the spinal cord and in the cerebral ventricles is epiblastic. (*d*) The epiblast gives origin to the epidermis and to all structures of epidermic nature, such as horn, hoof, nail, feather, etc., and also to the sebaceous and sweat glands. (*e*) The sensory expansions in the organs of special sense originate in the epiblast, either by involutions of the medullary or neural canal (retina and pigment epithelium of choroid), or by involutions of the external epiblastic layer covering the embryo (the sensory structures in the ear, in the nose, taste cells, touch corpuscles). (*f*) The crystalline lens is formed of infolded epiblast. (*g*) The epithelium lining the cavity of the mouth and anus, the epithelium of the glands and other structures related to these cavities, the enamel of tooth and the salivary glands are derived from the epiblast. (*h*) Lastly, the pituitary body belongs to this layer.

2. The *Mesoblast* gives origin to (*a*) the connective tissues; (*b*) the muscles; (*c*) the bones and cartilages; (*d*) the heart, arteries, veins, capillaries, lymphatics, and serous membranes, with their lining cells or endothelium; and (*e*) the embryonic blood and lymph corpuscles. From it also arise (*f*) the generative organs, their epithelium, and the generative elements, ova, and spermatozoids; (*g*) the urinary organs and a

part of the epithelium of the tubules of the kidney; and (*h*) the muscular, vascular, and connective tissue elements in the wall of the alimentary canal and in the skin. The muscular fibres of the sweat glands of the skin are said to be epiblastic. (*i*) The spleen and other blood glands also spring from this layer.

3. The *Hypoblast* is the layer from which most of the epithelial structures are derived, such as (*a*) the epithelium of the alimentary canal; (*b*) the epithelium of the respiratory organs, trachea, bronchial tubes, and pulmonary air cells; (*c*) the epithelium of the Eustachian tube and tympanum of the ear; (*d*) the epithelium of the ducts of the liver, pancreas, and of glands opening into the alimentary canal; (*e*) the hepatic cells; (*f*) the secreting cells of the pancreas; (*g*) the epithelium of the thyroid body and of part of the thymus gland; and (*h*) the epithelium of the urinary bladder and ureters and of a portion of the tubules of the kidney; (*i*) the hypoblast is also the parent of the notochord.

Chap. V.—THE MICROSCOPE AND THE METHODS OF MICROSCOPICAL RESEARCH.

A knowledge of the minute structure of the tissues and of the organs of the body is acquired by the use of the *microscope*, one of the most important instruments of physiological research. The microscope, more especially in recent years, has been so much improved, both in its mechanical and in its optical appliances, that it may now be considered as the scientific instrument of all others that has almost, if not quite, reached perfection, or at all events has reached such a condition, more especially as regards its optical arrangements, that we can scarcely hope to witness any marvellous improvement. Further, from the time of the first enunciation of the cell theory of the origin of tissues in 1838, most of our histological knowledge has been gained by instruments of comparatively low magnifying power and with imperfect methods of illumination, and it is only during the last few years that physiologists have availed themselves of the use of the most improved lenses, of new methods of illumination, and of the art of photography as applied to the microscope. During these years, however, immense progress has been made in this direction and there is a feeling amongst physiologists that the time has come for a new departure in histological work and that in the future the histologist will call to his aid those magnificent instruments and lenses and methods of work which have hitherto received attention chiefly from those interested in the mere optical properties of lenses or in the resolving of the fine lines on the valve of a diatom. Just as the astronomer in a great observatory requires all the aid of the finest

telescopes, the most complete mechanical adjustments, the application of the spectroscope and of photography to enable him to resolve nebulæ into star clusters, so the histologist of the future, by applying the best methods and using the best instruments, hopes to detect details of minute structure in organic bodies which have hitherto been described as structureless or as consisting merely of granular or jelly-like matter. Nor is this a mere dream. Already in these last few years, by the labours of Flemming, Klein, Van Beneden, Carnoy, and many others, a new development has been given to our views of cell structure, of cell division, and of fecundation, by the use of high powers and elaborate mechanical and chemical methods. Nor need this view of the matter interfere with the appreciation of simple microscopes and comparatively low powers for ordinary work. It is quite true that the great majority of histological facts has been observed with the aid of such instruments, and that for ordinary purposes such instruments are of the greatest service, but we must not forget that these simpler instruments with low powers bear the same relation to the more complex instruments, fitted with immersion lenses and achromatic illuminating condensers, that an ordinary field glass does to the best kind of astronomical telescope in an observatory, and that for many investigations the finer instrument in both cases is absolutely necessary.

1. **The Microscope.**—The form of microscope most convenient for ordinary histological work is such as is represented in Fig. 123. It should be provided with two objectives (Nos. 3 and 7) and two eyepieces (1 and 2).[1] This will give a magnifying power of from 25 to 350 diameters. A section of an ocular or eyepiece is shown in Fig. 121, and a section of an objective in Fig. 122. The larger form of microscope as made by Zeiss, of Jena, and shown in Fig. 124, is necessary for more refined work, and especially for bacteriological investigations. Such a microscope is supplied with an Abbe's condenser, placed underneath the stage, by which strong illumination may be obtained. This important adjunct to the microscope is shown in Fig. 125, and in section in Fig. 126.

If high powers, ranging from 350 to 1000 diameters, are required, preference should be given to homogeneous oil immersion lenses, having a large angle of aperture.[2]

[1] The cost of such a microscope is £5 10s.

[2] A large microscope, similar to that shown in Fig. 124, with Abbe's condenser, objectives, Nos. 4 and 7a; eyepieces, Nos. 3 and 4; and a $\frac{1}{12}$-inch homogeneous oil immersion lens can be had from Reichert, of Vienna, for about £21.

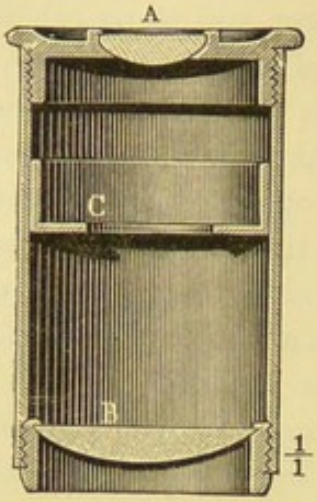


FIG. 121.—Section of Huygheuian Eyepiece or Ocular. A, eyeglass; B, field glass; C, diaphragm.

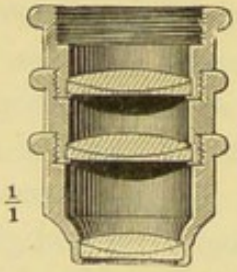


FIG. 122.—Section of Objective containing three compound achromatic lenses, each formed of a double convex lens of crown glass, cemented by Canada balsam to a plano-concave lens of flint glass.

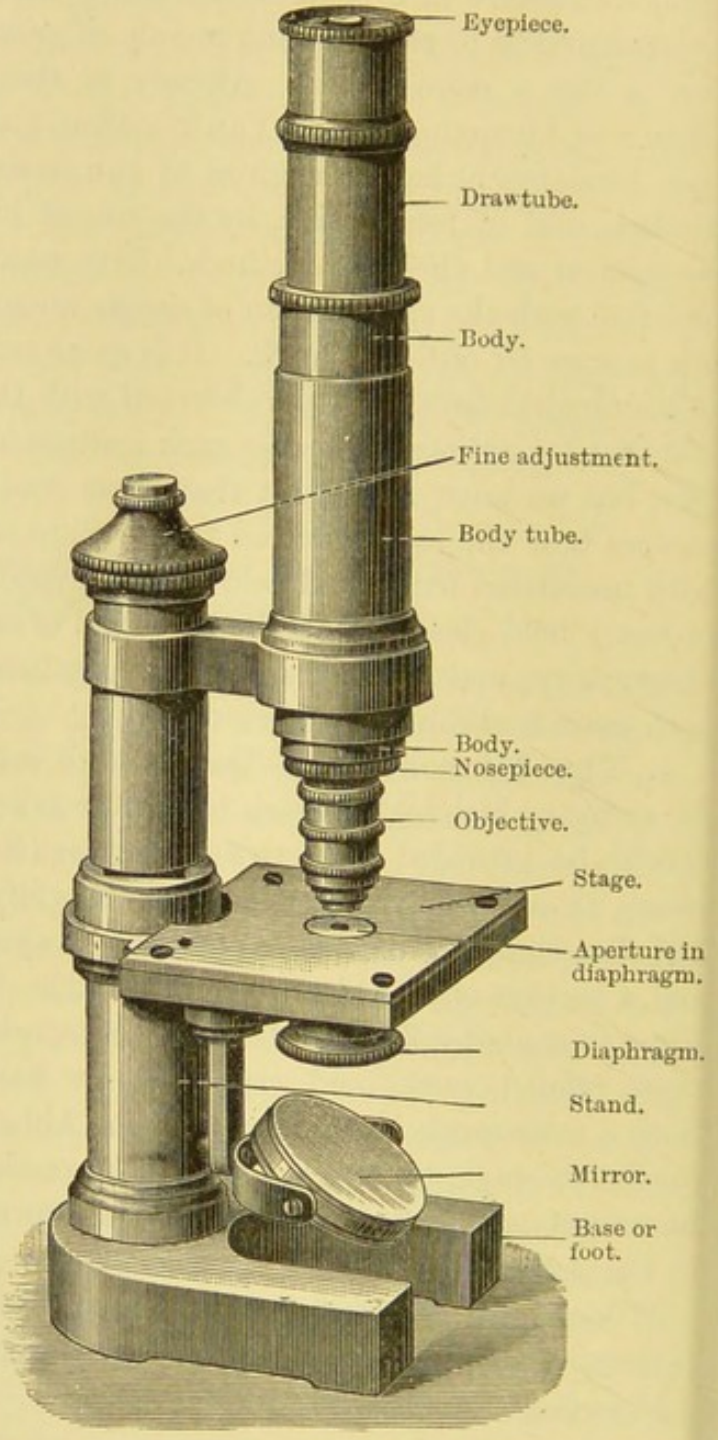


FIG. 123.—Leitz's Microscope, No. III. Half natural size.

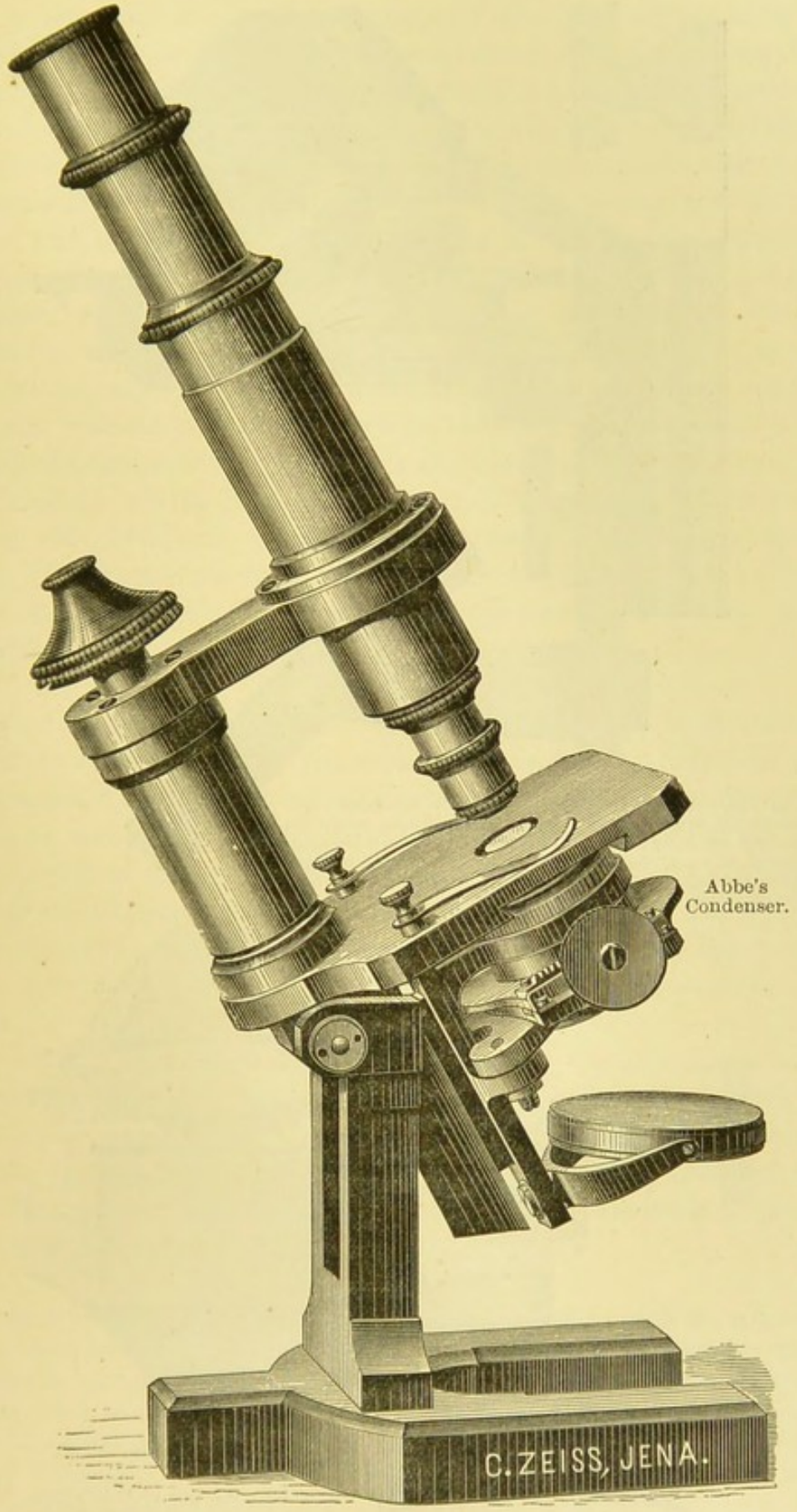


FIG. 124.—Zeiss' Microscope, Stand V.a, with Abbe's Condenser below the stage. Two thirds natural size.

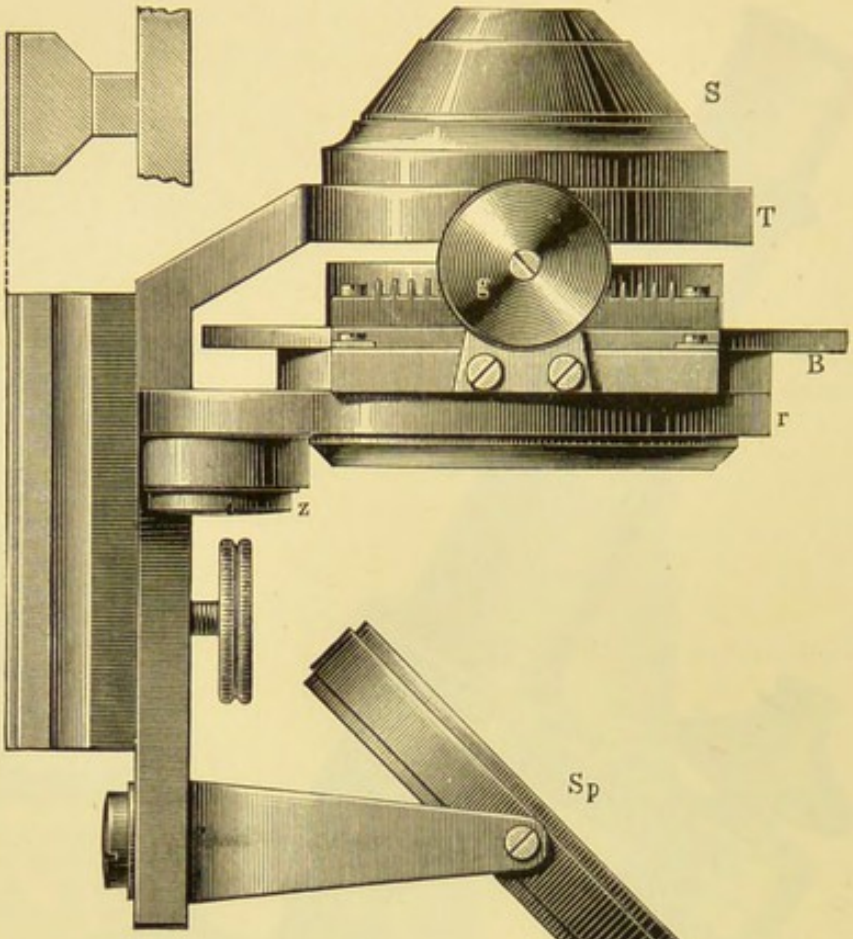


FIG. 125.—Abbe's Condenser, as made by Zeiss; Sp, mirror; B, brass framework containinglenses, as shown in the next figure; g, for rotating the diaphragm, placed at r; z, movement by which the frame bearing the diaphragm can be pulled out from below the condensing lens, S.

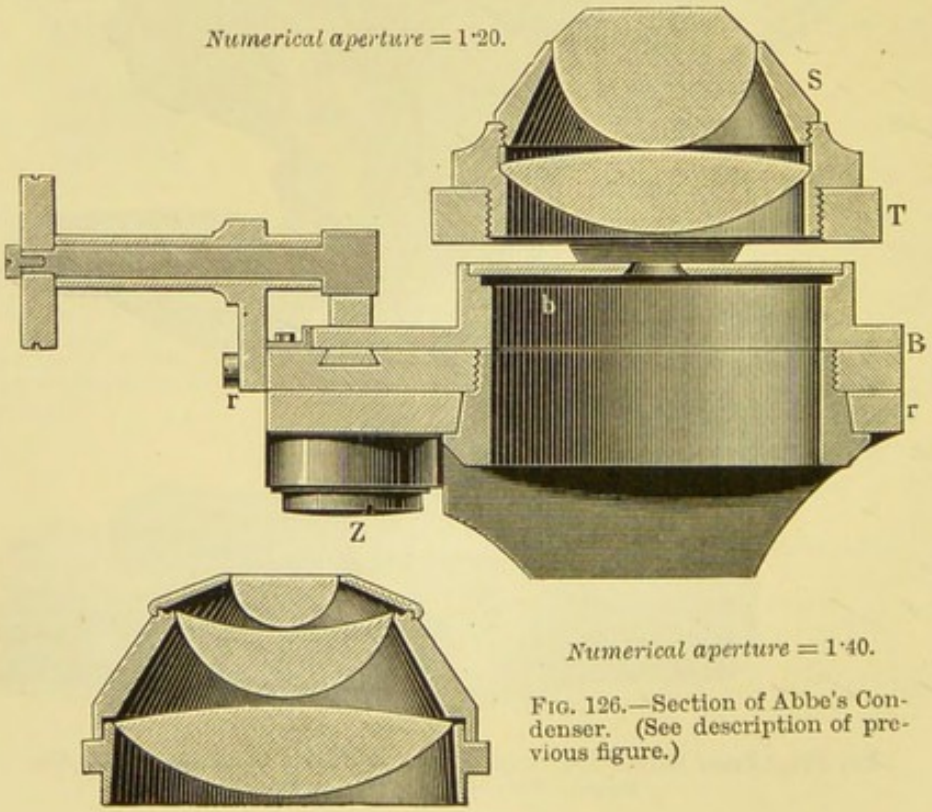


FIG. 126.—Section of Abbe's Condenser. (See description of previous figure.)

It is essential to the maintenance of the good condition of the microscope that it should be protected from dust when frequently employed, and it is best to preserve it under a bell glass in a place not exposed to the rays of the sun.[1] The dust, etc., which accumulates on the tube can be wiped away with a small dry piece of soft filter paper: impurities of the lenses[2] and of the mirror are to be removed with soft leather, and if this fails to attain the end in view (as, for instance, in the case of soiling with Dammar varnish), a small soft linen duster, moistened with a drop of pure alcohol, may be employed. In the process last mentioned, one must be very careful that the spirit of wine does not in any way penetrate into the setting of the lenses and dissolve the Canada balsam, with which they are fixed in their places. Rub off the stain quickly with the moistened part of the duster, and then carefully dry the lens. The screws of the microscope should be cleaned with petroleum. Take particular care of the fine adjustment screw. This should never be screwed either too far up or too far down, and it should only be employed for delicate focusing, never for *finding* the object.

Mode of Using the Microscope.—The first condition is the complete clearness of the various lenses. The mirror, objectives, and eyepieces must not be touched on their upper surface with the fingers. Hold the object-glasses by the under end up against the window and test the clearness of the reflected image. The screwing on to the tube is effected by holding the objective fast and turning the tube round. The eyepiece is then inserted; impurities on it can be recognized by turning it round in the tube; if any impurity adheres to the eyepiece, it revolves along with it.

Now find light. To do so, draw the body of the microscope out of the draw tube for a distance of about half an inch, look through the microscope towards the opening in the diaphragm and move the mirror until the light is reflected upwards. The rays of light reflected from the mirror so placed strike the object vertically. This mode of illumination is termed *central illumination.*

[1] In the preparation of the portion of this chapter dealing with the microscope and with histological methods, I have largely availed myself of Professor Stöhr's directions in his work, *Lehrbuch der Histologie*, and with the greater confidence, as the methods therein described are substantially those followed in the laboratory of the University of Glasgow, in teaching practical histology. I purchased the right of translation of this work from the publisher, Gustav Fischer, of Jena, with Professor Stöhr's consent.

[2] The lenses of the objective must never be screwed from each other.

In order to recognize finer differences of surface, we may advantageously employ the *oblique* or *lateral* illumination, in which the mirror is pushed on its side in such a manner that the rays of light reflected from it fall obliquely on the object. As sources of light, a white cloud illumined by the sun, or white curtains on which the sun shines, are to be chosen; less good, but still serviceable, is the blue sky; direct sunlight must be avoided. If in the evening we work with artificial light, we must derive the light from the inner surface of a lamp shade, not directly from the flame. A green plate of glass, placed before the mirror, tones down the artificial light without essentially injuring the distinctness of the images. It is self-evident that the investigator with the microscope should not sit in the sunshine; he should place the microscope at a distance of about two metres from the window.

The investigation may now begin. Always investigate, *first of all, with weak, and then with strong magnifying powers, and be very specially warned against the use of strong oculars.* The weakest oculars given with the usual microscopes (and even the medium ocular, Oc. 1 of Leitz) are adequate for the majority of cases: too strong oculars lessen and darken the field of vision, and render the investigation difficult in a high degree. Also the drawing out of the tube is usually unnecessary. When using low powers, have the largest aperture of the diaphragm under the opening in the stage; and when using high magnifying powers, have the smallest aperture in this position. For ordinary objectives (No. 3 and No. 7), the concave mirror is to be used. In finding the object, do not force the tube straight down, but lower it by revolving it spirally until the object on the stage comes indistinctly into view. Then use the fine adjustment until complete distinctness of the object has been attained. Thereupon let the left hand hold the slide, and the right hand rest on the micrometer screw. As we see clearly only the points of the preparation lying in *one* plane, scrutinize the preparation during the delicate raising and lowering of the tube, *i.e.* during the slow turning of the micrometer screw. In addition to this, keep both eyes open during microscopic investigation.

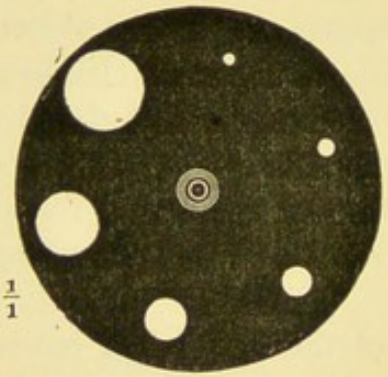


FIG. 127.—Diaphragm.

The *delineation* or sketching of microscopic objects is an invaluable aid to correct observation. Thus many details are shown which might otherwise have been completely overlooked. Even the most attentive contemplation cannot supply the advantages which drawing the objects

commands. Endeavour also to sketch objects seen by low and high powers. In drawing, place the paper on the level of the stage, look into the microscope with the left eye, and with the right look on the paper and the point of the lead pencil. At first this occasions a little difficulty; after some practice we rapidly acquire the necessary dexterity. When it is necessary to make an exact *fac-simile* drawing of an object, a *camera lucida* should be used, of which by far the most convenient is that shown in Fig. 128.

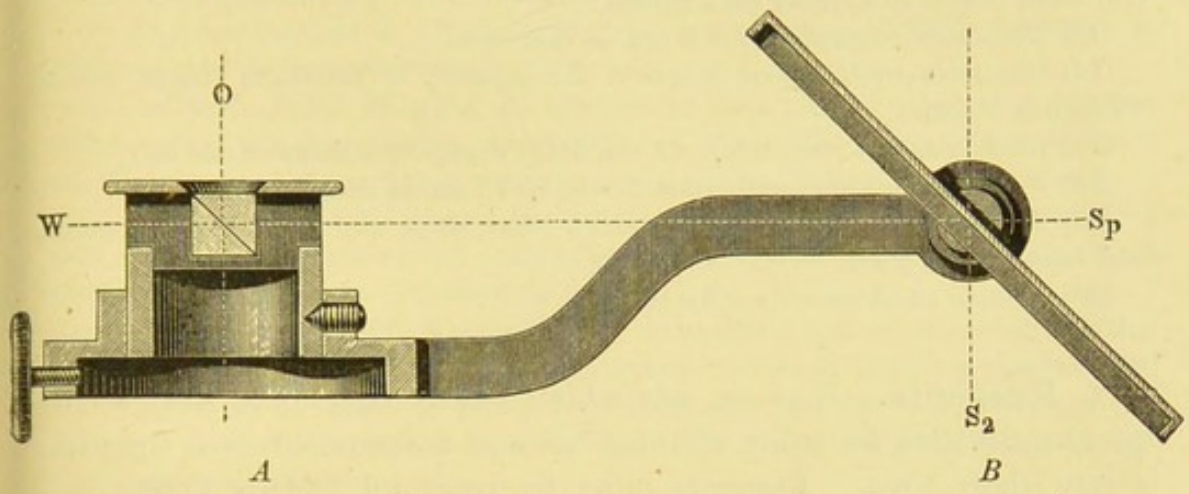


FIG. 128.—Abbe's Camera Lucida, made by Zeiss. *A*, Part screwed over the eyepiece. *B*, Mirror, reflecting light, coming from paper in direction S_2, at right angles in direction *Sp—W*. This light is caught by the prism and reflected in direction of *O*, the same direction as that followed by the rays coming from the object in the direction *AO*.

2. **Accessory Appliances.**—The following are convenient requisites for histological work—

(1) A good *razor*, the blade of which has been flattened on one side. The blade must always be kept sharp and must, previous to actual use, be passed over the strop. Let the razor be employed *exclusively* for the cutting of fine sections.

(2) A *fine whetstone.*

(3) A neat straight *pair of scissors.*

(4) A delicate, easily shutting *pair of forceps*, with smooth or only slightly indented prongs.

(5) Four *needles with wooden handles.* Let two of them be heated until they can easily be bent; let them be again heated, and then stuck into solid paraffin, by which they will again be hardened. In fine teazing, the needles must be sharpened and polished first on the whetstone, and then on the strop. The cataract needles of oculists are very useful.

(6) *A flat spatula*, made of German silver, for lifting the sections from fluids on to the slide. Instead of this, a broad-bladed knife may be used. One of those in a case of anatomical instruments suits quite well.

(7) *Pins*, *hedge-hog bristles*, a small *paint brush*, a *brush* for dusting glasses.

(8) *Slides* (of the usual shape) should be made of pure glass, and not too thick (1-1·5 mm.).

(9) *Cover-glasses* of 15 mm. broad are for most objects sufficiently broad; their thickness may vary from 0·1 to 0·2 mm.

(10) *Glass phials* to the number of a dozen, with a wide neck, of the capacity of 30 c.c. and upwards.

(11) *Some larger preparation glasses*, with ground-glass stoppers; height, 7-10 cm.; diameter, 6-10 cm.

(12) *A graduated cylindrical glass*, holding 100-150 c.cm.

(13) *A glass funnel* of 8-10 cm. at its upper diameter.

(14) *Pipette.* The student can supply himself with pipettes if he draws off to a point at one end, in the blow-pipe flame, a small glass tube about 1 cm. in diameter and 10 cm. in length, and fixes on the other end a piece of india-rubber tubing, 6 cm. long, tied with a strong pack thread.

(15) *One dozen watch glasses* of 5 cm. in diameter.

(16) *One dozen small reagent glasses* of the capacity of 10 cm. in length, and of 12 mm. in width.

(17) *Glass rods* of 3 mm. thick, 15 cm. long, drawn to a point at one end.

(18) *Preparation dishes* with glass lids of 10-12 cm. in diameter.

(19) *Two sheets of blotting paper, a cork plate, large and small gummed cards, soft linen rags, a towel.*

(20) *A large earthenware pot* for refuse.

3. **Reagents.**—It is not advisable to keep reagents in store in too great quantities, for many of them become decomposed in a comparatively short time. Reagents must be procured shortly before use. Every flask must be provided with a large label indicating its contents. It is recommended that not only should the prescription of the particular fluid be indicated on the label, but also the method of employing it. All the flasks should be closed tightly with corks or with good glass stoppers. The fluid must not reach to the under surface of the cork.

(1) *Distilled water*, 3-6 litres.

(2) *Solution of common salt*, 0·75 per cent.: distilled water, 200 c.cm.; common salt, 1·5 grms. The cork of the flask must be provided with a glass rod reaching down to the bottom of the flask. The liquid decomposes readily, and must be frequently prepared anew.

(3) *Alcohol*—(*a*) *Absolute alcohol.* Of this, it is necessary to have 200 c.cm. in readiness. The absolute alcohol of commerce is of the strength of 96 per cent., and it is, in the majority of cases, at once available for microscopic purposes. If, however, anyone wishes to preserve in alcohol completely free of water, he must throw into the flask some small pieces (15 grms. to 100 c.cm. of alcohol) of dried sulphate of copper ($CuSO_4$) that has been heated to a white heat. In the event of its turning blue, it must either give place to a fresh supply, or be burned anew. Newly burnt chalk likewise serves a like purpose, only its operation is more protracted. (*b*) *Pure spirit* (strength, 90 per cent. of alcohol), 3-5 litres; may be called 90 per cent. alcohol. (*c*) 70 *per cent. alcohol.* 500 c.cm. are to be made by the mixture of 390 c.cm. of 90 per cent. alcohol with 110 c.cm. of distilled water. (*d*) *Ranvier's third-part alcohol*, or, shortly, *Ranvier's alcohol.* 45 c.cm. of 90 per cent. alcohol + 85 c.cm. of distilled water.

(4) *Acetic acid*, 50 c.cm. The kind used by chemists is 33 per cent. strong.

(5) *Acetate of iron* (that purchasable in chemists' shops is 96 per cent. strong) must be inspected shortly before use (amount, 10 c.cm.).

(6) *Nitric acid.* It is necessary to possess a flask containing 100 c.cm. of concentrated nitric acid of specific gravity, 1·18.

(7) *Pure hydrochloric acid*, 50 c.cm.

(8) *Chromic acid.* Make a 10 per cent. solution (10 grms. of newly procured crystallized chromic acid to be dissolved in 90 c.cm. of distilled water). Of this, make (*a*) a solution of chromic acid, ·1 per cent. (10 c.cm. of the strong solution to 990 c.cm. of distilled water), and (*b*) a ·5 per cent. solution of chromic acid (50 c.cm. of the first solution to 950 c.cm. of distilled water).

(9) *Bichromate of potash.* Dissolve 25 grms. in 1,000 c.cm. of distilled water. It dissolves slowly (from three to six days).

(10) *Müller's fluid.* 30 grms. of sulphate of soda and 60 grms. of pulverized bichromate of potash are dissolved in 3,000 c.cm. of distilled water. The solution is effected slowly at the ordinary temperature of a room (from three to six days).

(11) *Picric acid.* Keep 50 grms. of crystals, and also a saturated aqueous solution (500 c.cm). The crystals must always be in a layer, from 2 to 3 mm. in depth, at the bottom of the flask. It dissolves easily.

(12) *Sulpho-picric acid* (Kleinenberg). Into 200 c.cm. of saturated solution of picric acid, pour 4 c.cm. of pure sulphuric acid; a dense precipitate immediately forms. After the lapse of an hour, this mixture is filtered, and the filtrate is diluted with 600 c.cm. of distilled water. The residuum left behind in the filter is to be thrown away.

(13) *Osmic acid.* Purchase 50 c.cm. of 2 per cent. aqueous solution. It must be kept in a dark place or in a dark glass, and, when well closed up, it keeps for many months.

(14) *Chromo-osmium acetic acid.* Prepare a solution of chromic acid, 1 per cent. strong (5 c.cm. of a 10 per cent. solution to 45 c.cm. of distilled water), and pour into it 12 c.cm. of 2 per cent. solution of osmic acid, and add to it 3 c.cm. of acetic acid. This mixture need not be kept in the dark, and is available for use for a long time.

(15) *Nitrate of silver.* Procure, a short time previous to using it, a solution of nitrate of silver, 1 grm. in 100 c.cm. of distilled water. The fluid must be kept in the dark or in a black bottle. It keeps for a long time.

(16) *Chloride of gold.* Procure shortly before use a solution of 1 grm. of chloride of gold in 100 c.cm. of distilled water. Must be kept in the dark or in a black or dark brown flask.

(17) *Formic acid*, 50 c.cm., for chloride of gold staining.

(18) *Concentrated* (35 per cent.) *caustic potash*, 30 c.cm. The bottle must be closed with an unvulcanized India-rubber stopper which is penetrated by a glass rod.

(19) *Glycerine.* It is necessary to have at hand 100 c.cm. of pure glycerine, as well as a solution of 5 c.cm. of pure glycerine in 25 c.cm. of distilled water. As a safeguard against fungi, which speedily appear in this mixture, there may be added to it 5 to 10 drops of a pure 1 per cent. solution of carbolic acid. The cork of the bottle must be provided with a glass rod, as also in the case of—

(20) *Lavender oil*, 20 c.cm. Oil of cloves serves the same purpose.

(21) *Dammar varnish.* It can be bought in bottles, containing 50 c.cm., from drysalters, and, when too viscid, be diluted with pure oil of turpentine. It possesses the requisite degree of consistency when, if a glass rod be dipped in it, the drops fall off without dragging long threads after them. Dammar

varnish is to be preferred to the too strongly refractive Canada balsam (which is diluted with chloroform), but it possesses the drawback of drying very slowly, while Canada balsam dries quickly. The cork of the flask must be provided with a glass rod.

(22) *Cement for cover-glass.* Venetian turpentine may be diluted with sulphuric ether until the whole forms a fluid trickling easily in drops; next it is filtered while hot in a funnel surrounded by hot water, and the filtrate is thickened on the sandbath. The proper consistence has been attained when a drop of it placed by a glass rod on a slide immediately becomes stiffened to such an extent that it can no longer be compressed by the finger nail. It may be prepared in a chemist's as a safeguard against danger from burning. *Zinc-white* is also an excellent cement. *Asphalt-varnish* cannot be recommended.

(23) *Hæmatoxylin.* (1) 1 grm. of crystallized hæmatoxylin is dissolved in 10 c.cm. of absolute alcohol. (2) 20 grms. of alum are dissolved in 200 c.cm. of distilled water, when warm, and filtered after cooling. On the following day, both solutions are mixed, and they are allowed to stand for eight days in a wide-mouthed vessel. The mixture is then filtered,[1] and is ready for use. Diminution of transparency from development of fungi does not in the least injure its usefulness.

(24) Weigert's *hæmatoxylin* for the demonstration of medullated nerve fibres of the brain and spinal marrow. 1 grm. of crystallized hæmatoxylin is placed in 10 c.cm. of absolute alcohol + 90 c.cm. of distilled water, and then boiled. After cooling, there is added to it 1 to 2 c.cm. of a solution—saturated when cold—of carbonate of lithium. The employment of this reagent demands previous treatment with (*a*) *Saturated solution of neutral acetate of copper*, 300 c.cm., and a subsequent treatment with a (*b*) *Solution of ferridcyanide of potassium and of borax.* 2 grms. borax and 2½ grms. of ferridcyanide of potassium are dissolved in 100 c.cm. of distilled water.

(25) *Neutral solution of carmine.* 1 grm. of the best carmine is dissolved cold in 50 c.cm. of distilled water + 5 c.cm. liquor ammoniæ. The brilliant cherry-red fluid remains exposed until it no longer yields an odour of ammonia (two days) and it is then filtered. To be kept ready at hand. The odour of this solution may become very offensive, but its colouring power is not thereby injured.

(26) *Picrocarmine.* Pour into 50 c.cm. of distilled water 5 c.cm. of liquor ammoniæ, and throw into this mixture 1 grm. of the best carmine. Stir round with a glass rod. After the solution of the carmine has been completed (in about five minutes), pour into it 50 c.cm. of a saturated solution of picric acid, and then let the whole stand for two days in a wide-mouthed vessel. Then filter it. Even abundant development of fungi does not injure the colouring power of this excellent reagent.

(27) *Borax carmine.* 4 grms. of borax are dissolved in 100 c.cm. of warm distilled water. After the solution has cooled, 3 grms. of good carmine are added during stirring, and then 100 c.cm. of 70 per cent. alcohol are poured in. After the lapse of twenty-four hours, filter the fluid, which drops very slowly, taking twenty-four hours and even longer in passing through the filter. The staining by borax carmine demands subsequent treatment with 70 per cent. hydrochloric-acid-alcohol,

[1] After the cooling of the alum, as well as after the hæmatoxylin-alum mixture has stood exposed for eight days, there are found (especially at a lower temperature) alum crystals at the bottom of the vessel, which are not of further use.

which is prepared by means of the addition of 4 to 6 drops of pure hydrochloric acid to 100 c.cm. of 70 per cent. alcohol.

(28) *Saffranin.* 2 grms. of the dye are to be dissolved in 60 c.cm. of 50 per cent. alcohol (33 c.cm. of 90 per cent. alcohol + 27 c.cm. of distilled water). Staining with saffranin requires subsequent treatment with absolute hydrochloric-acid-alcohol (8 to 10 drops of pure hydrochloric acid to 100 c.cm. of absolute alcohol). Both may be kept in readiness.

(29) *Eosin.* 1 grm. of the dye to be dissolved in 60 c.cm. of 50 per cent. alcohol (33 c.cm. of 90 per cent. alcohol + 27 c.cm. of distilled water).

(30) *Vesuvin*, or

(31) *Methyl-violet*, B., may be kept in readiness in saturated watery solutions (1 grm. to 50 c.cm. of distilled water).

4. **Preparation of Tissues and Organs.**—The minutest organs of the animal frame are so constituted that they are immediately accessible to microscopical investigation. They must possess a certain degree of transparency, which we effect either by dividing the organs into small portions—*isolating* the elements, or by cutting them into sections—*dissecting* them. But on the other hand the minutest organs may not possess a consistency which permits the immediate preparation of fine sections. They may be too soft (in which case they must be hardened), or too hard, from the presence of earthy matter (in which case they must be decalcified). As *hardening* and *decalcification* cannot be exercised on objects which are fresh without injuring their structure, both methods must be preceded by a process which renders possible a rapid hardening or stiffening and also a permanent hardening of the minutest particles. This process is termed *fixing*. The preparation of fine sections is accordingly possible, only after the previous fixing and hardening of the objects concerned (decalcification eventually following). But the sections also demand further manipulation. They may either be made transparent immediately by means of appliances for rendering objects clear (which are even applied with success to fresh objects), or they may be *coloured* or *stained* before clearing. Colouring matters furnish invaluable aids to microscopic research, as they admit of application to fresh, and even to living, organs. A great number of the most important facts have been discovered only by means of their help. Colouring matters syringed into vessels (*injected*) show their mode of division and the course of the finest ramifications of the capillaries.

1. *Nature of the Material.*—For studies of the elements of form and of the so-called "simple tissues," we recommend amphibia, such as frogs and salamanders (the spotted salamander, whose elements are very large, being the best). On the other hand, the mammalia are recommended for the studies of the organs. In many cases rodentia suffice (rabbit, guinea-pig, rat, mouse), or the tissues and organs of

young dogs, cats, etc., may be employed. Nevertheless no one should neglect any opportunity of acquiring the organs of the human subject. Fresh material may be obtained from the surgical clinic.

In general, it is advisable to collect the organs before the vital heat has departed. In order to do this with the utmost possible despatch, it is recommended to fill, first of all, with the suitable fluid, the jars selected for the reception of the objects, and to provide labels indicating, along with the object, the name of the fluid and the date. In the next place, let the instruments for dissection be arranged, and not until then should the animal be killed.

2. *Killing and Dissection of Animals.*—In the case of amphibia, the operator should, with a strong pair of scissors, sever the vertebral column at the neck, and destroy the brain and the spinal marrow by means of a needle thrust from the wound upwards into the cavity of the skull, and downwards into the vertebral canal. In the case of mammals, the operator should cut through the neck with a vigorous incision reaching to the vertebral column, or he may kill them with chloroform, which is poured upon a cloth and pressed against the noses of the animals. Small animals (4 cm. large), or embryos, may be thrown entire into the fixing fluid. After the lapse of six hours, open the abdomen and thorax by incisions. It is easy with a strong pin to fix the bodies of small animals by the sole of the foot on to plates of cork or wax. The organs must be neatly removed, best with forceps and scissors; squeezing and pressing of the parts, and taking hold of them by the fingers, are to be altogether avoided. The forceps should grasp the edge only of the object. Adhering impurities—mucus, blood, and the contents of the intestines—must not be scraped off with the scalpel, but must be removed by slowly swinging the part in the fixation-fluid used in the process.

By the methods specified in the sequel, it is unavoidable that scissors, forceps, needles, glass rods, etc., should be wetted by the most diverse kinds of fluids—with acids for example. The instruments should be cleaned immediately after use by being rinsed in water, and wiped dry. The operator should avoid above everything dipping into another fluid a glass rod soiled, for example, with an acid or with a colouring material. Overlooking this circumstance often leads to the reagents being decomposed, or it may entirely nullify the success of the subsequent preparations. Glasses, watch glasses, etc., are easily cleaned when it is done immediately after use. If, on the other hand, the operator allows, for example, the residuum of a colouring material to dry up in a glass, the cleaning of the latter always entails a tedious consumption of time. The operator should never neglect to clean glasses immediately after use; watch glasses at least may be thrown into a vessel of water. All vessels in which the operator isolates, fixes, hardens, colours, etc., must be kept close (watch glasses may be

covered with a second watch glass if the duration of the manipulations lasts more than ten minutes), and should not be exposed to the sun.

3. *Isolation.*—We isolate either by teazing fresh objects, or by teazing, after previous treatment of objects with fluids which dissolve some elements of the tissue. Sometimes such fluids render teazing altogether or partially unnecessary. It is a difficult undertaking to make a good preparation by teazing. Much patience and scrupulous fulfilment of the following directions are indispensable. The needles must be sharp at the point and quite clean; previous to use, they should be sharpened and polished on the moistened whetstone. The small object (at most 5 mm. broad) is now placed in one small drop upon the slide, and, if it is colourless, torn asunder on a black background, but if it is dark (coloured somewhat) the operation is performed on a white background. If the object is fibrous (*e.g.* a bundle of muscular fibres), the operator should place both needles on the one end of the bundle, and teaze, in the direction of its length, into two bundles; one of these bundles is in the same way divided in turn into two bundles by means of placing the needles on the end, and so on, until very fine single fibres are reached. By observation of the (uncovered) preparation with a low magnifying power, we can ascertain whether the necessary degree of fineness has been reached. As isolating fluids there are to be recommended—

(*a*) *For Epithelia.* Ranvier's alcohol is a useful fluid for isolation. Place sideways into 10 c.cm. of this fluid a small piece, say of the mucous membrane of the intestines, of 5 to 10 mm. After the lapse of five hours (in the case of stratified pavement epithelium, after the lapse of ten to twenty-four hours or more), the small pieces are cautiously and slowly lifted out with a pair of forceps, and twice dashed gently against the slide, which has been covered with one drop of fluid. In consequence of the impact, many epithelium cells fall off isolated, or in groups, which require only to be gently stirred round with the needle to secure complete isolation. Place a cover-glass over the preparation, and examine with the microscope. If the experimenter wishes to colour the object, he takes the small pieces *en masse* carefully out of the alcohol and places them into 6 c.cm. of picrocarmine. After two to four hours, the small piece is placed very carefully into 5 c.cm. of distilled water, and five minutes after is transferred to a slide and placed in a drop of diluted glycerine. Then put on the cover-glass. The preparation may be preserved.

(*b*) *For Muscular Fibres or Glands.* These require 35 per cent. solution of caustic potash. Small pieces from 10 to 20 mm. broad are immersed in 10 to 20 c.cm. of this fluid. After the lapse of nearly an hour, the small pieces have fallen asunder into their elements, which are fished out with needles or with a pipette, and examined in a drop of similar caustic potash under a cover-glass. Diluted caustic potash operates in an altogether different manner. The elements are soon destroyed in dilute caustic potash. If the caustic potash is too old, isolation

does not succeed; instead of it a pulpy softening of the small pieces sometimes occurs. For this reason, freshly-prepared solutions should always be employed. Even successful preparations of this kind cannot be preserved.

There is further required a mixture of chloride of potassium and of nitric acid. This is prepared by throwing into 20 c.cm. of pure nitric acid such a quantity of chloride of potassium (5 grms.) that an undissolved sediment remains at the bottom. After the lapse of fourteen hours (sometimes earlier, often later), the object is sufficiently loosened, and it is now transferred into 20 c.cm. of distilled water, in which it remains one hour, although it may remain in it even eight days without injury. It is then transferred to a slide, where it may with ease be teazed in a drop of dilute glycerine. If the nitric acid has been well washed out, the preparations admit of preservation, and even of being coloured under the cover-glass. Placing small pieces not yet teazed in picrocarmine does not succeed, as this colouring liquid makes objects brittle.

(c) *For the Ducts and Ductlets of Glands.* Place small pieces (from 1 cm. broad) into 10 c.cm. of pure hydrochloric acid. After the lapse of ten to twenty hours, the small pieces are immersed in 30 c.cm. of distilled water, which must be changed several times within twenty-four hours. The isolation is then successfully accomplished by means of the cautious spreading out of the small piece with needles in a drop of dilute glycerine. The preparation may be preserved.

4. *Fixing—General Rules.*—(1) For fixing, there must always be employed a copious amount of fluid—more than 50 to 100 times the volume of the object to be fixed. (2) The liquid must be always clear; it must, immediately after it has become turbid, be changed, *i.e.* its place must be supplied by fresh fluid. The turbidity often, indeed, occurs one hour after immersion. (3) The objects to be fixed should be of the smallest possible size; they should in general not exceed 1 to 2 c.cm. Should the preservation of the entire object be necessary, (say for subsequent adjustment, so that sections may be cut of the complete organ in its natural position) make in it several deep incisions five to ten hours after the first immersion. In the case of the fixing of delicate objects, the experimenter should place a thin layer of wadding (1 cm. in thickness) at the bottom of the vessel.

1. *Absolute alcohol* is very well adapted for glands, skin, and blood-vessels. It is used similarly for hardening. Objects placed in absolute alcohol may be cut after twenty-four hours.[1] On this account it is specially adapted for the rapid mounting of preparations. The following directions must be observed—(1) The absolute alcohol must be changed after three or four hours, even if it is not turbid; (2) the operator must take care that the immersed objects are not packed closely or allowed to stick fast to the bottom of the glass.[2] On this account we either suspend the objects in alcohol by a thread, or place a small pad

[1] The working up of objects fixed in absolute alcohol should not be delayed too long, as the elements gradually deteriorate. Cut after the lapse of one to eight days.

[2] The places in question, on being cut, appear strongly compressed.

of cotton wool at the bottom of the glass; in many cases frequent shaking of the glass suffices.

Alcohol not absolute (say 90 per cent.) operates differently, as it shrivels objects, and cannot on that account be employed as a substitute for absolute alcohol.

2. *Chromic acid* is employed principally in two aqueous solutions—(*a*) as a solution from 0·1 to 0·5 per cent. It is especially adapted for organs which contain much loose connective tissue. This powerful solution gives to connective tissue a strong consistency, but it labours under the disadvantage that it renders colouring difficult. Objects remain in it from one to eight days; they are then placed from three to four hours under a water tap, or if that is impossible, they are placed for the same period in water, which must then be three or four times changed; they are then transferred for some minutes into distilled water, and lastly they are hardened, while daylight is excluded, in alcohol of gradually increasing strengths.[1] (*b*) As 0·05 per cent. solution, which is prepared by diluting the 0·1 per cent. solution with an equal amount of distilled water. Procedure as with solution (*a*); objects may remain twenty-four hours in solution (*b*).

Chromic acid solutions penetrate slowly; consequently it is necessary, in a process of twenty-four hours' duration, that only small pieces (5 to 10 mm. broad) should be immersed in them.

3. *Kleinenberg's sulpho-picric acid* (p. 261). Delicate objects (embryos) are immersed for five hours in this fluid; the more solid parts for twelve to twenty hours. Then, with a view to hardening, transfer *without* the previous thorough washing in water to alcohols of increasing strength.

4. *Müller's fluid* (p. 261). Objects are placed in large quantities (400 c.cm.) of this solution from one to six weeks. Thereafter, for four to eight hours, they are thoroughly washed in water (flowing when possible), rinsed for a short time in distilled water, and lastly, daylight being excluded, they are placed within alcohols of increasing strength. "*He who does not with scrupulous conscientiousness follow the general rules above given for hardening fails to attain success, for which failure the innocent Müller's solution is then made responsible even by otherwise experienced microscopists.*" (Stöhr.)

5. *Osmic acid solution* (p. 261). By the use of this solution the operator avoids inhaling vapours very irritating to the mucous membranes. We fix, either by means of the immersion of very small (5 mm. broad) pieces in the acid (mostly employed in the 1 per cent. solution), which is to be diluted to the extent of one half with distilled water, and which is usually employed only in small quantities; or we fix by exposing the moistened object to the vapour of osmic acid. To attain this, pour 1 c.cm. of 2 per cent. solution into a small test-tube to the height of 5 cm.; add the requisite amount of distilled water, and fasten the object with hedgehog bristles to the lower surface of the cork with which the test-tube must be firmly closed. After the lapse of ten to sixty minutes (always according to the size of the object) the small piece is taken out and at once thrown into the liquid contained in the small glass. In both cases the objects continue for twenty-four hours in the acid; at the same time the glasses must be well closed and kept in the dark. The objects are then taken out, rinsed for a couple of minutes in distilled water, and hardened in alcohols of increasing strength.

[1] By this term is understood first, Ranvier's third-part alcohol; then 70 per cent. alcohol; then 90 per cent. alcohol, and lastly, if necessary, absolute alcohol.

6. *Chromo-osmium acetic acid* (p. 261) is an excellent fluid for the fixation of the parts of the nucleus. Place small pieces (2 to 5 mm. broad), quite fresh, and still *warm with vital heat*, into 4 c.cm. of this liquid, in which they remain one day (two days are preferable), but in which they may continue even longer. The small pieces are then thoroughly washed for the space of one hour or longer in flowing water (where possible), rinsed in distilled water, and hardened in alcohols of increasing strength.

The liquids once used for fixation are of no further use: they should be thrown away.

5. *Hardening.*—The best fluid for hardening is alcohol of gradually increasing strengths. Here also the rule that we should employ liquid copiously holds good—as well as that of changing alcohol which has become turbid or coloured.[1] The exact process is as follows—After the objects have been fixed (in one of the fluids above enumerated) and thoroughly washed in water,[2] they are transferred for twelve to twenty hours into 70 per cent. alcohol, and after the lapse of this period they are placed in 90 per cent. alcohol, in which the hardening is completed after the further lapse of twenty-four to forty-eight hours; objects may continue in this alcohol for months. The 90 per cent. alcohol that has been employed for hardening may be afterwards used for the hardening of the liver (to be used as an embedding substance to facilitate the cutting of sections), or for burning in a spirit lamp.

6. *Decalcification.*—Objects to be decalcified must not be immersed fresh in the decalcifying fluid; they must, on the contrary, be previously fixed and hardened. For this end, place small bones (up to the size of the metacarpus) and entire teeth, and pieces sawed off from the larger bones (from 3 to 6 cm. long), into 300 c.cm. of Müller's fluid, and thence, after the lapse of two to four weeks (and after previous thorough washing), into 150 c.cm. of alcohol of gradually increasing strength. After the small bone has continued for three days or longer in 90 per cent. alcohol, it is transferred to the decalcifying solution, viz., diluted nitric acid (pure nitric acid, 9 to 27 c.cm. added

[1] The pieces that have been fixed in chromic acid and in Müller's fluid give off even while in alcohol, substances which, by the simultaneous influence of daylight, appear in the form of precipitates. If, on the other hand, the alcohol is kept in the dark, no precipitates are produced, but the alcohol assumes a yellow colour, remaining free from turbidity. On this ground the exclusion of daylight has been recommended; it is sufficient to place the glasses in a dark part of the room. The 90 per cent. alcohol also, so long as it still remains intensely yellow, must be changed once every day.

[2] Objects fixed in sulpho-picric acid are excepted; these are directly transferred from this fluid into 70 per cent. alcohol. In this case the 70 per cent. alcohol must be changed several times during the first day.

to 300 c.cm. of distilled water). Here also, large quantities (300 c.cm. at least) must be employed, which, at the beginning, must be changed daily, but, subsequently, every four days until the decalcification is accomplished. We hasten the process by means of puncturing with an old needle, and incisions with a scalpel.[1] Decalcified bone is flexible, soft, and admits of being easily cut. Fœtal bones, heads of embryos, etc., are decalcified in nitric acid of a weaker kind (1 c.cm. of pure acid to 99 c.cm. of distilled water), or in 500 c.cm. of saturated aqueous solution of picric acid. The process of decalcification requires several weeks in the case of thick bones; in the case of fœtal and small bones, three to twelve days. When decalcification is completed, the bones are thoroughly washed, from six to twelve hours, in water, and again hardened in alcohols of increasing strengths. It not unfrequently happens, in the case of beginners, that the bone is placed in alcohol when the decalcification is yet incomplete, and then turns out to be useless for histological investigation. In such cases, the entire process of decalcification must be repeated. If objects lie too long in the decalcifying fluid, decomposition is induced.

7. *Sections.*—The razor must be sharp; the success of the preparations depends on the sharpness of the blade. In section-cutting, the blade must be moistened with alcohol (not with water, which moistens the blade only to an imperfect extent). For this end, dip the blade, before every third or fourth section, into a flat glass dish filled with 30 c.cm. of 90 per cent. alcohol, which serves also for the reception of the prepared sections. The blade must be held in a horizontal position, lightly grasped, with the thumb pressed against the edge, the fingers against the hinder part of the blade, and the back of the hand directed upwards. First of all, cut a slice from the object so as to expose a smooth surface, and then separate from the object, with one stroke, a piece of suitable thickness. Now begins the making of successive sections; this must always be done as smoothly as possible, with symmetrical thinness, and with one light, not too rapid, stroke.[2] We then place the dish on a black background, and pick out

[1] The needle and the scalpel must be carefully cleaned immediately after use.

[2] The blade is not to be *pressed* through an object, but to be *drawn* through it. It is wise to make always a very large number (10 to 20) of sections, which are then to be transferred into the glass dish with a needle or by the immersion of the knife.

Fine sections, which either have not been stained, or have already been stained throughout, can best be cut and floated off by the edge of the razor being inclined directly on the slide.

the best sections. *The thinnest sections are not always the most useful;* for many preparations, *e.g.* a section through the coats of the stomach, thicker sections are more to be recommended. For specimens intended for general inspection with low powers, let the operator produce large, thick sections; for small structures suitable for high powers, he must cut thin ones. For high powers, the very smallest fragments of 1 to 2 mm. broad, obtained by means of the superficial manipulation of the knife, or marginal parts of somewhat thicker sections, often suffice. If the object to be dissected is too small to be held by the fingers, it ought to be imbedded. The simplest method is that of *imbedding in liver.*

Take either ox's liver or, what is better, human fatty or amyloid liver (to be obtained from the pathological department), and cut it into thick pieces (3 cm. in height, 2 in breadth, and 2 in thickness); these are immediately thrown into 90 per cent. alcohol, which must be changed the next day. After three to five days longer, the liver possesses the requisite hardness. Now, cut one of these pieces from above down to the half of its height, and squeeze the object to be cut into the fissure so produced. If the object be too thick, the operator can, with a small scalpel, cut furrows in the liver, into which the object is fitted.

Most objects may be cut in liver, and very delicate sections can thus be obtained after some practice, regularly carried on for a few weeks.

8. *Colouring, Staining.*—The particular solution of the colouring matter must always be filtered before use. Small funnels are made of double thicknesses of filter paper (5 c.cm.), and fastened in a cork frame which has been prepared by means of the excision of a piece 2 cm. broad out of a cork plate 5 cm. broad. The frame of cork is fixed on four long pins. Such funnels can be used several times. Funnels and frames are to be employed only for one and the same fluid.

1. *Nucleus staining with hæmatoxylin* (p. 262). Filter 3 to 4 c.cm. of colouring solution into a watch glass and introduce the sections into it. The time in which the sections acquire colour is very various. Sections in alcohol of fixed and hardened objects acquire colour in one to three minutes. If the fixation has been effected with Müller's fluid, the objects must be immersed somewhat longer (five minutes) in the fluid.

[Sections of objects fixed in strong chromic acid, or of those not entirely free from acid, often take on colour very slowly, and sometimes not at all. We can remedy this inconvenience either by means of protracted preservation for two or three months in 90 per cent. alcohol, to be changed two or three times, or by immersing such sections, before they are brought into the hæmatoxylin, for five to ten minutes, in a watch glass, along with 5 c.cm. of distilled water, to which three to seven drops of 35 per cent. caustic potash are added. We then transfer the sections for one to two minutes into a watch glass with pure distilled water, and thence into the hæmatoxylin. Such sections also become coloured after five to ten minutes.]

The sections must next be transferred from the colouring matter into a watch glass containing distilled water, rinsed, *i.e.* moved somewhat with the needle, in order to free them from the surplus colouring matter, and, after the lapse of one to two minutes, transferred into a large dish filled with 30 c.cm. of distilled water. The sections must remain here at least five minutes, during which their purple colour gradually passes over into a beautiful dark blue, which becomes all the more pure the longer (twenty-four hours) we allow the sections to be in the water.

The sections at first appear entirely blue. About five minutes after, in many cases only after the lapse of hours, the differentiation is first effected, so as to admit of many details being perceived even with the naked eye.

The colouring matter which has been used is again poured back through the filter into the bottle containing the hæmatoxylin solution. The watch glass must be immediately cleaned. It is recommended to beginners to leave the sections in the dye for a time, ranging variously from one, three, to five minutes, and then to watch what length of time is adapted for successful staining. In the case of hæmatoxylin colouring, the principal thing is careful washing.

2. *Diffusive staining*—for the staining of protoplasm and of the intercellular substances. (*a*) *Slow colouring.* A small drop of neutral solution of carmine (p. 262) is placed by a glass rod in a dish filled with 20 c.cm. of distilled water; at the bottom of this dish there lies a small piece of filter paper.[1] The sections must lie in the fluid over night. The more rose-tinted the fluid is, the longer the colouring process lasts, and the brighter the section becomes. The beginner is always inclined to regard the pale-rose tint as unlikely to produce a good stain, until, on the next day, the dark-rose sections turned to red teach him a better lesson. But this mode of colouring is seldom applicable for its own sake; on the other hand, it is to be recommended for compound staining. Colour first of all with carmine solution, then with hæmatoxylin. (*b*) *Rapid colouring.* Pour 10 drops of eosin solution (p. 263) into 3 to 4 c.cm. of distilled water. The sections remain therein one to five minutes, they are then for a short time "rinsed out" (as in the case of hæmatoxylin colouring) in a watch glass with distilled water and transferred for ten minutes into 30 c.cm. of distilled water. The colouring may be employed alone or combined with hæmatoxylin. The entire process of hæmatoxylin colouring is to be completed first, and the eosin colouring next.

3. *Staining of the chromatic substance in nuclear division.* The objects are placed, from sixteen to forty-eight hours (the longer the better), in only 3 c.cm. of solution of saffranin (p. 263). Then the solution, together with the sections, is poured out into a plate containing distilled water; entirely opaque sections are lifted out with the needle and, in order to change their colour, placed in 5 c.cm. of hydrochloric-acid-alcohol. If the section does not yield much colour (after a half to two minutes), it is transferred into 5 c.cm. of pure absolute alcohol, and after a minute cleared up and mounted. Too long continuance in hydrochloric-acid-alcohol (just as in absolute alcohol)—may lead to a too great change of colour of the preparation. We employ the saffranin colouring only after preceding fixation with chromo-osmium acetic acid.

4. *Staining in mass.* Colouring of the nucleus in entire pieces of organs previous to their separation into sections. Fixed and hardened objects are, when small (5 mm. broad), transferred for twenty-four hours to 30 c.cm. of borax

[1] If this is neglected, the sections acquire colour only on one side.

carmine; when larger, for two to three days; they are transferred out of this directly into 25 c.cm. of hydrochloric-acid-(70 per cent.)-alcohol (p. 263) —(the borax carmine, after being used, is poured back into the flask). After the lapse of a few minutes, the alcohol is red, and must now be replaced by new hydrochloric-acid-alcohol. After nearly a quarter of an hour, the alcohol is again changed, and this changing is continued without intermission until the alcohol is no longer coloured.[1] The piece is then transferred into 90 per cent. alcohol, and if it has not, after twenty-four hours, become hard enough for cutting, it is transferred for twenty-four hours or more into absolute alcohol.

5. *Picrocarmine.* Double dyeing, nuclei and connective tissue red, protoplasm yellow. Filter 5 c.cm. of the fluid into a watch glass. The period of time in which picrocarmine operates varies according to the tissue, and can only be approximately given. After the colouring has been completed, the colour is again filtered back into the flask, and the object transferred for ten to thirty minutes into 10 c.cm. of distilled water. It, of course, loses colour under the cover-glass. If the object, *e.g.* a section, is to be placed in absolute alcohol, it must not remain there long (one to two minutes), as the alcohol extracts the yellow colour.[2] Picrocarmine is especially employed in investigations of fresh objects. If the solution is good, we secure a beautiful colour, which comes out brilliantly, especially with the subsequent application of glycerine acidulated with a few drops of acetic acid.

6. *Colouring of nuclei with aniline dyes.* The best aniline dyes for this purpose are vesuvin and methyl-violet B. Filter 4 c.cm. of the fluid into a watch glass, and place the sections in it. They become, after two to five minutes, coloured quite dark; they are then, for a short time, washed in 5 c.cm. of distilled water, and transferred to a watch glass containing absolute alcohol, where they lose much colour. After a few minutes (three to five), when the sections have become lighter, we can recognize individual parts (*e.g.* the glands in the case of the skin) with the naked eye. The sections are now transferred into a second watch glass with 5 c.cm. of absolute alcohol, and, after two minutes, cleared up and mounted in dammar varnish (p. 261). The result is a very beautiful colouring of the nucleus. Vesuvin should be kept in glycerine. A disadvantage lies in the large consumption of absolute alcohol.

7. *Silver staining*—for the representation of cell boundaries and colouring of cement substances. The use of metal instruments is to be avoided; use a glass rod. Instead of pins, use hedgehog bristles. The object is immersed in 10 to 20 c.cm. of 1 per cent., or weaker, solution of nitrate of silver (p. 261, par. 15). After half a minute to ten minutes (always according to the thickness of the object) it is taken out of the fluid—which has meanwhile assumed an almost milky hue—by a glass rod, rinsed, and exposed to direct sunlight in a large white dish (a porcelain plate) containing 100 c.cm. of distilled water. After the lapse of a few minutes, a slight browning will supervene, the sign of the successful reduction of the silver. Immediately on the object becoming a dark

[1] This may occupy one to three days; during the first day, change every two hours; during the subsequent time, every four hours. If the operator wishes to be economical, he may slowly, with a needle, push the object out of the layer of the red fluid in which it lies, and bring it to another uncoloured part of the fluid.

[2] This deprivation of colour can be avoided, if we throw into the watch glass a small crystal of picric acid along with the absolute alcohol.

reddish-brown (usually after five to ten minutes), it is taken out and transferred to a watch glass containing distilled water, to which a couple of grains of common salt are added, and, after five to ten minutes, it is put for preservation in 30 c.cm. of 70 per cent. alcohol, in darkness. After three to ten hours, substitute 90 per cent. alcohol for the alcohol of 70 per cent. The substance is immersed in the solution of silver in the dark. The reduction, on the contrary, must be accomplished in the sunlight.[1] If there is no sunshine, lift the object (after it has been taken out of the silver solution and washed thoroughly for a short time in distilled water) into 30 c.cm. of 70 per cent. (later on into 90 per cent.) alcohol, in the dark, and expose it, while in the alcohol, to the light at the first blink of sunshine.

8. *Gold staining*—for demonstrating the terminations of the nerves. Steel instruments must not be immersed in the gold solution. All manipulations in the gold solution are to be undertaken with glass rods or with small wooden rods. Heat in a small test-tube, to boiling, 8 c.cm. of 1 per cent. solution of chloride of gold + 2 c.cm. of formic acid (p. 261). The mixture must boil up thrice. In the cooled mixture, very small pieces of tissue (at the most 5 mm. broad) are immersed for the space of an hour, and kept in the dark ; they are then for a short time thoroughly washed with distilled water in a watch glass, and exposed to the light (not necessarily sunshine) in a mixture of 10 c.cm. of formic acid with 40 c.cm. of distilled water. The reduction by which the small pieces become dark violet on the outside is accomplished very slowly (often after twenty-four to forty-eight hours at the earliest). The pieces are then transferred to 30 c.cm. of 70 per cent. alcohol, and, on the following day, to 90 per cent. alcohol, in which, for the prevention of further reduction, they must remain for eight days.

9. *Injection.*—The filling of the blood vessels and of the lymphatic vessels with coloured substances is a peculiar art, which can be acquired only by means of much practice. Knowledge of the many little artifices employed can scarcely be acquired by lectures or even with all the help of copious directions. Practical instruction is here indispensable.

Anyone wishing to inject must have a syringe, working well, and provided with an easily movable piston and nozzles of various diameters. As injection material use Grübler's Berlin blue, 3 grams. dissolved in 600 c.cm. of distilled water. Begin with the injection of single organs, *e.g.* the liver, which possesses the advantage that even an incomplete filling of its vessels yields useful results. Place the injected object for two to four weeks in Müller's fluid, and harden it in alcohol. The sections must not be too thin.

10. *Mounting and Preservation of Preparations.*—The prepared sections, etc., are now, with a view to microscopical investigation, transferred to

[1] The reduction doubtless succeeds also in ordinary daylight, but it is effected slowly, and yields less distinct forms.

a slide and placed under a cover-glass. The *media* in which the sections are examined are either (1) water; or, if it is desired to brighten up and preserve the sections, (2) glycerine; or (3) dammar solution.

Transference to the slide is effected thus—the investigator places first of all a small drop of the requisite fluid on the middle of the slide; he then lifts the section with the spatula and lays it on the slide. It is a good method to lift very delicate sections with the end of a glass rod, and to lay them on the slide by rolling it round. When the section lies smoothly, place a cover-glass on it.[1] The latter must be grasped by the edge. In the act of covering, the cover-glass is placed on the slide, resting on the left edge, and then it is slowly lowered on to the preparation, while the under surface of the cover-glass is supported by a needle held in the right hand. It is still simpler to place a drop of the requisite fluid on the lower surface of the cover-glass and then to allow the cover-glass to fall gently on the preparation. The fluid in which the section happens to be must fill up the entire space between the cover-glass and the slide. If a sufficiency of fluid is lacking (which is recognizable by large air bubbles appearing under the cover-glass), place, with the point of the glass rod, one drop more of the fluid on the rim of the cover-glass. If there is a superabundance of fluid—and this usually occurs, especially with beginners—the liquid which has oozed out from under the rim of the cover-glass must be absorbed by filter paper. *The upper surface of the cover-glass must always be dry.* Remove small air bubbles under the cover-glass by means of frequently carefully raising and lowering it with the needle.

1. Never neglect to examine uncoloured as well as coloured sections in water or in solution of common salt, as in these many details of structure, *e.g.* connective tissue formations, distinctly emerge to view, whilst the same, under the brightening influence of glycerine or of dammar varnish, almost entirely elude observation. Objects mounted in water or even in a solution of common salt cannot be preserved.

2. Preparations mounted in glycerine may be preserved. In order to prevent removal of the cover-glass, fix it with cover-glass cement. As a previous condition of success, *the rim of the cover-glass must be completely dry*, for cement adheres only to dry surfaces. To dry the slide round the cover-glass, first absorb with filter paper the glycerine oozing from under the rim of the

[1] Investigations with low magnifying powers without a cover-glass are only admissible in the most superficial examinations, as, for example, to ascertain if object has been thoroughly teazed out. In all other cases, the cover-glass is indispensable. In order to be persuaded of this, let anyone examine an uncovered section, then let him cover it with a cover-glass and examine it again. Many a good preparation, the covering of which has been neglected, appears unfit for use. Investigations with high powers without a cover-glass are in general inadmissible.

cover-glass, and then with a cloth moistened with 90 per cent. alcohol (the cloth being drawn over the point of the finger), carefully wipe the slide all round the cover-glass, without touching the latter. Then heat a glass rod and plunge it into the cement,[1] and bring four drops on to the four corners of the cover-glass. Then lead the cement round the margins of the cover-glass, so as to cover the cover-glass on the one hand and a portion of the slide on the other, to the breadth of 1 to 3 mm. Finally, smooth with the heated rod the upper surface of the layer of cement.

Preparations preserved in glycerine are often beautifully transparent on the second or third day. Hæmatoxylin and other colouring matters grow dim in glycerine after some time; on the contrary, picrocarmine and carmine retain their colour.

3. Mounting objects in dammar varnish is an excellent method of preservation. Dammar varnish has, as opposed to glycerine, the advantage of retaining colours, but with the drawback that it renders objects more transparent than dilute glycerine and frequently causes delicate structures to disappear.

Sections which happen to be in water or alcohol, cannot be placed at once into dammar varnish; *they must be previously entirely freed from water.* To this end, the sections are transferred to a covered watch glass containing 4 c.cm. of absolute alcohol, in which they may remain for two minutes in the case of thin sections, and for ten minutes in the case of thicker ones.[2] Next we fish out the sections with the needle (very delicate sections with spatula or section lifter) and transfer them, with a view to clearing up, into a watch glass containing 3 c.cm. of lavender oil or oil of cloves.[3] Place the dish on black paper so as to observe the sections becoming transparent. Avoid breathing into the watch glass, as immediate turbidity of the lavender oil is the result. If individual portions of the sections do not become transparent after two or three minutes (in this case the portions appear a dirty white when the light falls on them and dark brown when the light passes through them), the section has, in that case, not been freed from water and it must once again be transferred to absolute alcohol. After the clearing up has been completed, the section is transferred to a dry slide and the superfluous oil[4] carefully wiped off with filter paper, or with

[1] Glass rods crack very easily when heated, and metal rods are preferable, but they cool too rapidly. We can to some extent avoid the cracking by heating the glass rods, while they are being continually turned round, up to a red heat. Glass rods heated for only a short time crack immediately on being plunged into the cement.

[2] Beginners should transfer the sections out of the water, first of all into 4 c.cm. of 90 per cent. alcohol, and then quickly into the same quantity of absolute alcohol.

[3] We can also bring the section directly out of the absolute alcohol on to the slide, wipe off the superfluous alcohol and place upon it a drop of lavender oil. At first the oil always trickles off from the section and it must be guided back again by a needle; after complete clearing up, which we can observe under the microscope with a low power, the oil is as soon as possible wiped off and a cover-glass with dammar varnish placed over it. If we examine the uncovered section in oil, section and oil may become obscured by the breath; in such cases, let the turbid oil trickle off and substitute a drop of fresh oil.

[4] The oil in the watch glass used in clearing up may again be poured back into the bottle.

a linen duster wrapped round the forefinger, and a cover-glass is placed on it, to the under surface of which a drop of dammar varnish has been attached. When several sections are brought under one cover-glass, in the first place arrange the sections in close proximity by means of the needle, then with the glass rod spread the dammar varnish over the under surface of the cover-glass, in a layer proportionately thin throughout, and place the cover-glass over the sections. Large air bubbles are got rid of by placing a small drop of dammar varnish near the rim of the cover-glass.

It not unfrequently occurs in the case of beginners that the varnish becomes turbid and finally renders the entire preparation, or parts of it, opaque. *The reason is, that the section has not been freed from water.* In the case of slight turbidity (which reveals itself under the microscope as consisting of minute drops of water), slight heating of the slide may be a remedy. In the case of denser turbidity, place the entire slide in oil of turpentine, carefully lift off the cover-glass half an hour afterwards, place the sections in oil of turpentine for two minutes to dissolve the adhering varnish, and lastly place it, with a view to the complete removal of the water, into 4 c.cm. of absolute alcohol, which must be changed after five minutes. The lavender oil and dammar varnish process is then repeated.

Dammar varnish dries very slowly, and therefore the slides must not be placed on their side.

The successive processes through which a fresh object has to pass until it is preserved as a finished stained section is a very long one. When, for example, it is prescribed in the appendix detailing special methods thus—"Fix in Müller's fluid fourteen days, harden in gradually concentrated alcohol, colour the section in carmine and hæmatoxylin, mount in dammar varnish"—the process is as follows:—The fresh object, 1 c.cm. in size, is placed in 200 c.cm. of Müller's fluid,[1] which, immediately after turbidity has set in (usually after one hour), is changed. After twenty-four hours the fluid is again changed; the object now remains in it for a period of fourteen days. After the expiration of this, it is washed in flowing water for one to four hours; then placed in 20 c.cm. of distilled water for fifteen minutes; then in 50 c.cm. alcohol (70 per cent.) and in the dark for twenty-four hours; then in 50 c.cm. of 90 per cent. alcohol for twenty-four hours, when the 90 per cent. alcohol is changed.

The fixed and hardened object may now be cut after a suitably long time, during which the 90 per cent. alcohol is perhaps once more changed. The section is taken out of the alcohol and transferred into 30 c.cm. of dilute carmine solution for twenty-four hours; then into 5 c.cm. of distilled water for fifteen minutes; then into 3 c.cm. of hæmatoxylin for five minutes; then into 20 c.cm. of distilled water for ten minutes to two hours; then into 5 c.cm. of absolute alcohol for ten minutes; then into 3 c.cm. of lavender oil or oil of cloves for two minutes, and lastly the section is mounted in dammar varnish.

11. *Investigation of Fresh Objects.*—This is placed at the end of all the other methods because it is the most difficult, and presupposes a somewhat practised eye. Experience is most easily acquired by the investi-

[1] The directions for size are calculated only for one piece 1 c.cm. large. When the piece is larger, or when several pieces are submitted to the process, more fixing and hardening fluid must, of course, be employed.

gation of objects already *prepared* (hardened, coloured, etc.). If anyone has once distinctly seen and studied details of structure seen in stained specimens, it is not so difficult to make out these even in fresh objects. That which now follows requires attention. Slides and cover-glasses must not be greasy. Clean them with alcohol and dry them with a clean cloth. Then place *one* drop of ·75 per cent. solution of common salt on the slide, next place in it a *small* piece of the object to be investigated, and cover the latter with a cover-glass. In this process, all pressure must be carefully avoided. In the case of very delicate objects, lay two small strips of paper on the slide so that the cover-glass will rest on them without pressing the object itself. If the object requires no further manipulation, the observer should run round the edge of the cover-glass a thin layer of paraffin to prevent evaporation. Melt on an old scalpel a large piece of paraffin, nearly as large as a lens, and let it run, not from the point, but from the edge of the scalpel, on to the rim of the cover-glass. Gaps, here and there, may be filled up by applying the heated end of the scalpel. It is easy to subject fresh objects to the operation of certain reagents (acetic acid, caustic potash, colouring matters) while under the microscope. One may remove a part of the medium in which the object lies (as, for example, the solution of common salt), and substitute another fluid in its place. To do this, place a drop, *e.g.* of picrocarmine, with a glass rod on the right rim of the cover-glass. If the drop does not reach entirely to the rim of the cover-glass, do not incline the slide to it, but guide the drop with a needle to the margin of the cover-glass. We see now that a little of the colouring matter mixes with the solution of common salt, but that a regular flow of the colouring fluid does not take place. In order to secure this, place a little bit of filter paper on the left rim of the cover-glass,[1] and the picrocarmine will soon occupy the entire under surface of the cover-glass.[2] Lay the filter paper aside, and allow the stain to operate. When the colouring has been completed—a fact which can be always observed under the microscope—place on the right rim of the cover-glass a drop, *e.g.* of glycerine, to which add, in cases of colouring by picrocarmine, as much acetic acid as will drop from a needle dipped once into it (thus a small drop), and then lay the filter paper again on the left side of the rim. In this manner, we can conduct an entire series of fluids under the cover-glass, and test their action on the tissues. Certain of the fluids, picro-

[1] I cut out a piece 4 cm. long, 2 cm. broad, notch it transversely, and place the paper roof so formed on the slide in such a manner that it touches the left rim of the cover-glass with the rim entirely and straightly cut, and 2 cm. broad.

[2] When the first drop has penetrated, place (always in proportion to what is needed) two or three further drops on the right of the cover-glass.

carmine for example, must, after preceding fixation with osmic acid, remain very long in contact with the objects. Evaporation is prevented by leaving the preparation in a damp chamber. Moist chambers are made by means of a porcelain plate and a small glass lid, of 9 cm. diameter at the least.[1] Pour water to the height of 2 cm. into the plate, then place in the middle a small glass basin, or a cork plate standing on four wooden feet. The slide, along with the preparation, is placed on this, and the whole is covered with the glass lid, the free edge of which is immersed in water.

12. *Preservation of Preparations.*—Finished preparations must immediately be labelled. Do not employ gummed paper tickets, but those of pasteboard 1·2 mm. thick, which are fixed on the slide by means of the cement called water-glass.[2] Special boxes for holding specimens are thus superfluous. The slides may be placed over each other without pressure on the preparations. The labels should be as large as possible (2 cm. broad in the case of slides of the English size), and they should have recorded on them the name of the animal, the organ, and, where possible, a short indication of the method. Use boxes or cabinets which allow the slides to lie flat, not those in which they stand on their sides.

Chap. VI.—Microtomes and Serial Section Cutting.

A *microtome* is an instrument by which extremely thin sections of organs and of tissues may be cut. There are many varieties of such instruments, and most of them have certain points of excellence that commend them to their inventors, or to those who acquire dexterity in their use. I shall only refer to those of which I have had special experience. There are essentially five methods of imparting to tissues the proper consistence for section cutting by means of microtomes: (1) embedding the tissue or organ in paraffin or in a mixture of white wax and paraffin; (2) infiltrating the tissue with gum and then freezing it by the application of freezing mixtures; (3) freezing the tissue directly, and often in the fresh state, by the use of ether which, by its evaporation, produces the requisite degree of cold; (4) infiltrating the tissue with paraffin dissolved in chloroform or other solvent; and (5) holding the hardened tissue firmly by means of celloidin or other substance capable of giving it sufficient resistance to the pressure of the knife.

[1] A pot, a larger preparation glass, etc., serve the same purpose.

[2] It is a liquid of the viscidity of syrup, to be had at all druggists, and must be preserved in a well-stoppered vessel.

1st Method.—Paraffin and Wax Embedding.

The microtome used in this method consisted of a strong brass cylinder fixed into a horizontal brass plate having a smooth upper surface. The bottom of the cylinder consisted of a flat plate exactly fitting the cylinder, and attached underneath to a thick screw having a very fine thread. A well about 30 mm. in depth, and 45 mm. in diameter, was thus formed, the bottom of which was moved upwards by a slight turn of the screw. The tissue, previously hardened, was immersed in melted spermaceti, and then in a mixture of melted white wax and paraffin, or in paraffin alone. When the mass became solid, by turning the screw it was pushed upwards, and a section was made by pressing a razor or broad knife over the surface of the broad and smooth plate, in the centre of which the well opened, the tissue and razor being kept moist with alcohol. This method had many disadvantages, as, for instance, the shrinkage of the tissue from the paraffin, so that it became loose; but it did good service in its day, especially in the hands of such a man as the late Mr. A. B. Stirling, assistant curator of the Anatomical

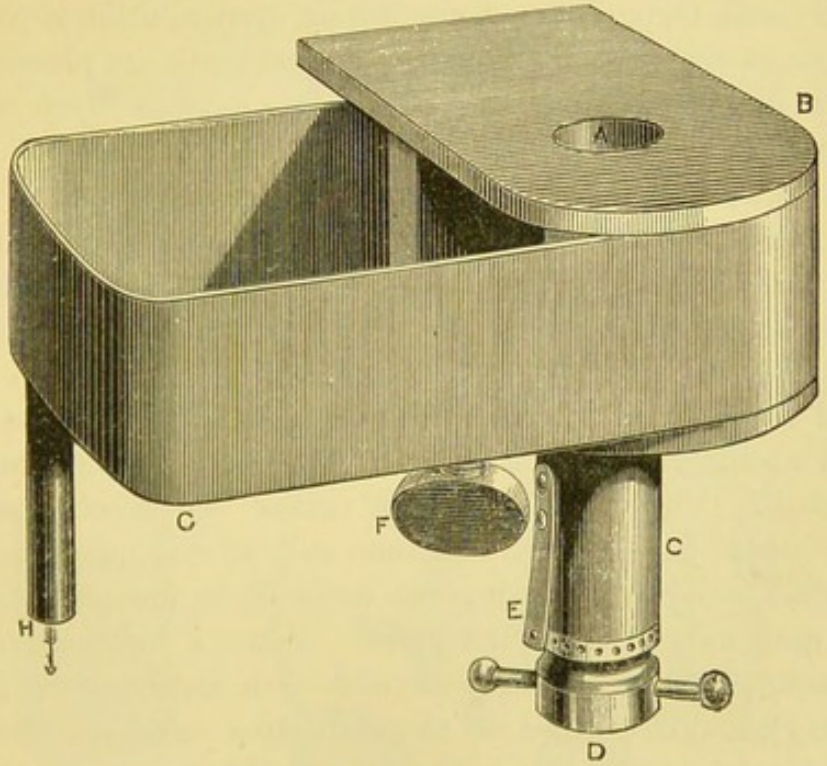


Fig. 129.—Rutherford's Microtome, arranged for freezing with ice and salt.

Museum of the University of Edinburgh, who prepared histological specimens with great skill and success. It is now almost entirely abandoned.

2nd Method.—The Ice Freezing Microtome of Professor Rutherford and Allied Instruments.

In 1871, Professor Rutherford,[1] of the University of Edinburgh, invented and described this instrument, and it is not too much to say that it revolutionized the teaching of histology in this country. By means of it, 400 or 500 thin sections can be cut in the course of an hour. This has made it invaluable in the teaching of large classes of students. Its simplest form is seen in Fig. 129. It consists of a stage or plate *B*, in the centre of which is a well of considerable depth, the bottom of which is movable by the screw *D* having a fine thread. An indicator *E* is a little spring having a small point on the inner aspect of its lower end which, by sliding with a turn of the screw into the small holes, serves to make the turns of the screw equal in amount and consequently secures sections of uniform thickness. Surrounding the well is the ice box *C*, having a tube *H* for the escape of water when the ice melts. The bit of hardened tissue is immersed in a strong solution of gum arabic (with a few drops of a solution of carbolic acid added to prevent the growth of fungi in the gum) for several days, so that the gum may thoroughly soak through it. Some strong gum solution is poured into the bottom of the well, finely pounded ice and salt are placed in layers or mixed in the freezing box, and when a thin layer of ice has formed round the interior of the well, the bit of tissue is placed in position. A sufficient amount of gum solution is poured in to fill the well, the mouth of the well is then closed with a bit of gutta-percha sheeting, and the whole apparatus is wrapped in a piece of thick flannel cloth, and left until freezing has been thoroughly established. The wrappings are then removed, the bit of gutta-percha taken from the mouth of the well, and with the knife held firmly so that it glides uniformly on the plate *B*, the mass of frozen gum at the mouth of the well is cut off. The screw *D* is then turned and another section cut until the tissue is reached. It is then easy to cut numerous sections quickly after each other, turning the screw *D* to the requisite amount between each movement of the knife. When a sufficient number of sections accumulate on the knife (or with each section when great care is necessary), they are wiped off the knife by a camel-hair brush into a flat vessel containing water. The water dissolves the gum. After some time, the sections are gently collected on a straight needle, transferred to another vessel of water, and from that into a third; at the end of half an hour, they are gathered in the same way and transferred to a

[1] William Rutherford, *Journal of Anatomy and Physiology*, 1871; see also *The Lancet*, 3rd January, 1885.

stoppered bottle containing methylated spirit, where they may be kept till required. The most useful form of knife is one with a blade 200 mm. in length and 30 mm. in breadth. The back of the knife should be 6 mm. in thickness to prevent bending of the blade, and the side of the blade which touches the microtome should be ground flat, while the upper surface is bevelled.

Another form of freezing microtome is that of Williams, made by Mr. Swift of London. It is a circular box of considerable capacity, having a metal pillar in the centre bearing on its upper end a brass plate 30 mm. in diameter. This box is packed with powdered ice and salt, and then the lid is pressed on, so that the brass plate just mentioned passes through a hole in the centre of the box and is on a level with the upper surface of the lid. The upper surface of the lid is made of glass. The bit of tissue, soaked in gum, placed on the brass plate, soon becomes frozen. The sections are then cut by a razor mounted in a frame supported on three fine screws passing through it, and by lowering the edge of the razor by turning the screw placed *beyond the edge of the razor*, and pushing the frame, carrying the razor, somewhat

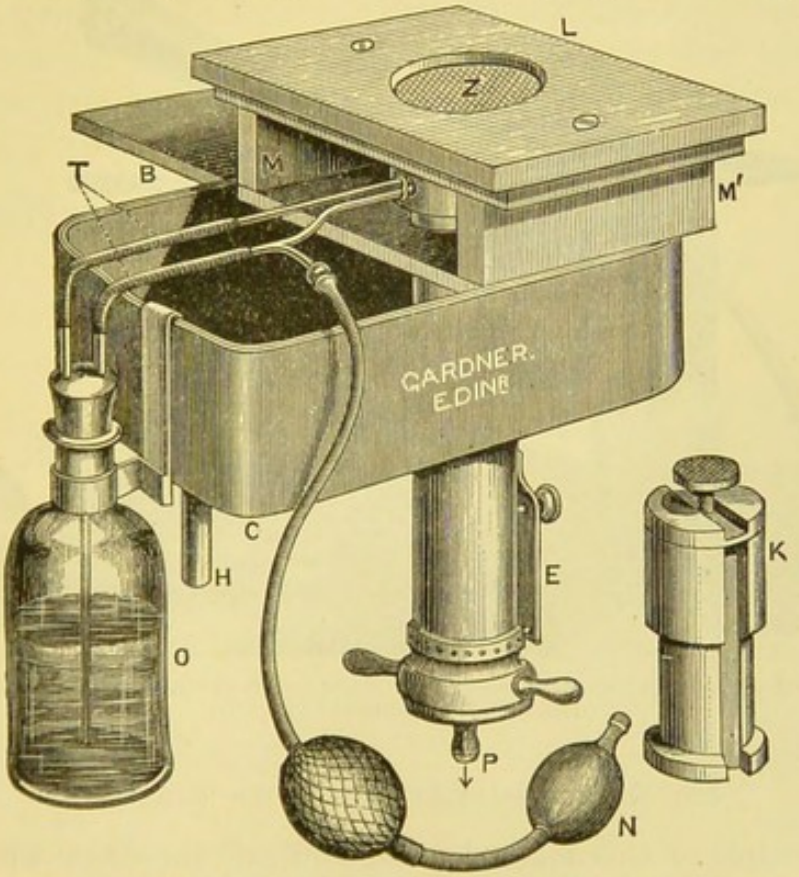


FIG. 130.—Rutherford's Microtome, arranged for freezing with ether spray.

obliquely across the frozen tissue, thin sections can be readily made. This form of freezing microtome is serviceable for private use, and for cutting sections of small organs.

3rd Method.—Freezing by Means of Ether.

The production of cold by the evaporation of ether has been applied to many microtomes. The first microtome in which it was used was the invention of Dr. Bevan Lewis in 1877.[1] Dr. Rutherford[2] has applied the method to his own microtome, as seen in Fig. 130, and he gives the following directions as to its use—

"The tissue, which should not be more than a third of an inch thick, is laid on the roof of a zinc box *Z* and covered with gum. Ether, which must be anhydrous, is then blown from the bottle *O* by the elastic bellows *N* against the lower surface of the zinc plate. The condensed ether flows down through the tube *P*, and is collected in a vessel. The spray-producing tubes *T* can be readily pulled out of the slot under *Z* for examination. The tissue is soon frozen, and it remains frozen for about five minutes in a cold room without any further production of spray."

A very convenient form of microtome in which ether spray is used as the freezing agent is that invented by Professor Charles S. Roy, shown in Fig. 131. With this instrument, the consumption of ether is small, and the mechanism works with great smoothness.

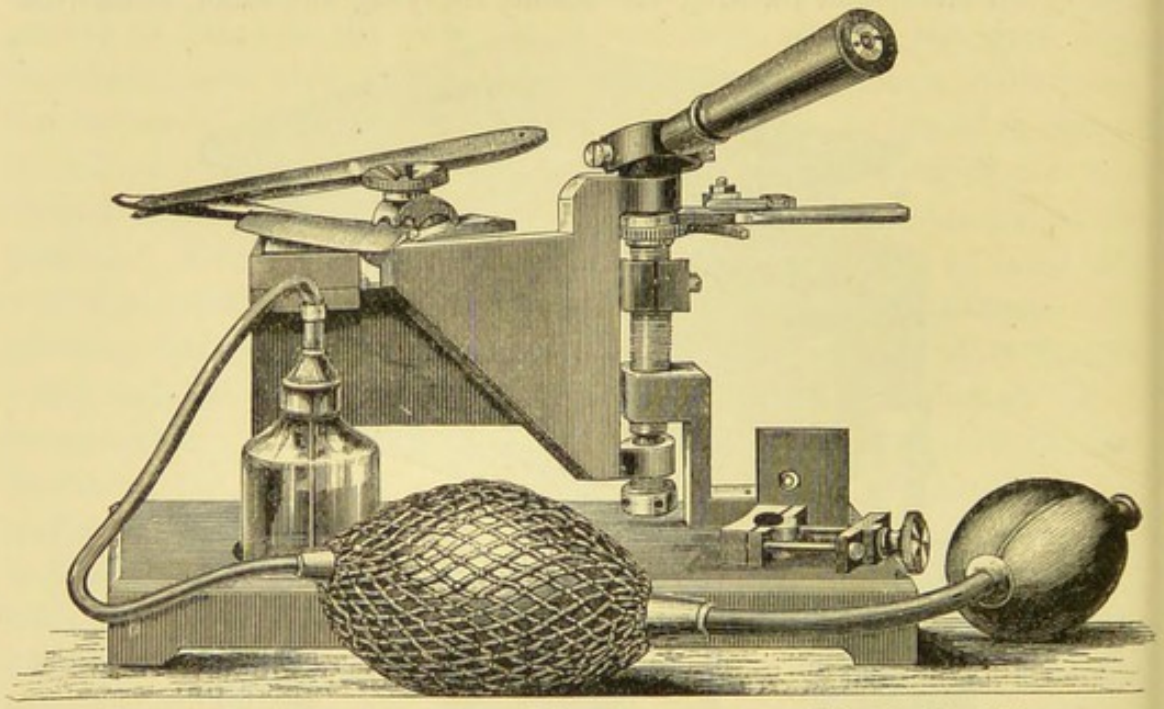


Fig. 131.—Roy's Microtome, arranged for ether spray, as made by the Cambridge Scientific Instrument Company.

4th Method.—Infiltration with Paraffin.

The principle of this method is to infiltrate the tissue with paraffin, so that the minute cellular elements may be so supported as to remain in their natural position even when they have been cut in section. One of

[1] *Journal of Anatomy and Physiology*, 1877, vol. xi.

[2] *Lancet*, Jan. 3, 1885.

the simplest methods is the following—The bits of tissue to be embedded, after having been fixed and hardened according to any of the common methods, are thoroughly dehydrated with absolute alcohol. On a suitable water bath, the temperature of which can be kept uniform by a mercurial regulator governing the gas flame underneath, the student should have a few shallow vessels, each capable of holding 40 c.c. of fluid. Chloroform, to which a little sulphuric ether has been added, is poured into one of these, and one or two bits of tissue are transferred from the absolute alcohol to the chloroform. This is then gradually heated to the melting point of the paraffin employed. Two kinds of paraffin should be at hand, one melting at 50° C., and the other at 36° C., and paraffin masses having different melting points can be readily made by mixing these in various proportions. Suppose the temperature of the water bath is regulated to stand at 38° C., then pieces of paraffin are by degrees added to the chloroform, until the bubbles at first given off cease to appear, indicating that the paraffin dissolved in chloroform has thoroughly penetrated the object. In this gradual infiltration there is scarcely any shrinkage. The paraffin is kept at its melting point for some time, so that the chloroform is completely driven off. The mass is allowed to cool, and then sections may be cut in the dry condition, either with a razor or by means of a suit-

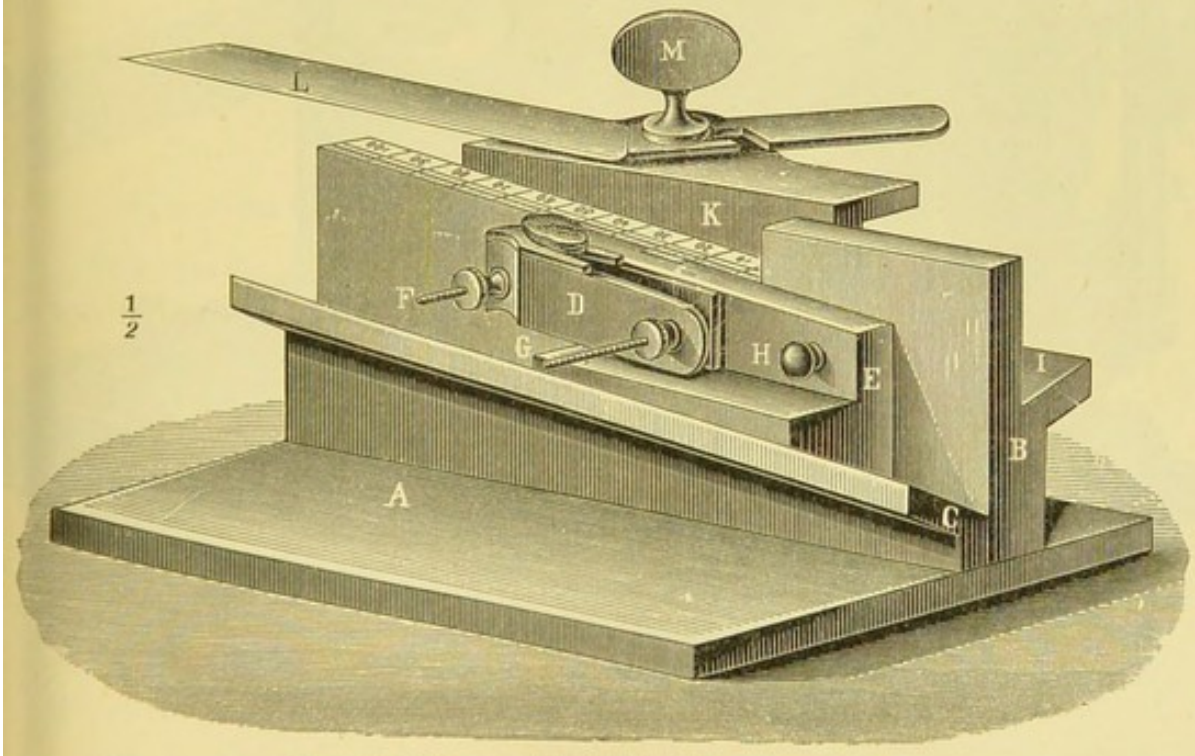


FIG. 132.—Microtome for Cutting Objects in Paraffin.

able microtome. The sections are placed on a slide, and the paraffin is dissolved by pouring over the slide a little turpentine, benzin,

naphtha, or xylol. The section may then be stained, and ultimately mounted in Canada balsam or dammar according to the usual methods.

Various forms of microtomes are now employed for cutting sections thus infiltrated with paraffin. One of the most convenient of these is shown in Fig. 132.

The paraffin block is held firmly by the clip D, and the knife L is moved by the block K, somewhat like a carpenter's plane, cutting a thin section at each stroke. With each section, the paraffin block is gradually raised to an extent varying according to the required thickness of the section. This is done by pushing the block E along the inclined plane C. In other microtomes, the paraffin block is raised automatically by the same movement as determines the horizontal movement of the knife, and thus a series of sections may be cut in quick succession. A microtome of this construction is shown in Fig. 133

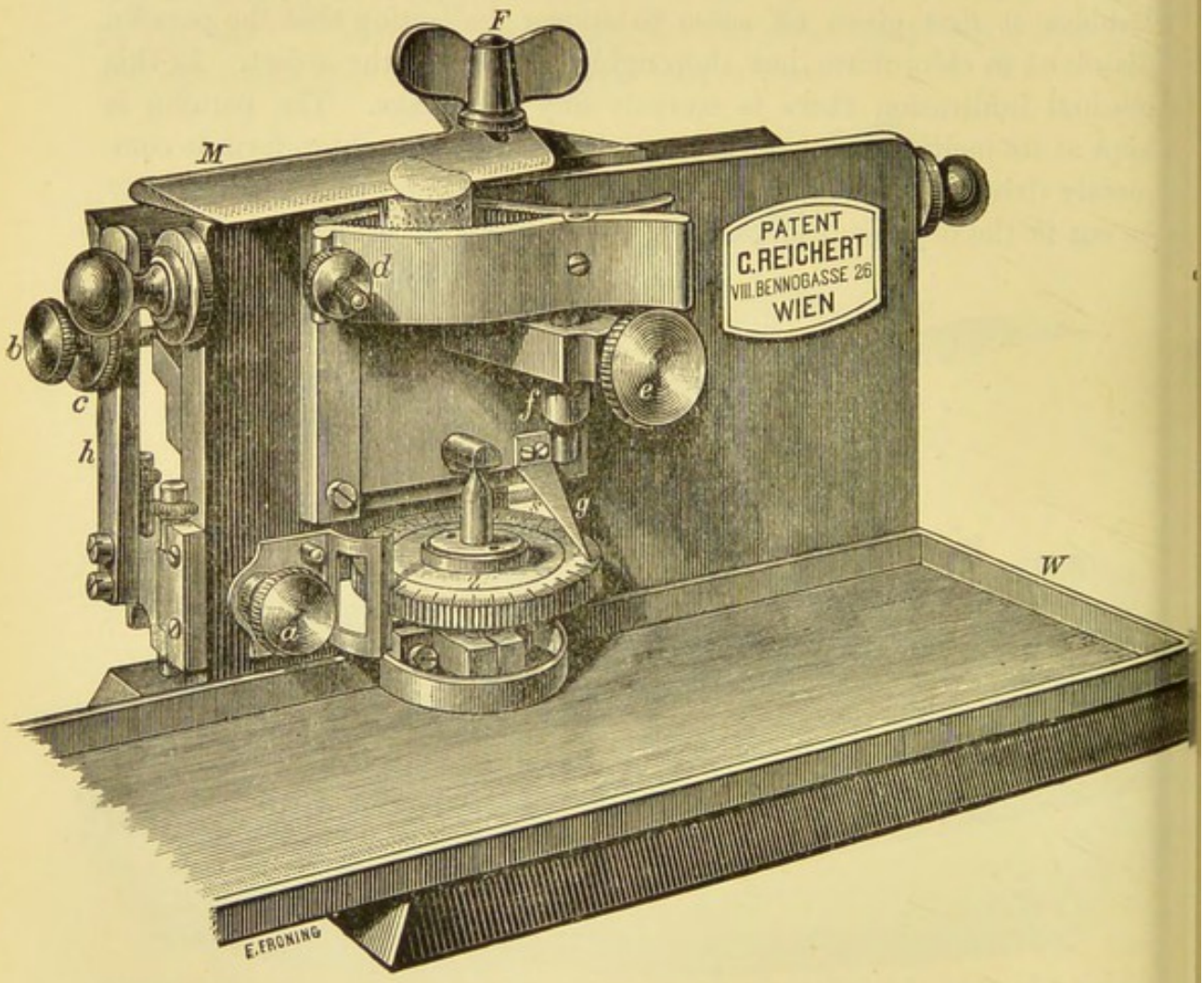


FIG. 133.—Rivet's Microtome, as modified by Reichert.

In this instrument, the clip *d* holding the paraffin block is moved slightly upwards by means of the toothed wheel *Z* with each horizontal

movement of the knife *M*, and there are arrangements by which the amount of upward movement, and consequently the thickness of the section, may be determined.

For certain kinds of work, such as cutting sections of embryos or sections of particular parts of the brain and spinal cord, it is often important to secure a consecutive series of sections, so that a number may be mounted on one slide for comparative examination. Various contrivances have been designed to secure this end. The most serviceable and simplest of these is shown in Fig. 134.

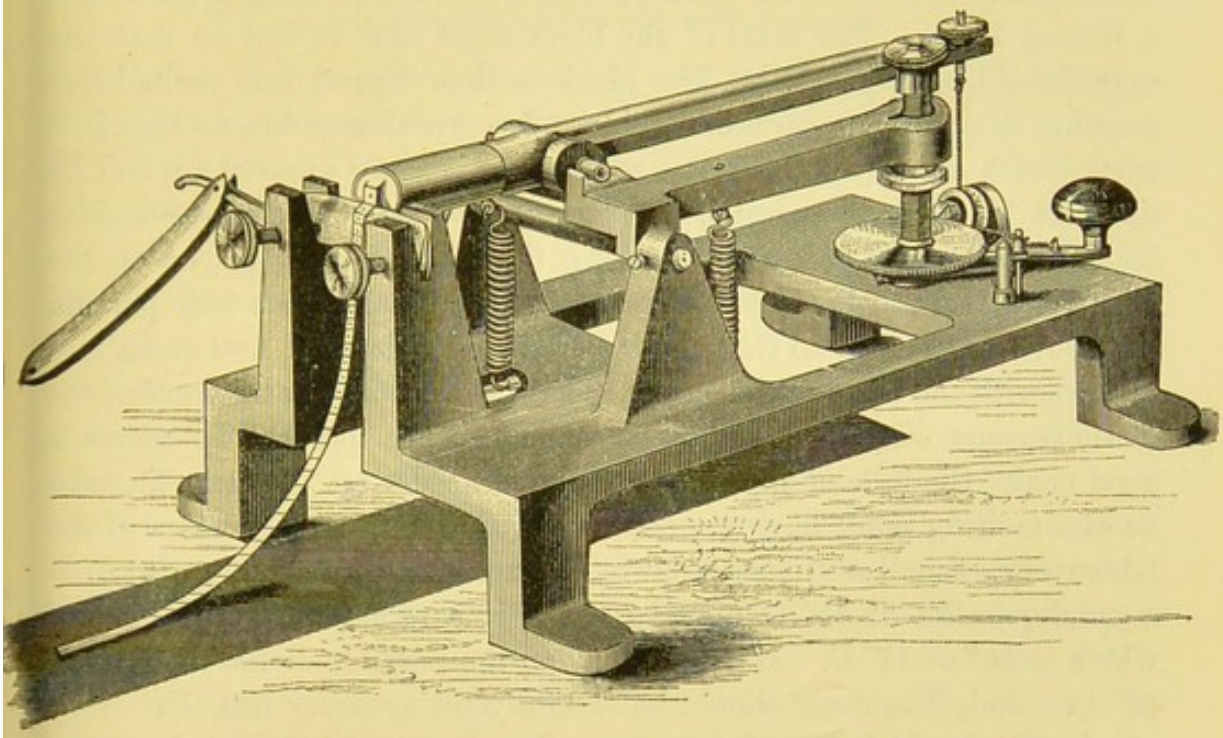


FIG. 134.—The Rocking Microtome of the Cambridge Scientific Instrument Company.

The paraffin block is fixed to the end of the horizontal bar. This bar moves in a V-shaped pivot on another rectangular bar placed underneath, which also moves on a V-shaped pivot. A glance at the diagram shows that the rectangular bar is connected with a screw bearing a disk having a toothed edge, whilst a cord is attached to the end of the upper bar and passes round a pulley connected with the knobbed arm seen at the end of the instrument. When we move this knobbed arm, the toothed disk is pushed round a little by a small catch, the horizontal rectangular bar is slightly raised, and the upper bar is also moved so as to bring the paraffin block down on the edge of the razor.

" The distance between the centres of the two pivoted systems is 1 inch, and the distance of the screw from the fixed rod is 6¼ inches. The thread of the screw is 25 to the inch; it follows that if the screw is turned once round, the object to be cut will be moved forward by $\frac{1}{25}$ of $\frac{1}{6\frac{1}{4}}$ or $\frac{1}{156}$ inch. The feed can be varied from

0 to $\frac{5}{32}$ of a turn, hence the thickness of the sections cut can be varied from a minimum, depending on the sharpness of the razor, to a maximum of $\frac{5}{32}$ of $\frac{1}{25}$ of $\frac{1}{64}$, or $\frac{1}{10000}$ of a turn. The practical minimum thickness is $\frac{1}{40000}$ of an inch approximately. The value of the teeth on the milled disk are as follows—1 tooth = $\frac{1}{40000}$ of an inch; 2 teeth = $\frac{1}{20000}$; 4 teeth = $\frac{1}{10000}$; 8 teeth = $\frac{1}{5000}$; and 16 teeth = $\frac{1}{2500}$ of an inch."

To use this instrument effectively requires careful attention to details, but when this is given, its performance is very satisfactory. The object is embedded in paraffin. The block is then cemented to the paraffin contained in the socket of the microtome, its surface being melted with a heated knife. The sides of the block must now be cut so that the opposite sides are parallel. The block is then dipped into melted soft paraffin, that is into paraffin having a low melting point, so that it is coated with a layer of this soft paraffin. It is again squared up and its opposite sides made parallel, the coating of two of the opposite sides being removed. The socket carrying the block is now placed on the microtome, care being taken that the sides coated with soft paraffin are above and below, so that the lower coated side shall first come into contact with the edge of the razor. Then, as we work the microtome, the sections come off like a ribbon, as shown in the figure. To secure a good chain, the following points must be noticed—1st, The paraffin must be of a melting point having a certain relation to the temperature of the laboratory. Small sections can always be made to "chain" when cut from a good paraffin of 45° C. melting point, in a room the temperature of which is 16° or 17° C. If at 22° C., then the paraffin should melt about 48° C. 2nd, The razor should be carefully set square. 3rd, The block of paraffin should be pared down very close to the object, and should be cut so as to present a straight edge to the knife edge, and the opposite edge should be parallel to this. 4th, The sections should be cut rapidly, by swift, sudden strokes.

To mount sections thus cut in ribbons various methods may be employed.

(*a*) *Giesbricht's method.* Make a moderately strong solution of brown shellac in absolute alcohol; filter; warm the slides to remove all moisture, and spread over them by a glass rod a thin layer of shellac. Allow the slides to dry. Moisten the shellac surface with a little creasote, and then lay the ribbons on the sticky surface thus produced. Heat the slide on a water bath at the melting point of paraffin for a quarter of an hour; the paraffin melts, and the sections come thus into contact with the shellac. The creasote also evaporates, and when the slide cools, the sections will be adherent to the shellac. Then drop turpentine on the sections to dissolve off the paraffin, and lastly mount in Canada balsam.

(*b*) *Gaule's method.* Moisten the slide with alcohol, and arrange the ribbons on it with a camel hair pencil; heat the slide slightly so as to cause the section to stick to the glass; put on a cover-glass, and run in below it a solution of equal

parts of Canada balsam and xylol. If the sections are thick, the process of adding a drop of pure xylol may have to be repeated until all the paraffin has been dissolved.

(*c*) A mixture of equal parts of egg-albumin and water may be employed, to which a little glycerine should be added to prevent too rapid drying. This is spread in a thin layer upon the glass, and when the section is laid upon the slide it is advisable to breathe upon it, thus rendering the albumin moister, and slightly heating the paraffin with which the tissue is impregnated, and so making it more pliable and adhesive.

(*d*) A mixture of one part of collodion with four parts of oil of cloves may be employed.

5TH METHOD.—CUTTING IN CELLOIDIN.

Celloidin is pure pyroxylin, and is sold in the form of tablets of a tough gelatinous consistency. It is soluble in ether and alcohol. Cut the celloidin into small bits, and dissolve in equal parts of absolute alcohol and ether. Soak the tissue to be cut in absolute alcohol, transfer it to the celloidin, and leave it there for two or three weeks. When thoroughly permeated with celloidin, the preparations are transferred to a paper tray, and are surrounded with celloidin, until a skin forms round the mass, and then the mass is thrown into a considerable quantity of alcohol of 82°, in which it may be kept for any length of time. After twenty-four hours' immersion in this alcohol, the sections may be cut in alcohol. Gudden's microtome should be used. This consists of a cylindrical well with movable bottom and horizontal plate, as in Rutherford's microtome, but fixed in the centre of an iron trough, so that the surface of the tissue may be bathed in methylated spirit while the sections are being cut. The sections may be cleared in glycerine, which makes celloidin perfectly transparent, or they may be mounted in Canada balsam by the following process: dehydrate with 95 per cent. alcohol, clear with oil of bergamot, and mount in balsam. Oil of cloves cannot be used as it dissolves celloidin. Many other methods of using celloidin have been devised, but the above is simple and convenient. To mount sections that have been cut in series in celloidin, the following is the method recommended by Minot: the sections are arranged on a slide in 95 per cent. alcohol; the alcohol is poured off, and a drop of alcoholic shellac (made by dissolving 10 grms. of bleached shellac in 100 c.c. of absolute alcohol, and filtering) placed over each section; the slide is placed over a water bath at 40° C. for five or ten minutes, until dry; it is then cleared up with oil of cloves, and finally mounted in balsam.

For full details regarding histological methods, reference is made to Whitman's *Methods of Research in Microscopical Anatomy and Embryology*, Boston, 1885; to Arthur Bolles Lee's *Microtomist's Vade-Mecum*, London, 1885; and to *Manuel de Technique Microscopique*, by Dr. Paul Latteux, Paris, 1887.

Chap. VII.—PROTOPLASM AND CELLS.

1. **Protoplasm.**—The general characters of protoplasm have already been described (p. 205). It is a jelly-like substance, colourless, or faintly yellow, amorphous, or showing a network of fine fibres, or having embedded in it molecules or granules. It is difficult to observe it in the ultimate structures of the higher animals, and our knowledge of it has been derived chiefly from observations on microscopic animals and plants. It may be met with in nature in two conditions—(1) free, or (2) in the interior of cells. Free protoplasm may occur in masses of jelly-like matter, called *plasmodies*, as may be seen in *myxomycetes*, which are the fungi found on old leather, or on tan. Such masses, when examined under the microscope, are granular, and show not only changes of form slowly occurring, but also currents flowing through the jelly-like matter in various directions. In a state of rest, protoplasm is alkaline in reaction, but during activity it becomes acid. Thus Engelmann observed that granules of blue litmus became red in the bodies of small infusoria. It has been ascertained that these movements are affected by external agents. Thus, the protoplasm moves towards light; heat quickens while cold retards the movements, and extreme cold or extreme heat will arrest the movements altogether; electricity excites contraction; oxygen, as in atmospheric air, is necessary for the movement, and excess increases its activity; and finally, the movements are arrested by carbonic acid, ether, chloroform, solutions of sulphate of quinine, and by other poisons.

Another form of free protoplasm is seen in the *amœbæ* of stagnant pools. These consist of a mass of protoplasm, sometimes homogeneous, but usually containing granules. These bodies slowly change their form, pushing out one part of the body in a particular direction as a perfectly hyaline substance, and afterwards slowly retracting it. Sometimes numerous processes are thus protruded simultaneously, or one after the other, so as to give the mass of protoplasm an irregularly stellate appearance. By these changes of form the amœba moves from place to place. It may also surround by these processes any particle of nutrient matter with which it may come into contact, and afterwards absorb it into its substance. Bodies exactly similar to the amœba, and therefore called *amœboid*, exist in the body, as, for example, the colourless corpuscles of the blood and connective tissue corpuscles.

Protoplasm may also exist in the interior of vegetable and animal cells. Thus, its movements may be watched in the hairs of *Tradescantia* (Fig. 135), of *Urtica*, the common nettle, and in the cells of *Chara* and *Vallisneria*. The existence of protoplasm may also be

demonstrated in many animal cells, such as in the cartilage cell, in the pigment cell of the frog's skin, in the ovum, and in the bodies of many cellular infusoria.

The jelly-like matter which forms the organic basis of protoplasm is nitrogenous, and is probably of an albuminous nature, while the granules consist of fats or starch. The identity of protoplasm in animal and vegetable cells has been assumed, but has never been proved. Protoplasm is permeable by water. The molecular changes occurring in it are very active. It assimilates and excretes, and it absorbs oxygen and eliminates carbonic acid. So far as our present methods of research carry us, the liberation of energy in protoplasm occurs under the form of movement. This movement presents itself under two aspects—(1) a kind of liquefaction, as it has been called, which produces the optical appearance of a current in the mass; and (2) a change of form which, in some cases, produces a movement of progression. In certain masses of protoplasm there exist small cavities filled with water, called *vacuoles*, the walls of which frequently show rhythmical contraction.

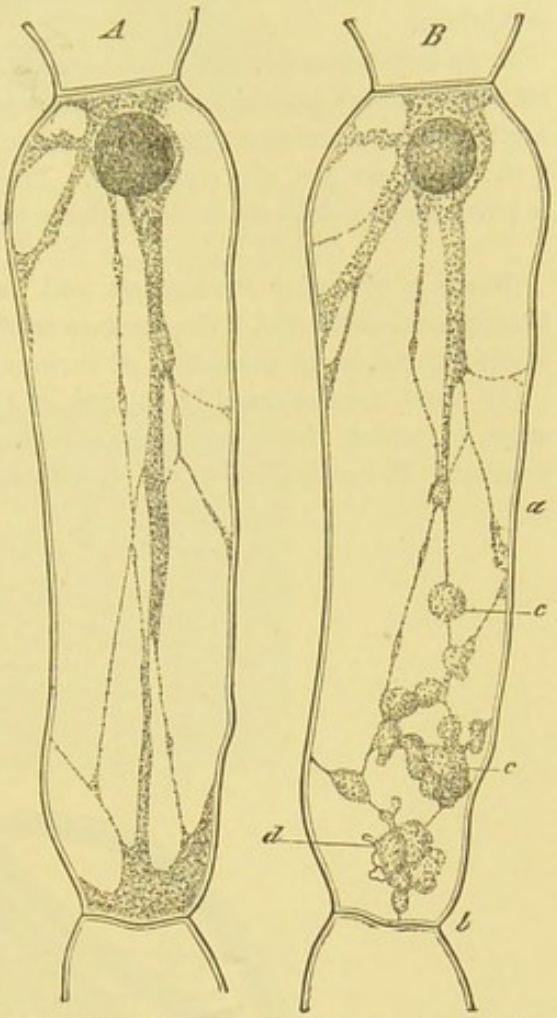


FIG. 135.—Cells of Tradescantia. *A*, fresh in water; *B*, the same cell after strong electrical irritation. The irritated protoplasm assumes the forms of little lumps or nodules, *c*, *c*, united by slender filaments and also small club-shaped projections, *d*.

Heitzmann, whose researches on the structure of the cell and of nuclei have been already referred to, in a recent communication,[1] makes the following remarkable statements regarding the nature of protoplasm—

1. Protoplasm is not structureless, but is reticulated. The nucleus is not solid, but is also reticulated, and the intersections of reticulations are the nucleoli. Protoplasm is the same substance as that which forms the nucleus and reticulum. This reticulum is the real living matter; it is in constant motion; all new growth starts from it.

2. All varieties of connective tissue, myxomatous, fibrous, cartilaginous, bony,

[1] Heitzmann, *Transactions of the New York Academy of Medicine*, vol. iv. p. 167.

and the intercellular substance, have also a reticulum of living matter. In the tissue-elements (cells), the meshes of the reticulum are filled with liquid, but in the meshes of the reticulum of the intercellular matter it is "glue yielding." There are no individual cells in a strict sense, as all are united by threads of reticulum.

3. In inflammation, not only do cells grow and proliferate, but the basis-substance furnishes a considerable amount of inflammatory elements. Pus corpuscles are the inflammatory corpuscles torn asunder and isolated.

4. Epithelia are also reticular. The cement substance joining epithelial cells is traversed by delicate conical offshoots.

Modes of observing Protoplasm and Cells.—It is sometimes necessary to submit protoplasm, cells, and living tissues to the action of physical agents, such as heat or electricity, or to the action of various gases and vapours.

(1) *Heat* may be readily applied by the use of the arrangement termed *a hot stage*, of which there are many forms. The hot stage shown in Fig. 136 is convenient. It consists of a flat hollow box, through which a stream of hot water

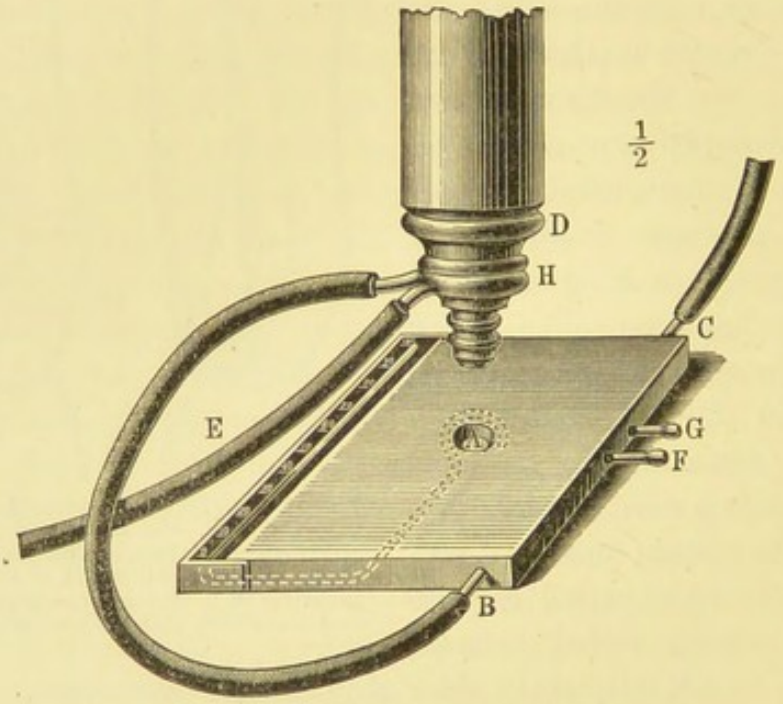


FIG. 136.—Hot Stage.

can be carried by the entrance tube, C, and the exit tube, B. In this form the hot water, after issuing from B, is carried to a small circular chamber, H, above the objective, so as to bring the latter to nearly the same temperature as the stage, and then the water flows off by the tube, E. In the centre of the stage there is a well, A, the bottom of which is formed of glass. A drop of the fluid containing the living structure to be submitted to the action of heat is placed on the centre of a cover-glass, and the latter is then inverted over A. The temperature at which the living structure is submitted to examination is ascertained by the thermometer on the left of the stage.

(2) *Electricity* may be applied, either in the form of shocks or of a continuous current by the simple arrangements shown in Figs. 137 and 138.

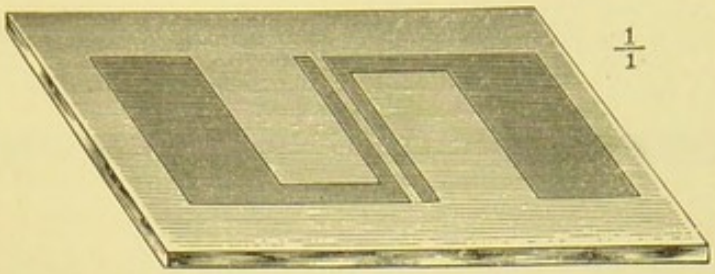


FIG. 137.—Arrangement for electrical stimulation of objects under microscopical examination, consisting of strips of tinfoil cemented on a glass slide. The object to be examined is placed on the centre of the narrow strips that are close together.

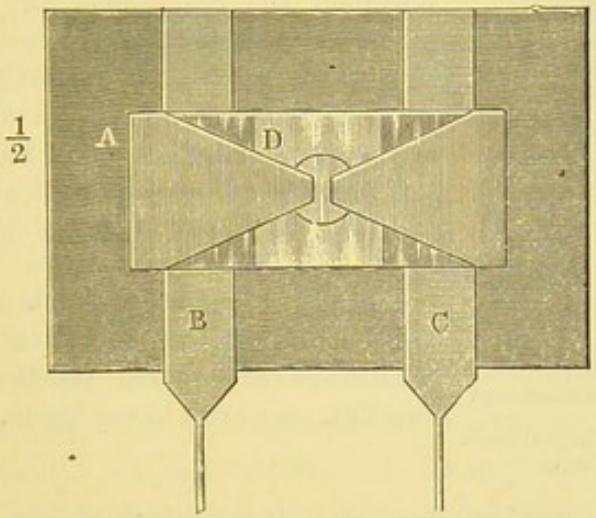


FIG. 138.—Arrangement for electrical stimulation of objects under microscopical examination. A, triangular strip of tinfoil cemented to glass, and bent round the ends of glass D. Observe a similar piece on the other side. Object is placed in centre of circle between pointed ends of tinfoil. B and C, terminals from battery lying between glass, D, and the large slide, and touching the tinfoil bent round ends of D.

(3) *Gases and Vapours.*—These may be conducted into a small chamber containing the living tissue by means of simple arrangements, such as are shown in Figs. 139 and 140.

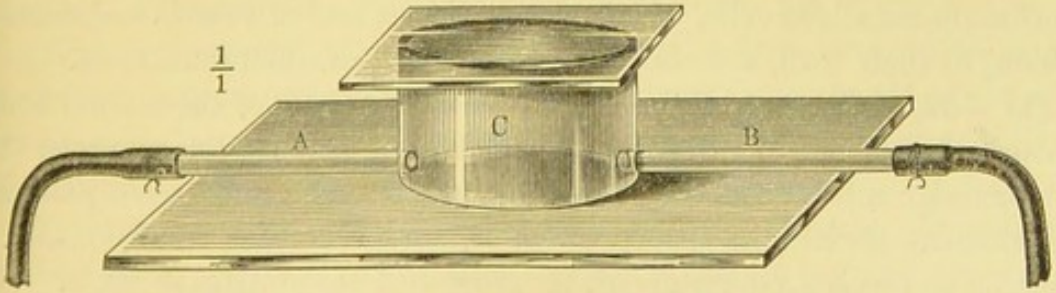


FIG. 139.—Arrangement for observing action of gases and vapours on living tissues. C, glass cylinder cemented to slide; on the under surface of the cover, the object may be placed. A and B, entrance and exit tubes.

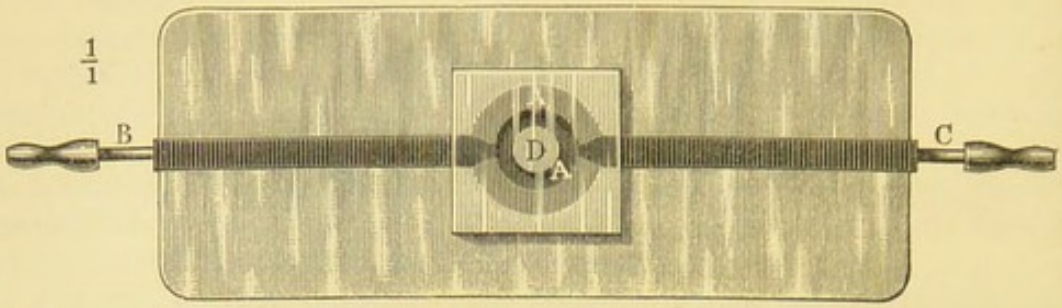


FIG. 140.—Arrangement for observing action of gases and vapours. A A, metal ring resting on slide and covered by square cover-glass; B, C, entrance and exit tubes, passing into chamber D.

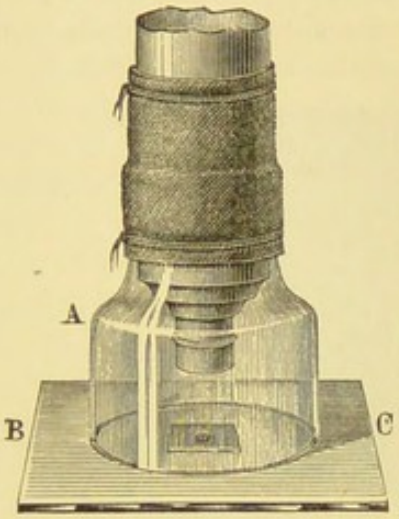


FIG. 141.—Von Recklinghausen's Moist Chamber.

For the examination of tissues or fluids in a chamber, the atmosphere of which contains so much moisture as to prevent evaporation from, and drying of, the tissue or fluid, the arrangement termed Von Recklinghausen's *moist chamber* is very convenient. It is shown in Fig. 141. The chamber consists of a cylindrical glass, A, narrow at the top, cemented to the slide, B C. Into the upper and narrow end of the glass chamber, the lower end of the body of the microscope, bearing the objective, passes, and the glass and end of the microscope are bound together by india-rubber sheeting, as shown in the figure.

2. **Cells.**—Many tissues consist almost exclusively of one species of cell. Such are termed *simple tissues* to distinguish them from other tissues, in the structure of which various species of cells are concerned; in the latter case, the term *complex tissues* is employed. By the term simple tissue, epithelial tissue, connective tissue, muscular tissue, and nerve tissue are represented. By the term complex tissue, we designate the structures resulting from the union of various simple tissues—structures which receive the more appropriate term of Organs. It must also be borne in mind that in point of fact the simplest tissues of all are composed of several tissues. For example, muscular tissue consists of muscular cells, of connective tissue, and of vessels and nerves, which, in their turn, are themselves composed of different tissues.

(1) *General Theory of Cells.*—The collective organs of the animal body consist of cells and of substances formed from cells as, for example, the intercellular substances. By the term cell is understood a morphological element limited in space, which is, under certain conditions, in a position to receive nourishment, to grow and to propagate itself. By reason of these powers, the cell may bear the designation of an Elementary Organism.

The morphological properties of cells have already been described, but a brief recapitulation may here be given. The essential constituents of a cell are—(1) protoplasm, a fine granular, yielding substance, which, insoluble in water, is easily capable of a flowing motion, and consists principally of albuminous bodies, water and salts; and (2) the nucleus, an almost clear, sharply defined vesicle, which is confined within a thin membrane, the nuclear membrane, and contains a network of minute threads of different degrees of fineness. Thickenings of these fibres are termed nuclear corpuscles or nucleoli. In the meshes of the reticulum there exists fluid, viz., the nuclear fluid. The reticulum and the nucleoli are readily coloured by means of many dye stuffs, on account of which the substance composing them is termed chromatin. The nuclear fluid is incapable of taking on colour. The nuclei also consist of albuminous bodies, of water and salts. To these there is also added a substance peculiar to nuclei, nuclein. The majority of cells contain one nucleus; only isolated cells possess several nuclei (giant cells). Cells without nuclei (the horny cells of the epidermis, the coloured blood corpuscles of mammals) originally possess one nucleus, but lose it in the process of development. As unessential constituents of cells may be mentioned—the cell membrane or cell wall, which is wanting in many cells, and in cases in which it is present, it is either the firm peripheral layer of protoplasm or it appears as a thin, almost structureless, cuticle; as constituents of secondary importance, may also be classed minute grains of pigment and drops of fat, of watery and mucous moisture, which occur in the protoplasm of individual cells. In very young cells sometimes the cell wall possesses a certain amount of elasticity so as to mould itself upon the surface, when the included protoplasm changes its form, but at other times it is stiff and rigid. It is permeable by water and by aqueous solutions of acids, bases, and salts, but it will not permit the passage of oils and fatty substances. Its chemical constitution is not the same in the animal and vegetable kingdom. In plants it is formed of cellulose, a non-nitrogenous substance; but in animals it is always nitrogenous. It contributes only to the life of the cell by its physical property of permitting the passage of fluid by osmosis, but it is not connected in any other way with the vital phenomena of the cell. Occasionally, it may become hard and impermeable by the deposit in it of calcareous salts and of silica.

(2) *Form of Cells.*—The form of cells is very various. It may be: (1) Spherical, which is the form of all cells in the embryonic period, but in the case of the adult, the white blood corpuscles, for example, are spherical; (2) Disc-shaped, as, for example, the coloured blood corpuscles;

(3) Polyhedral, the cells of the liver; (4) Cylindrical, the epithelial cells of the small intestine; (5) Cubical, the epithelial cells of the capsule of the lens; (6) Flattened, for example, endothelial cells; (7) Spindle-shaped, such as many connective tissue cells; (8) drawn out into long fibres, for example, smooth muscular fibres; and (9) those which are star-shaped, such as many ganglion cells. The form of the nucleus is in most cases adapted to the form of the cells. It assumes an oval shape when surrounded by cylindrical, spindle-shaped, and star-shaped cells; it is spherical when surrounded by round and cubical cells. Irregularly shaped or polymorphic nuclei occur in the case of leucocytes (white blood corpuscles) and giant cells.

The form would appear to depend largely on the amount and direction of the pressure to which the cell is subjected. The most important physical character of the active cell is its permeability to fluids. If cells are placed in distilled water, they swell out by imbibition; on the other hand, if placed in a fluid of greater specific gravity than that of their contents, they may shrivel and become irregular in form by the passage of a portion of their contents into the surrounding fluid.

(3) *Size of Cells.*—The size of cells fluctuates from microscopically small forms, 4 μ in diameter (certain coloured blood cells), up to bodies visible to the naked eye, such as the ova of birds, amphibia, etc.

(4) *Nutrition of Cells.*—The nutritional changes of the cell consist of *assimilation* and *disassimilation.* By assimilation the cell receives from the medium which surrounds it certain materials which it converts into its own proper substance, or which it may utilize in various ways. There appear to be two phases of the assimilative process: (1) one in which the cell transforms the matter which it receives, and the other (2) in which the substances transformed become an integral part of the cell. The first phase is well marked in the life of the vegetable cell, but is not so distinct in the animal cell, which in a manner lives upon materials previously formed by the plant; the second phase, on the contrary, exists to an equal extent both in the animal and vegetable cell. The assimilating process in the living portion of the cell has received the name of *metabolism*, while the chemical change by which matters taken up by the cell may be converted into other matters is called *metastasis.* Thus a cell may, by metabolic processes, convert dead matter into living matter like itself, or it may, by metastatic processes, convert dead matter into another form. The disassimilative processes occurring in the living cell consist of chemical changes in the substance of the cell itself, or of materials in contact with the cell. Disassimilative processes constitute a marked feature in the life of animal cells. Certain cells have the property of separating or forming a special kind of material. Thus

some cells form fatty matter, others pigments, and a third, one or other of the substances existing in bile. This power has been termed *elective affinity*. Certain materials are separated from cells by a process which may be termed *cellular excretion*, and other cells may store up certain materials in their interior, a process called *cellular secretion*.

(5) *Irritability of Cells.*—By this term we mean the aptitude which a cell has of responding to a determinate stimulus, and which is an important condition of vital phenomena. The stimulus may be mechanical, chemical, physical, or vital. The same kind of stimulus may produce different results, varying according to the nature of the cell. Thus the response to a stimulus of a muscular cell is contraction; of a glandular cell, secretion; of an epithelial or connective tissue cell, cell multiplication; and of a nerve cell, some kind of activity resulting in sensation, perception, volition, or one or other of the stages of intellectual acts. It may be laid down as an axiom that no activity in a cell ever occurs without an antecedent stimulus.

(6) *Movement of Cells.*—One of the most evident phenomena in the life history of many cells is *movement*. This is seen in the form of amœboid motion, of ciliary motion, and in the contractions of certain fibres (muscular fibres). Amœboid motion is the most important. Widely spread, it has been observed in nearly all kinds of animal cells. In marked cases, in that of leucocytes (white blood corpuscles) for example, the protoplasm of the cells stretches out finer or coarser extensions, which sub-divide, again flow together, and produce in this manner many diverse forms. The extensions (pseudopodia) may be again retracted, or they fix themselves to some particular spot and draw to themselves in some measure the remaining part of the body of the cell. Thus there are changes of situation, which are termed the *migrations* of the cells, and which play an important part in the economy of the animal frame. The expansions of the protoplasmic body may flow round grains or small cells, and thus enclose them within the body of the cell (Fig. 155). Amœboid motions in the case of warm-blooded animals are very slow, and can only be detected by careful observation with the aid of the hot stage. They may be readily studied by watching the movements of the leucocytes, or white blood corpuscles, in the blood of amphibia (frog or newt), when such forms as are represented in Fig. 142 may be observed.

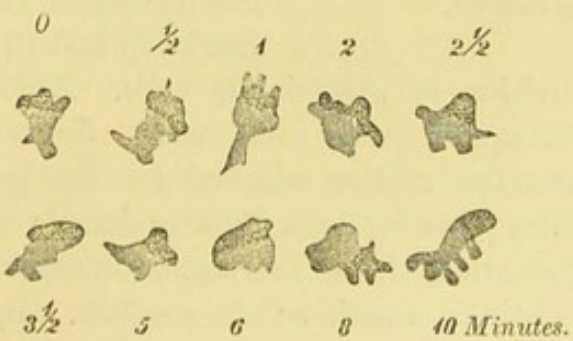


FIG. 142.—Leucocytes in frog's blood. ×560 d. Changes of form observed during ten minutes. *0*, beginning of observation, then at intervals of ½, 1, 2 minutes, etc. (Method No. 11, Appendix.)

A remarkable example of movement in cells was described by Lister as occurring in the pigment cells of the skin of the frog. These cells contain fine molecules of a black pigment. Under the influence of light, these molecules collect towards the central part of the cell, leaving light-coloured areas around each dark mass of molecules. In these circumstances, the frog's skin, to the naked eye, is pale. In darkness, the molecules pass out towards the circumference of the cells, and consequently the skin of a frog, from which light has been excluded for some time, appears dark, if examined before light has had time again to make it pale. Von Wittich found in the tree-frog (*Hyla arborea*) that contraction of the pigment cells followed mechanical irritation of the skin or irritation by electricity or by turpentine, and that the same result followed irritation of the motor nerves passing to the limb. Undoubtedly, in this case, the movement of the protoplasm in the cell is due to a reflex nervous influence, as division of the nerves passing to the limb causes the skin to appear dark, and the darkness does not then disappear on exposure to bright light. The motor impulse passing to the pigment cells must therefore travel from the nerve centres along the ordinary motor nerve trunks. On the other hand, the sensory impulses which affect the centres so as to cause the motor impulses to pass out, travel from the retina along the optic nerves to the nerve centres, as it has been ascertained that, after blindness, exposure to light no longer causes the skin to become pale. Thus, whether the play of light and shade in the skin of such amphibians is due to a streaming of the molecules to and from the centre of the cell, or to a contraction of the protoplasm in which the molecules are embedded, there is here an automatic mechanism by which the tint of the animal's skin can be brought into harmony with that of its surroundings, a fact of great general significance.

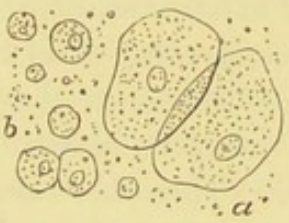


FIG. 143.—Drop of saliva, × 250 d., showing *a*, squamous epithelial cells; and *b*, salivary corpuscles, in which the Brownian movement may be observed.

There is yet another phenomenon of movement which is observable not only in the living but also in the dead cell. It is the so-called *molecular motion* or Brownian movement, an oscillation of the minutest granules in the cell, which is the result of fluid molecular currents. It is readily observed in salivary corpuscles (Fig. 143).

(7) *Formation and Reproduction of Cells.*—A distinction was at one time drawn between two kinds of cell formation, viz., (1) free cell formation (*generatio æquivoca*) and (2) the origination of cells through division of pre-existing cells. According to the theory of free cell formation, the cells were supposed to have originated in soft granular matter adapted for the purpose, called *kytoblastema.* This theory has been totally abandoned; we now know only *one* kind of cell formation, viz., the formation of cells through the *division* of cells already existing. *Omnis cellula e cellula.* In the division of a cell, the nucleus plays an important part. The division is effected, not often in the simple way of the division, first of the nucleus, and then of the protoplasm into two equal parts, termed *direct division* or *karyostenosis*;

but it occurs as a complex process, termed *indirect division* or *karyokinesis*. This latter process has already been fully described at p. 213. As special modifications of cell division, the so-called *endogenous* cell formation and the process of *budding* furnish good examples. Endogenous cell formation is seen in cells which possess a firm covering, such as ova (Fig. 111, p. 234) and in cartilage. (See Cartilage.) The process of division is exactly the same as that above described, with this difference that the daughter cells, originating from one cell (the mother cell) through continued division, remain enclosed within a common covering. The process of cell multiplication is termed *gemmation* or *budding*, when a cell sends forth shoots or buds which gradually separate from the parent and grow into independent cells. Young cells always exhibit the character of the mother cell. The development of connective tissue cells, for example, from the division of an epithelial cell never occurs.

(8) *Phenomena of Secretion in Cells.*—This process is manifested by differences seen in the form and contents of the gland cell when it is in a state of rest and when it is in a state of activity. In many, as, for example, in the cells of a serous gland, these differences are shown in a small bulk and a dark appearance of the cells in the state of rest, and an increased bulk and clearer appearance in the state in which the process of secretion is active. In the case of other gland cells, such as those of mucous gland cells in man, the formation of the secretion admits, on the other hand, of being more exactly described. We begin with the secretionless

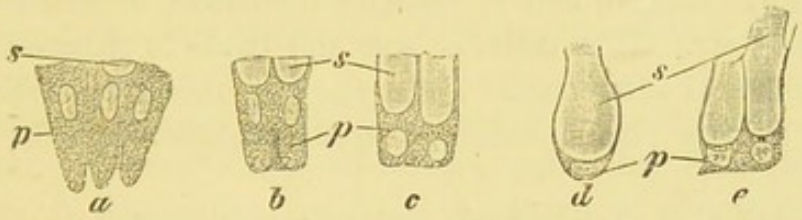


FIG. 144.—Secreting Epithelial Cells. From a thin section through the mucous membrane of the human stomach, × 560 d. *p*, Protoplasm; *s*, secretion; *a*, two cells in a resting state. The cell situated between these shows the beginning of the mucous metamorphosis. *e*, The upper wall of the cells on the right has burst, the contents have escaped, the granulated protoplasm has again increased, and the nucleus has again become round. (Method No. 12, Appendix.)

condition in which the cylindrical cell contains a granular protoplasm, and an oval nucleus placed almost in the middle of the cell. The formation of the secretion now appears on the free side of the gland cell, and is shown by the alteration of the granular protoplasm into a clear mass (Fig. 144, *b*, *s*). As the process advances (*c*) larger masses of protoplasm are converted into secretion, and the nucleus and the remainder of the granular protoplasm are pressed against the base of cell, whereby

the nucleus gradually becomes round or even flattened. The entire cell, filled with secretion, has now become larger. Finally, the wall of the cell ruptures on the free upper surface. The secretion issues out gradually, while the protoplasm regenerates itself and the nucleus resumes its previous oval form. The cell thus passes back to the state of rest. Several of the stages of the process of secretion are illustrated in Fig. 145. The majority of gland cells do not perish in the act of secretion, but they are in a position to repeat the same process. The cells of the sebaceous glands are excepted from this rule, as secretion is formed in these by the cells falling in pieces. (See also Secretion.)

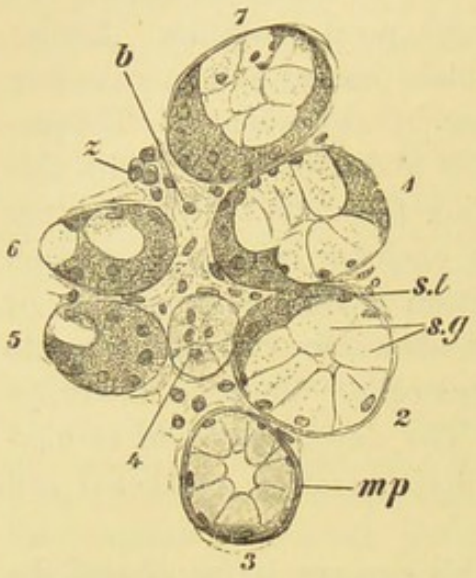


FIG. 145. — Section of sub-lingual gland of man, × 240 d. Opposite 2, six cells are seen full of secretion, *s*, *g*; two cells, containing no secretion, *s*, *l*, are thrust away from the centre of the pouch of the gland to form a half-moon or crescentic mass. This figure will be repeated and described fully in treating of salivary secretion.

(9) *Growth of Cells.*—The growth of cells is determined by the activity of the protoplasm contained in them. Seldom do they grow equally in all directions, so as to preserve the original form of the cell. As a rule, disproportionate growth occurs. The original form of the cell is thereby changed, and it becomes distended, or flattened, or put out of shape, etc. Most cells are soft and liable to change their shape under mechanical influences. Thus, the cylindrical epithelial cells in the empty urinary bladder assume flattened shapes when the bladder is distended.

(10) *Evolution of Cells.*—Each cell may be regarded as an organism which has a determinate period of existence. The duration of life is very variable. Some, such as epithelial cells, may pass through existence in from twelve to twenty-four hours; those of the mammary gland may have a more transitory existence; whilst such cells as those of cartilage probably exist during the lifetime of the individual. Cells die in various ways. They may be mechanically removed from the superficial surfaces, as is the case with epidermic cells; or they may undergo chemical transformations of such a nature as to be inconsistent with the vitality of the cell. For instance, as frequently happens in pathological conditions, the cell may become infiltrated with calcareous, fatty, or amyloid matter. Sometimes, also, cells may break down, molecule by molecule, undergo liquefaction, and be absorbed.

CHAP. VIII.—STRUCTURE OF THE VARIETIES OF CELLS AND OF CELLULAR TISSUES.

From the morphological point of view, all the tissues are composed of cellular elements, although in the fully developed condition of certain tissues it may be difficult to detect the cell form. Thus, while epithelial and cartilaginous tissue and the corpuscles in the blood show the usual cell type, so-called muscle and nerve fibres have become so much altered as to conceal the fact that they also are composed of cells. Modern physiology, however, attaches so much importance to the doctrine that all tissue elements are formed of cells—a doctrine also having a direct bearing on many pathological questions—as to make it desirable, in the first instance, to take a general survey of the cell-elements, leaving the functions of these cell elements to be afterwards discussed.[1]

1. **Leucocytes** are cells having no cell wall, and consisting of a granulated, glutinous protoplasm, and of one or more nuclei. A definite form cannot be ascribed to them, because, during life, they show amœboid motion; in a state of rest they are globular (Fig. 146, 8); their size fluctuates between 4 and 14 μ. Leucocytes are found in the lymph and chyle vessels (lymph and chyle corpuscles), in blood-vessels (white or colourless blood corpuscles), in the marrow of the bones (marrow cells), in adenoid tissue, and lastly, scattered in the connective tissue and between epithelial and gland cells, whither they have wandered by their amœboid motions. On this account they are sometimes called *wandering cells.*

2. **Coloured Blood Cells** (coloured blood corpuscles, Fig. 146) are soft, ductile, very elastic structures, possessing a smooth surface. In man and the mammalia they have the shape of almost flat circular discs,[2] which are hollowed out on each side, and consequently resemble biconcave lenses. The llama and the camel are exceptions to this rule, as their blood corpuscles are oval. In the case of man, their surface diameter is, on an average, 7·5 μ; their thickness 1·6 μ. The coloured blood corpuscles of our domestic mammalia are all smaller; the largest are those of the dog (7·3 μ). The coloured blood corpuscles consist

[1] Histological details regarding the various tissues shortly described in this chapter, which are of special physiological interest, will be given when we consider the special functions. For example, the structure, etc., of the blood corpuscles will be again referred to in the description of the characters of blood.

[2] Besides these, a few spherical coloured blood corpuscles are to be found (Fig. 146, *A* 7); they are smaller (5 μ) than the ordinary red corpuscles.

of a stroma (protoplasm), which is brought into close relation with the colouring matter of the blood. Hæmoglobin gives to the coloured blood corpuscles their yellow or greenish-yellow hue. They have no cell wall nor nucleus. The coloured blood corpuscles of fishes, amphibia, reptiles, and birds are distinguished from those of mammalia by their oval and biconvex shape, by their large size (in the case of the frog 22 μ long, 15 μ broad), as well as by the presence of a round or oval nucleus. In other respects, they display properties similar to those of the mammalia.

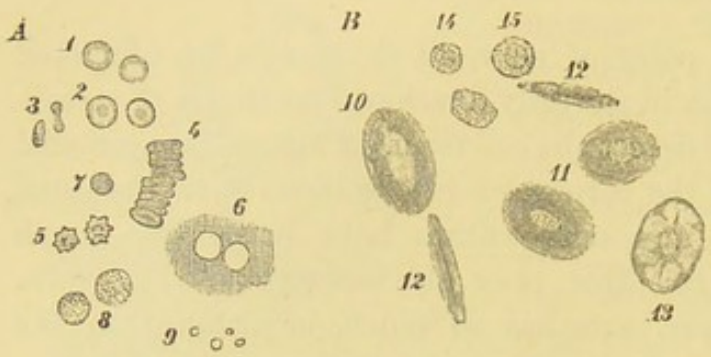


FIG. 146.—Blood corpuscles—A, of man; B, of the frog, 560 times magnified. *1-6*, disk-shaped coloured blood corpuscles. *1*, Deep focusing; *2*, high focusing of the objective; *3* and *4*, edgeways; *5*, become stellated by evaporation; *6*, after the addition of water. *7*, Spherical coloured blood corpuscles. *8*, Colourless blood corpuscles. *9*, Small blood plates. *10-13*, Coloured blood corpuscles of the frog. *10*, Entirely fresh, nucleus little distinguishable; *11*, some minutes later nucleus distinctly visible; *12*, seen on the side; *13*, after addition of water. *14*, Living. *15*, Dead colourless blood corpuscles. (Method No. 13, Appendix.)

The size of the coloured corpuscles varies greatly in different animals. Gulliver has given the following measurements in fractions of an inch—

	Diameter.
Man,	$\frac{1}{3200}$
Elephant,	$\frac{1}{2745}$
Musk Deer,	$\frac{1}{6300}$

	Long Diameter.	Short Diameter.
Camel,	$\frac{1}{3250}$	$\frac{1}{5921}$
Ostrich,	$\frac{1}{1049}$	$\frac{1}{3000}$
Pigeon,	$\frac{1}{2314}$	$\frac{1}{3429}$
Humming Bird,	$\frac{1}{2000}$	$\frac{1}{4000}$
Frog,	$\frac{1}{1108}$	$\frac{1}{1821}$
Crocodile,	$\frac{1}{1231}$	$\frac{1}{2280}$
Proteus,	$\frac{1}{400}$	$\frac{1}{727}$
Pike,	$\frac{1}{2000}$	$\frac{1}{3333}$
Shark,	$\frac{1}{1143}$	$\frac{1}{1934}$
Earth-worm,	$\frac{1}{110}$	$\frac{1}{1200}$
Leech (after addition of water),	$\frac{1}{3000}$	$\frac{1}{3600}$

The size of the coloured corpuscles bears a relation to the calibre of the ultimate capillary vessels, which are just large enough to admit of their passage in single file. Injections, therefore, of the blood-vessels are more easily made in reptiles than in any other animals.

The addition of *water* causes the coloured corpuscles to lose their colour, to swell out, and become globular. *Syrup*, *gum*, solutions of *albumin*, and dense *saline* solutions, render them flaccid, misshapen, irregular in outline, contracted, puckered, etc. *Acetic acid* appears at first wholly to dissolve mammalian cor-

puscles, but their form may be faintly recognized on adding to them tincture of iodine. But on the oval corpuscles of birds, reptiles, and fishes, the effect is simply to dissolve, or render very transparent the protoplasm, while the nucleus is unaffected, and even becomes more distinct. *Astringent* solutions, and especially a solution of nitrate of silver, cause puckerings and folds to appear in the cell. This is well seen in the corpuscles of the newt, where also a solution of *boracic acid* develops the nucleus in the form of an oval body, from which processes radiate outwards. A solution of *magenta* causes a minute molecule to appear on the external margin, as pointed out by Sir William Roberts. The same observer, also, was the first to describe the effect of a dilute solution of *tannic acid*, which causes one, and sometimes two, protrusions to take place from the corpuscle. A stream of *carbonic acid* passed into frog's blood is quickly followed by the appearance of the oval nucleus, which may again disappear under the action of air, or of oxygen.

3. **Epithelial Cells** are sharply-defined cells consisting of protoplasm and a nucleus. A cell membrane is frequently awanting, but it is often represented by a more consolidated portion of the peripheral layer of protoplasm. Most epithelial cells are soft, and readily adapt themselves to pressure brought to bear upon them; hence the numerous forms taken by epithelial cells. Two principal forms may be distinguished—(*a*) the flat, and (*b*) the cylindrical or prismatic. There are numerous transitional forms from the one kind to the other.

(*a*) *Flat Epithelial Cells*, flat cells, pavement cells, seldom possess regularity of shape. Pigment epithelium alone (see Fig. 150) consists of tolerably regular hexagonal cells; in the majority of cases the contour is very irregular.

(*b*) *Cylindrical Epithelial Cells*, cylinder cells, looked at from the side, are distended elements, whose height surpasses their breadth; looked at from above, they appear polygonal; they are thus in reality prismatic. Epithelial cells, which are as long as they are broad, are

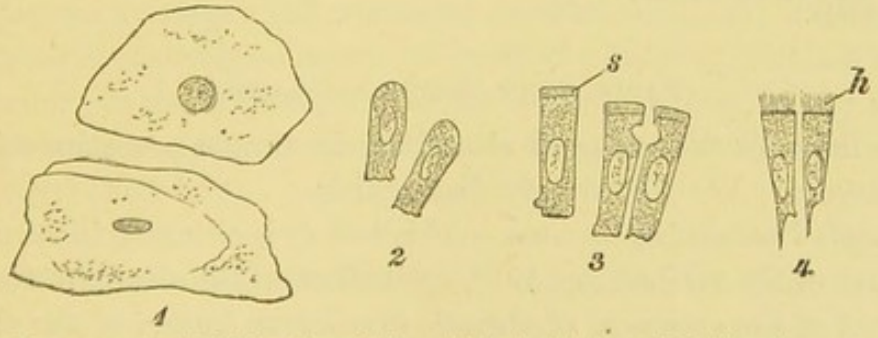


FIG. 147.—Epithelial cells of the rabbit, isolated, × 560 diameters. 1, pavement cells from the epithelium of the mucous membrane of the mouth; 2, cylindrical cells, corneal epithelium; 3, cylindrical cells, with cuticular edge, *s*, intestinal epithelium; 4, ciliated cells—*h*, cilia, bronchial epithelium. (Method No. 14, Appendix.)

termed *cubical cells*. Many cylindrical cells bear on their upper surface a striped border (Fig. 147, 3), which is a product of the cell—a kind of

cuticular structure. Other cylindrical cells have on their free surface delicate cilia, which during life show oscillating motion in a certain direction. We call these *ciliated* or *flagellate cells*.[1]

Epithelial cells are so bound together that they either touch each other with their smooth surfaces (*i.e.* through the interposition of an intermediate or cement substance present in very small quantity), or they are united by variously shaped prolongations. There are also included under such prolongations the delicate bristles and filaments which are visible on the upper surface of certain epithelial cells (Fig. 148). These are connecting threads which penetrate the cement substance between two epithelial cells, and effect a connection with neighbouring epithelial cells. Cells provided with such bristles and filaments are termed *bristle cells*; the bristles themselves have been indicated of late by the appropriate term, *intercellular bridges* (Fig. 149).

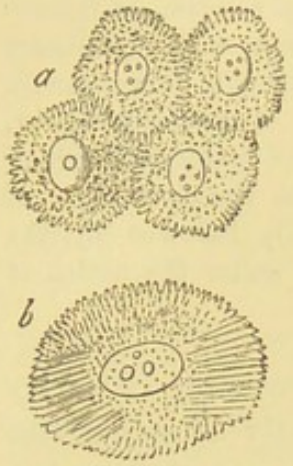


FIG. 148.—Spinous, bristle, or furrowed cells. *a*, from the undermost layers of the human epidermis; *b*, a cell from a papillary tumour of the human tongue.

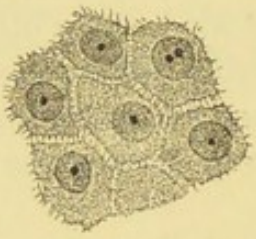


FIG. 149.—From a perpendicular section through the stratified pavement epithelium of the *stratum mucosum* of the epidermis, × 560 d. Seven pavement epithelium cells bound to each other by intercellular bridges. (Method No. 15, Appendix.)

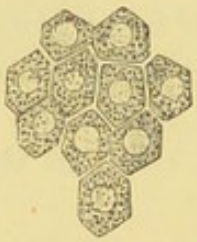


FIG. 150.—Simple pavement epithelium (pigment epithelium of the retina) of man, × 560 d. (Method No. 16, Appendix.)

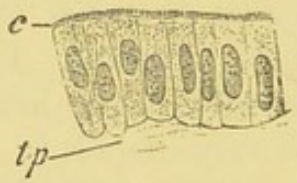


FIG. 151.—Simple cylindrical epithelium (intestinal epithelium) of man, × 560 d. *c*, Stripe-shaped cuticular edge, cylindrical cell; *tp*, tunica propria, from small intestine. (Method No. 17, Appendix.)

VARIETIES OF EPITHELIAL LAYERS.

Epithelial cells are arranged sometimes in simple, sometimes in compound, layers. We accordingly distinguish—

(1) *Simple Pavement Epithelium.*—This variety is observed in the pigment epithelium of the retina (Fig. 150), epithelium of the alveoli of the lungs, of the coat of the stomach, of the *rete vasculosum Halleri* of the skin and of the lining membrane of the labyrinth. The epithelium formed of one layer of cubical cells constituting the covering of the *plexus choroidea*, and the epithelium on the inner surface of the capsule of the lens, in

[1] The specially differentiated sense epithelium cells will be described in treating of the senses.

the thyroid gland, and in most other glands, also belong to this class of epithelium.

(2) *Simple Cylindrical Epithelium*, Fig. 151, such as the epithelium of the intestinal canal and of many ducts of glands.

(3) *Simple Ciliated Epithelium* exists in the most delicate bronchiæ, in the uterus, in the Fallopian tubes, in the nostrils, and in the central canal of the spinal cord.

(4) *Stratified Pavement Epithelium.*—In this variety, the cells are not all pavement cells, as the deepest layer consists of cylindrical cells. Above this, there are several layers of differently shaped bristle cells (mostly in the form of irregular polygons, Fig. 152), and on these are placed cells more and more flattened towards the surface of the epithelial layer. Stratified pavement epithelium is found in the mouth, in the cavity of the throat, in the œsophagus, on the vocal cords, on the conjunctiva, in the vagina, and in the female urethra. The epidermis is also covered over with stratified pavement epithelium; but the latter is characterized by the cells of the layer nearest to the surface being changed to small horny scales, while they have lost their nucleus. On the nails and hair also, horny, but in this case nucleated, scales are found.

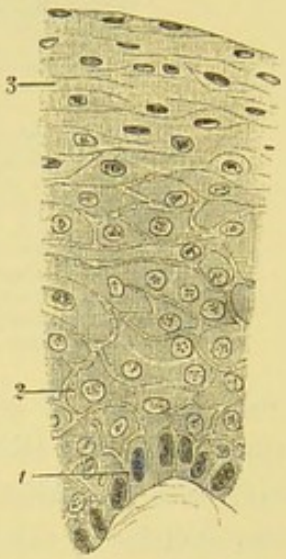


FIG. 152.—Stratified pavement epithelium (larynx of man) × 240 d. 1, cylindrical; 2, bristle cells; 3, flat cells. (Method No. 18, Appendix.)

(5) *Stratified Cylindrical Epithelium* is, in the case of man, found only on the *conjunctiva palpebrarum*. The arrangement of the layers is the same as in the next form.

(6) *Stratified Ciliated Epithelium.*—The cells next to the surface are cylindrical and they bear minute hair-like processes, *cilia;* in the deepest layers, roundish cell elements are met with, and in the middle layers, spindle-shaped ones occur. Stratified ciliated epithelium is found in the larynx, in the trachea, and in the large bronchiæ, in the nostrils, and in the upper part of the throat, in the Eustachian tube, and in the testicles[1] (Fig. 153).

4. **Connective Tissue Cells** are recognized as such only when found in the tissues, that is to say, an *isolated* connective tissue cell is indistinguishable from a leucocyte. In form they are very variable. Connective tissue cells are either flat, polygonal, bent, or twisted in various directions; or they are roundish, oval, spindle or star-shaped. (See Connective Tissue.) Their arrange-

[1] The embryonal origin of epithelial layers has already been indicated, p. 251.

ment is the reverse of that of epithelial cells, inasmuch as the rule is that they are separated from their neighbours by means of consider-

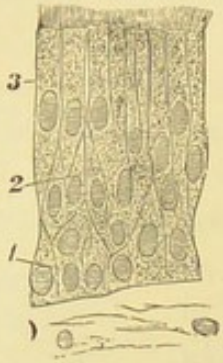


FIG. 153. — Stratified ciliated epithelium: 1, oval; 2, spindle shaped; 3, cylindrical cells. × 560 d. From the mucous membrane of the nose (*regio respiratoria*) of man. (Method No. 19, Appendix.)

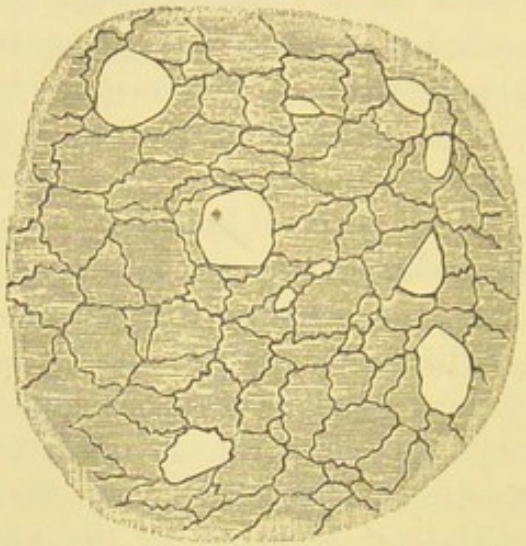


FIG. 154.—Endothelial cells on the serous covering of the diaphragm. (Some of the ragged openings are probably accidental.)

able masses of connective tissue. Endothelial cells are an exception to this as they have a great resemblance to simple flat epithelium and can be distinguished only by their anatomical position (in the cavities of the joints, in the sheaths of tendons, in the *bursæ mucosæ*, and on the inner surface of the heart, blood-vessels, and lymphatics). (Fig. 154.) The protoplasmic nucleated body of the cells of connective tissue may contain granules of colouring matter. The cells are thereby changed into pigment cells, which occur in man only in the eye, but in many of the lower animals they are widely diffused. Other connective tissue cells may contain small drops of fat, which, when they become large, give a spherical shape to the cell. Such are termed fatty cells. (See Fig. 155, *d*.) In fatty cells, the protoplasm forms only a narrow border, situated on the periphery of the cell. The flattened nucleus is found at the side of the cell. The border is frequently so thin that it sinks out of sight. (See Fig. 155.)

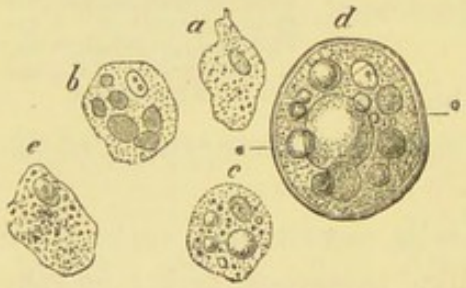


FIG. 155.—Cells with deposits of foreign matter in their protoplasm. *a*, A lymph corpuscle with granules of carmine embedded in it; *b*, another of the same, with included blood cells and fragments of the latter; *c*, an hepatic cell, containing fat globules and granules of biliary pigments; *d*, a cell with fat globules; *e*, another with granules of melanin.

5. **Fat** or **Adipose Tissue Cells** are nearly akin to the fatty cells above described, but they are nevertheless distinguished from the latter by the fact that they are united without the interposition of an intermediate substance, so as to form in certain parts of the body a tissue interpenetrated by numerous blood vessels, lymph vessels, and

nerves, namely, *fatty* or *adipose tissue*, which plays a very important part in various physiological processes. In extreme emaciation,

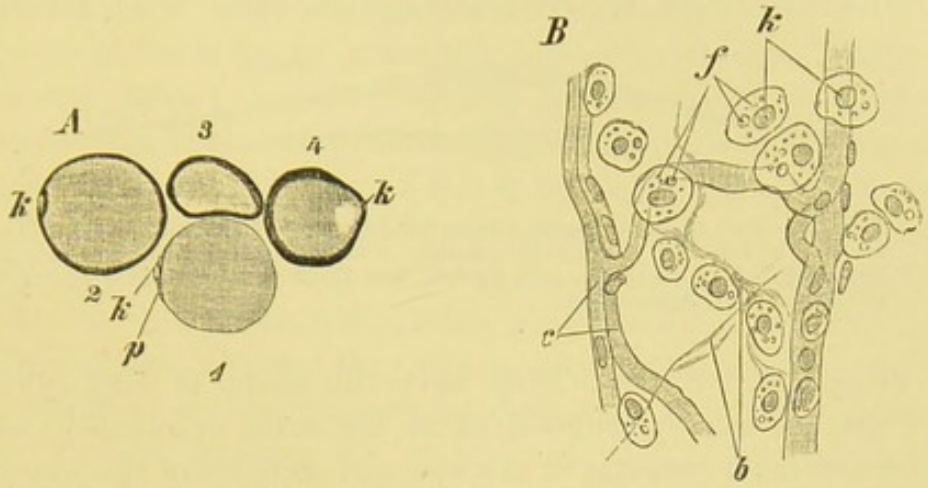


FIG. 156.—Fat tissue cells from the axilla, × 240 d. *A*, of an individual only a little emaciated; *1*, on focusing the objective on the equator of the cell; *2*, objective slightly raised; *3*, *4*, cells put out of shape by pressure; *p*, traces of protoplasm situated in the neighbourhood of the nucleus. *B*, of a highly emaciated individual; *k*, nucleus; *f*, small drops of fat; *c*, blood capillaries; *b*, bundle of connective tissue. (Method No. 20, Appendix.)

we find, in individual fat tissue cells, the fat shrunk to small drops, and its place occupied by a pale protoplasm, mingled with mucous fluid. The cell is no longer spherical, but has become flat. Such cells are termed *serous fat cells.*

6. **Muscle Cells.**—The fibres of the muscles appear in two forms, which we term the *smooth* and the *transversely striated.* Both are cells, of which the body is greatly extended in a longitudinal direction.

(1) *The smooth muscular fibres* (Fig. 157) (contractile fibre cells), are spindle-shaped, cylindrical, or slightly flattened cells, having pointed ends. Their length fluctuates between 45 and 225 μ; their breadth between 4 and 7 μ; in the pregnant uterus, still longer, smooth muscular fibres, measuring $\frac{1}{2}$ mm., have been found. They consist of a homogeneous protoplasm, containing a rod-shaped nucleus.[1] This nucleus is characteristic of smooth muscular fibres. The fibres are united firmly to each other. Smooth fibres are found in the intestinal canal, in the smaller bronchial tubes, in the gall bladder, in the pelvis, in the kidney, in the ureter, in the urinary bladder, in the sexual organs, in blood and lymph vessels, in the eye, and in the skin. As this kind of fibre is not subject to voluntary control, it is often called involuntary muscular fibre.

(2) *The transversely striated muscular fibres* are to be recognized as

[1] In individual smooth muscular fibres a longitudinal striation of the protoplasm has been observed.

cells only by studying their development. As the result of a colossal growth in length, of continued division of the nucleus, as well as of peculiar differentiation of their protoplasm, they have attained to

FIG. 157.—Two smooth muscular fibres from the small intestine of a frog. Isolated by means of 35 per cent. solution of caustic potash. The nuclei have, by action of the caustic potash, lost their characteristic form. (Method No. 21, Appendix.)

highly complicated forms. They have the shape of long cylindrical fibres, which are usually rounded off at the ends, or obtusely pointed, but in certain cases (muscles of the eye and muscles of the tongue) the fibres are branched (Fig. 158). Their length, only in rare cases, exceeds 4 cm.; their thickness fluctuates between 15 and 50 μ; and the muscles of the face have finer fibres than the muscles of the trunk.

The following table gives certain measurements of muscular fibres in fractions of an inch. (Allen Thomson.)

Muscle of	Diameter of Fibres.			Distance of Transverse Striæ.
	Greatest.	Least.	Average.	
Birds, - -	$\frac{1}{350}$	$\frac{1}{1500}$	$\frac{1}{810}$	$\frac{1}{10400}$
Mammalia, -	$\frac{1}{192}$	$\frac{1}{1100}$	$\frac{1}{560}$	$\frac{1}{10000}$
Man, - -	$\frac{1}{192}$	$\frac{1}{615}$	$\frac{1}{400}$	$\frac{1}{9400}$
Reptiles, -	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{480}$	$\frac{1}{11600}$
Fishes, - -	$\frac{1}{65}$	$\frac{1}{765}$	$\frac{1}{220}$	$\frac{1}{11100}$
Insects, - -	$\frac{1}{200}$	$\frac{1}{765}$	$\frac{1}{420}$	$\frac{1}{9500}$

Each transversely striated fibre consists of the following parts—(1) A structureless covering or membrane, termed the *sarcolemma*, which surrounds the fibre like a sac. (2) Oval *nuclei*, placed parallel to the axis of length of the muscular fibres, which nuclei lie, in the case of the mammalia, between the sarcolemma and muscle substance; but in other vertebrates, in the muscle substance itself, in which they are surrounded by a very small amount of protoplasm. This protoplasm is the remainder of the original cell protoplasm not spent in the up-building of the transversely striated muscle substance, and it is accumulated especially at the poles of the nuclei. The granulated accumulations of protoplasm are termed *interstitial granules*. (3) *The muscle substance.* It is trans-

versely striated, *i.e.* it shows alternately dark and narrower, and clear and broader transverse bands. The substance of the dark transverse bands is doubly refractive (*anisotropic substance*); that of the bright transverse bands is singly refractive (*isotropic substance*). Under strong magnifying powers, double transverse stripes (*transverse lines*) may be perceived (Fig. 164), which divide the clear transverse bands into two equal parts; in the case also of the dark transverse bands, another set of transverse stripes, the so-called *middle discs*, has been described. The collective stripes and bands (with the exception of the middle discs) penetrate the entire thickness of the muscular fibres, and they are thus in reality discs. Besides the transverse striation, there is also to be observed a more or less pronounced longitudinal striation of the muscular fibres. Certain reagents, for example, solution of chromic acid, cause this longitudinal striation to manifest itself still more distinctly, and effect even a falling asunder of the muscular fibre lengthwise into delicate similarly striped threads, which are termed *fibrils*. Other reagents, for example, solutions of hydrochloric acid, bring the transverse striation more distinctly into prominence, and they can, moreover, cause a falling asunder of the muscular fibres transversely into *discs*. Fibrils and discs can fall asunder into still smaller, roundish-edged, anisotropic portions, which are termed the primitive muscle particles or the *sarcous elements*.

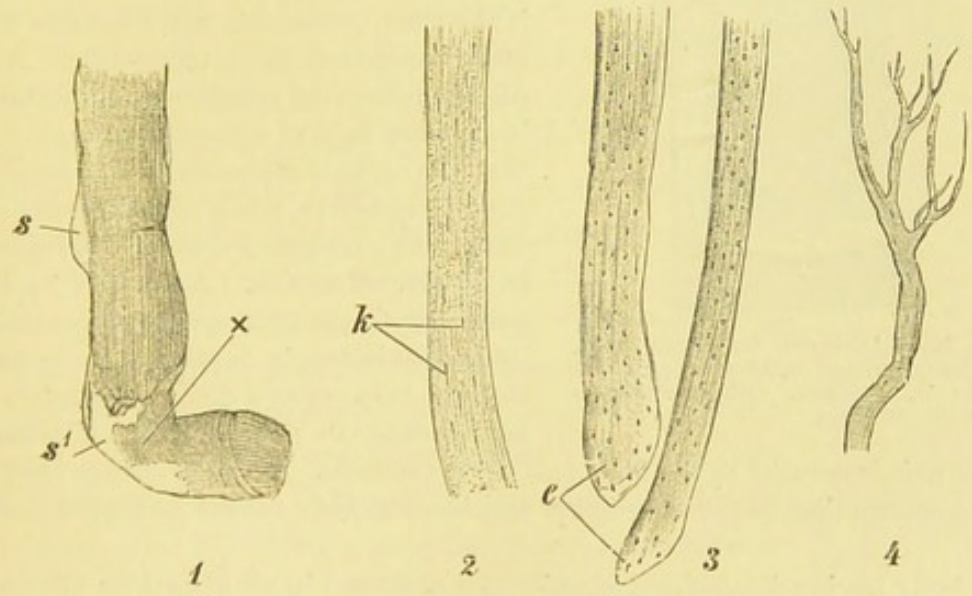


FIG. 158.—Portions of isolated transversely striped muscular fibre of a frog, × 50 d. 1. Effects of water; *s*, *s'*, sarcolemma. At × the muscular substance has been torn asunder, its transverse striation is not visible, but its longitudinal striation, on the contrary, is distinctly seen. 2. Action of acetic acid; *k*, nuclei. The delicate puncturing corresponds to the interstitial granules. 3. Action of a concentrated solution of caustic potash; ends rounded off, the numerous nuclei appear to have flowed into a vesicle or swelling *e*; the transverse striation of the substance of the muscles is in 2 and 3 invisible under this magnifying power (Method No. 22, Appendix). 4. Branched muscle fibre from the tongue of a frog (Method No. 23, Appendix)

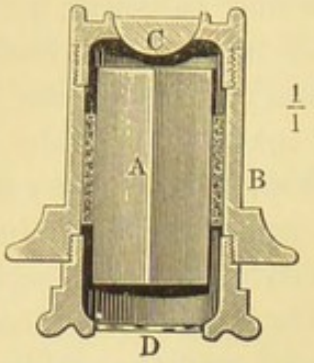


FIG. 159.—Polarizer for microscope. A, Nicol's prism; B, brass case; D, end next mirror; C, plano-convex lens —which is not necessary.

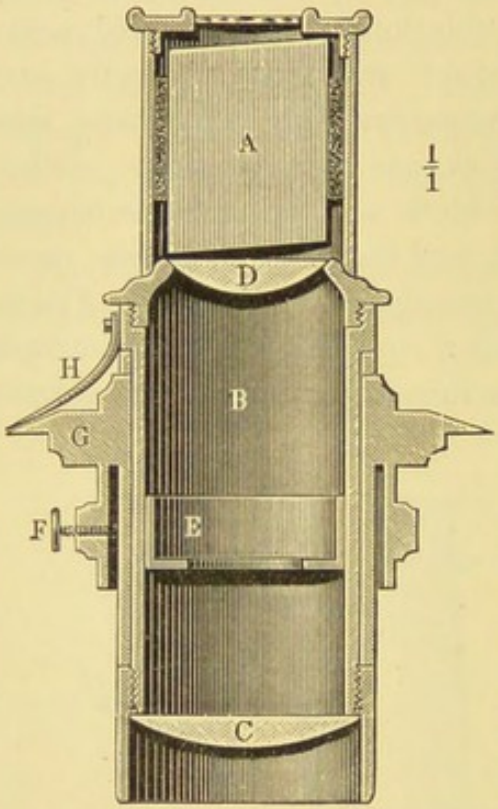


FIG. 160.—Analyzer for microscope. A, Nicol's prism; B, eyepiece; C, field glass; D, eyeglass. Other references of no importance.

Examination of muscular fibre by polarized light (see p. 66).—As already described, when examined by ordinary light, a muscular fibre is seen to consist (1) of a substance which forms the sarcous elements, highly refracting, and black when seen by transmitted light; and (2) of an intermediate or fundamental substance, which is much less refractive, and is clear by transmitted light. Suppose now that arrangements are made by which the fibre, whilst on the stage of the microscope, can be placed between crossed Nicol's prisms. This is accomplished by placing one Nicol's prism below the stage (the *polarizer*, Fig. 159), and another in the eyepiece of the microscope (the *analyzer*, Fig. 160). Either or both of these prisms are capable of rotation, and they may be so rotated with reference to each other that when we look through the microscope the field is quite dark. It will then be found that if a striated muscular fibre is in the dark field, the sarcous elements (represented by the dark transverse bands seen with ordinary light) shine very distinctly, while the clear band seen with ordinary light is now invisible. The observation is more striking if the slide containing the muscular fibre is placed on a thin plate of selenite. As only doubly refractive substances thus shine out in the dark field of crossed Nicol's, it follows that the sarcous elements are *anisotropic* or doubly refractive, while the substance which unites the contiguous discs is *isotropic*, that is, singly refractive. According to Engelmann, contractility is manifested exclusively by the anisotropic portion. Each sarcous element behaves as a doubly refractive body having only one positive axis,[1] of which the optical axis is parallel to the long axis of the muscle. As they change their form during contraction, becoming shorter and thicker, they cannot be regarded simply

[1] A body having its molecules uniformly arranged in all directions affects light only by *simple refraction*, that is, the incident ray gives rise to one refracted ray, and the crystal possesses one refractive index. When, on the other hand, the molecular structure of a body possesses parts of different density in certain directions, it manifests double refraction, that is, a ray of light traversing it is divided into two rays, one of which is bent more out of its course than the other. Common glass is simply refractive, and is said to be *isotropic;* but if it be compressed in one direction, it may become doubly refractive or *anisotropic*.

as double refractive bodies like crystals, but, as suggested by Brücke, they must consist of a great number of small doubly refractive particles, to which he has given the name of *disdiaclasts*, and which he supposes are irregularly distributed through the fundamental substance (Fig. 168). The latter, which may be termed *muscle-plasma*, is a viscous liquid capable of absorbing water freely, while the disdiaclasts are more dense and are less capable of taking up water. As has been remarked by Schäfer, Brücke himself pointed out that in living muscle at rest the whole of the muscular substance appears doubly refracting, and that it is only in contraction that the alternate stripes appear singly refracting.[1]

Some authors have claimed the fibrils, others the discs, and others again the sarcous elements, as the *primary form elements* of the fibres of the muscles. In the latter case, we would have to represent the building up in such a manner that the fibrils are formed from sarcous elements placed so as to form a row and the discs from the sarcous elements being placed side by side, in both cases assuming that the sarcous elements cohere.

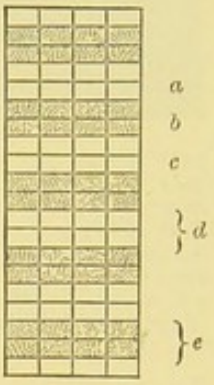


FIG. 161.—Showing hypothetical views regarding the structure of striated muscle. Four fibres side by side. *a*, clear bands or discs, each formed of two clear bands or discs, termed the *lateral discs of Engelmann*, separated by a thin dark line or band, known as *Dobie's line* or *Krause's membrane*; *b*, two discs of dark substance, constituting the *sarcous elements of Bowman*, having in the centre an ill-defined band or line, the *median disc of Hensen*; *c*, Dobie's line; *d*, isotropic or singly refractive portion; *e*, anisotropic or doubly refractive portion.

Various theoretical views have been advanced regarding the ultimate structure of striated muscular fibre, and the question must still be regarded as unsettled.[2] A view, regarded with favour for some time, was that the muscular fibre might be regarded as built up of small caskets (see Fig. 161), bounded at the two ends by a thin membrane or band in the centre of the clear band, called *Dobie's line* or *Krause's membrane*, *c*, and laterally by the sarcolemma. In this casket there were supposed to be (from above downwards in Fig. 161)—(1) a thin clear disc, the *lateral disc of Engelmann*; (2) a disc of dark substance, the *sarcous element of Bowman*; (3) in the centre of this disc, an ill-defined granular substance constituting the *median disc of Hensen*, *b*; and (4) another lateral disc of Engelmann. Further, the dark portions forming the *sarcous elements of Bowman* are the anisotropic or doubly refractive part, while the clear portion, formed of two discs of Engelmann separated by Dobie's line, is singly refractive or isotropic. These appearances have undoubtedly been figured and described, but they are to be regarded as optical expres-

[1] Brücke, *Physiologie*, p. 465.

[2] An excellent account of this subject is given by A. van Gehuchten, in *Étude sur la Structure Intime de la Cellule Musculaire Striée.—La Cellule*, tome ii. 2e Fascicule.

sions of layers of substances acting differently on light, and also sometimes superposed, so that the structure of one fibre shines through, and affects the appearance of, the one above it. There is also the further difficulty that if a living muscular fibre is examined under the microscope, especially with high powers, the amount or degree of relaxation or of contraction affects in a marked way the optical appearances, so that the fibre may present a different appearance while at rest from

FIG. 162.—Living muscular fibre from *Geotrupes stercorarius.*

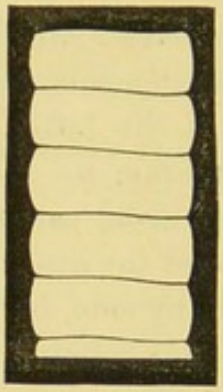


FIG. 163.—The same fibre seen by polarized light, with crossed Nicol's prisms.

what it shows in a condition of contraction. Thus, a fibre may show the appearance represented in Fig. 162, in which the clear bands are narrow, Dobie's line dark and well defined, and the dark band very wide: or a fibre may, in another condition, show more details of structure, as in Fig. 164. In Fig. 163 the same fibre as is represented in Fig. 162 is seen as it appears in the dark field of crossed Nicol's prisms, with polarized light. It will be observed that the dark anisotropic portion of Fig. 162 is clear and brilliant in Fig. 163, while Dobie's line, and the part on each side of it, is dark. I have recently convinced myself, after a study of muscular fibre with the use of chloride of gold, that the appearances shown in Figs. 162, 164, 165, and 167, can be seen, and they support the view that the muscle substance is composed of rod-like structures, as first described by Professor E. A. Schäfer.[1] Whether these rods, in turn, are only the optical expressions of a fibrillar net-work with elongated meshes, as advanced by Marshall, is in my opinion more doubtful. I have not been able to satisfy myself upon this point, and especially have I failed in detecting appearances favouring the view that there is a net-work with irregularly formed

FIG. 164.—Muscular fibre from *Hydrophilus piceus* (large water-beetle), showing all the details of structure represented in the diagram, Fig. 161.

[1] As to Schäfer's views, see *Philosophical Transactions*, 1873, and Quain's *Anatomy*, vol. ii. p. 123, *et seq.*

meshes in the clear disc. The conception of a muscular fibre as an elongated cell in which the fibrillar net-work assumes a regular arrangement is one of much significance, as it brings muscular fibres into the general category of cells.

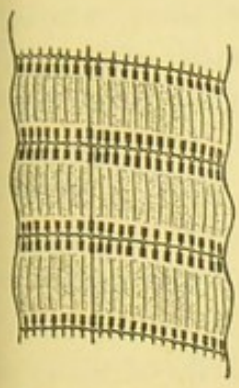


FIG. 165.—Muscular fibre of *Geotrupes stercorarius*, living, showing rod-like structures passing longitudinally in the dark substance, and rod or somewhat square-shaped points or dots in the clear substance.

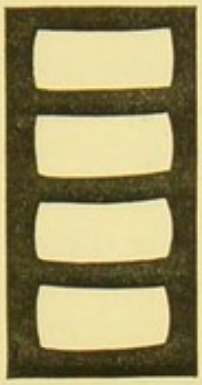


FIG. 166.—Same fibre as in Fig. 165, seen with polarized light. Dark bands of Fig. 165 are the clear bands of this Figure.

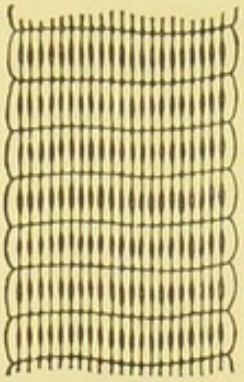


FIG. 167.—Muscular fibre from *Geophilus*, showing rod-like structures running longitudinally with dots or points at their ends.

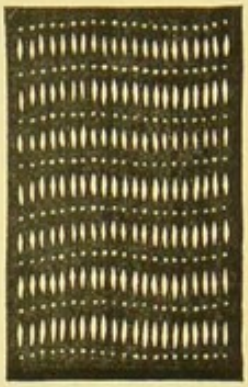


FIG. 168.—Same fibre as in Fig. 167, seen with polarized light. Observe that the rods and the dots of Fig. 167 are clear, and are therefore doubly refractive or anisotropic.

Striated muscle fibres are found in the muscles of the larynx, of the extremities, of the eye, and of the ear. They are also found in the tongue, in the pharynx, in the upper half of the œsophagus, in the larynx, and in the muscles of the genital organs and of the rectum. In the case of many animals, *e.g.* the rabbit, two different sorts of transversely striped muscles are found; red (for example, the semitendinosus and the soleus), and white (for example, the adductor magnus). The red muscles contract slowly, the white ones quickly, and they also show microscopic differences, inasmuch as the fibres of the red muscles possess a somewhat irregular transverse striping, a more distinct longitudinal striping, and a larger number of roundish nuclei than the pale variety. In some animals, the two kinds of muscular fibre appear to be separated from

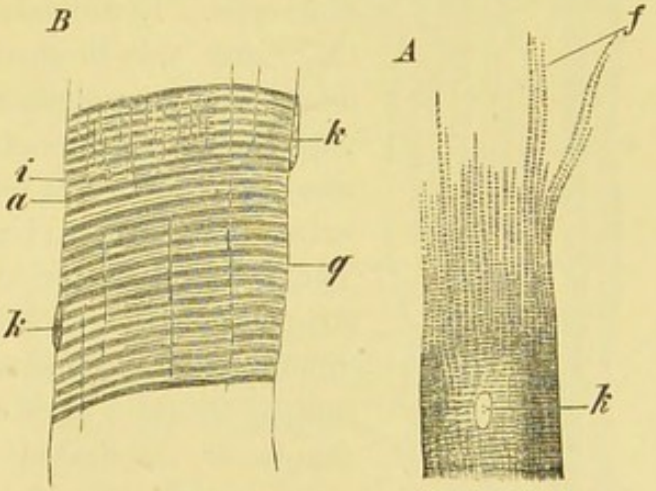


FIG. 169.—*B*. Portion of a human muscular fibre, × 560 d. (*a*) Anisotropic and (*i*) isotropic transverse bands; (*q*) transverse line; (*k*) nuclei. (Method No. 23 *a*, Appendix.) *A*. The end of the muscular fibre of a frog, × 240 d. Splitting into fibrils (*f*); (*k*) nucleus. (Method No. 24, Appendix).

one another in particular muscles; but in others, as in man, the two kinds of fibres are sometimes mixed in the same muscle. The contraction of the transverse striped muscles is quick and under the control of the will, and hence this kind of muscle is often termed *voluntary muscle*. When the fibre contracts, the contraction occurs in the broad, dark, transverse band, which, when contracted, increases in breadth, while the clear discs and the narrow, dark, transverse band play a purely mechanical part.

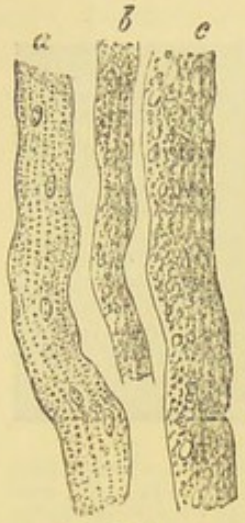


FIG. 170.—Fatty degeneration of human muscular fibres. *a*, low degree; *b*, a higher; and, *c*, the highest degree.

Muscular fibre is sometimes the seat of fatty changes, in which the striæ disappear, and the muscle-substance becomes crowded with small molecules of fat (Fig. 170).

The smallest blood-vessels, or capillaries of muscle, form a fine network of elongated meshes between the fibres, but not extending within the sarcolemma, as shown in Fig. 171.

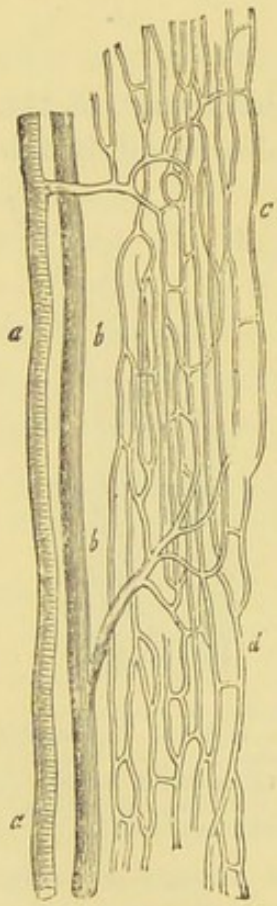


FIG. 171.—Capillary network of striated muscular fibre. *a*, arteriole; *b*, venule; *c* and *d*, capillary network

(3) *The muscular fibres* of the heart occupy a special position. They show transverse striation, but it is not so distinct as in ordinary striated muscular fibre. A consideration of their development as well as their behaviour under the microscope show that the muscular fibres of the heart are only modified contractile fibre cells. In animals low in the scale (*e.g.* the frog) they are spindle-shaped, provided with a large nucleus, the protoplasm is distinctly striped transversely as well as longitudinally (Fig. 172, *A*); in the mammalia the fibres take the form of short cylinders, whose ends are often irregularly dentate (Fig. 172, *B*). The protoplasm is differentiated into transverse striped fibrils, between which the remains of non-differentiated protoplasm may still be seen. Longitudinal striation is often very distinct. Around the simple or duplicated nucleus, there lie masses of homogeneous protoplasm, and granules exist in very large quantities. The sarcolemma is absent. The connection of the irregularly cubical-shaped fibres by means of short, crooked, or transverse processes is characteristic of the muscular fibres of the hearts of the higher animals (Fig. 172, *B*).

Muscular fibres are *developed*, as already indicated, from the elongation of cells and the multiplication of

nuclei. These cells are embryonic cells in the mesoderm. Striation appears first at one side and it gradually passes around the fibre and onwards to its centre. The nuclei are at first near the centre of the fibre, but they are pushed by degrees to the circumference until they rest below the sarcolemma, which is formed at a comparatively late stage in the development of the fibre.[1]

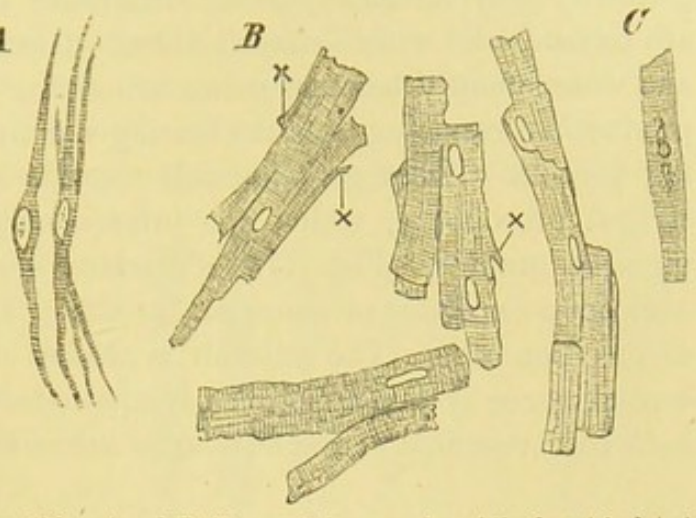


FIG. 172.—*A* and *B*, fibres of the muscles of the heart isolated in potash. *A*, of a frog; *B*, of a rabbit. × Crooked processes (Method No. 25, Appendix). *C*, from a longitudinal section through a papillary muscle of man. Fat granule on the poles of the nucleus. (Method No. 26, Appendix.) All the fibres ×240 d.

7. The Nerve Cells (Ganglion cells) are irregularly-shaped cells of very variable size (10 to 100 μ). There are spherical and spindle-shaped ganglion cells. Star-shaped cells are also very frequent, that is, the protoplasm sends out prolongations of irregular size and length. The majority of these subdivide continuously, and end in a complicated network of delicate fibrils. Such processes of nerve cells are called *poles* (Fig. 174, *p*). The pole, however, may run for a long distance, and is associated with a medullary sheath, by means of which it comes to be a *medullated nerve fibre*. The pole consequently corresponds to a part of the nerve fibre called the *axis cylinder*, and it is on that account termed an *axis cylinder pole* (Fig. 174, *ax*). Ganglion cells with two poles are termed *bipolar*, cells with several poles are termed *multipolar*; one may also speak of *unipolar* and *apolar* ganglion cells, but it can be shown that in spinal ganglia the single pole of unipolar ganglion cells consists in reality of

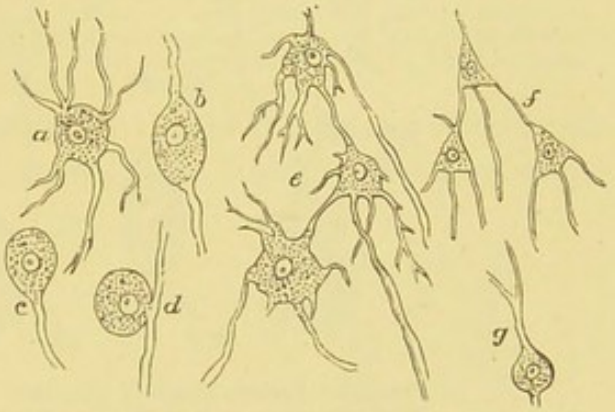


FIG. 173.—Various forms of nerve-cells. *a*, multipolar, from grey matter of spinal cord; *b*, *d*, bipolar, from ganglia on posterior roots of spinal nerves; *c*, *g*, unipolar (?), from cerebellum; *g*, shows indications of a process coming off at lower end; *e*, union of three multipolar cells in spinal cord; *f*, union of three tripolar, or pyramidal, cells in grey matter of central hemispheres.

[1] Other details of the structure of muscle, and more especially the mode of the termination of the nerves in muscle, will be described in treating of the functions of muscular fibre.

two poles, which run from the cell in the same direction, and then generally they turn away from each other at right angles. Such cells are termed cells with T-shaped fibres. Ganglion cells, which are apolar, and consequently have no poles, are either embryonic, or are artificial productions resulting from the tearing asunder of the poles by the isolating process. Every ganglion cell consists of a granular or delicately striped protoplasm, which not infrequently contains yellowish-brown pigment granules (Fig. 174, *A*) and a vesicle-shaped nucleus, which encloses a nucleolus of considerable size. This nucleus is characteristic of ganglion cells. The cell-wall is absent. Ganglion cells occur in the central nerve system and in the sympathetic ganglia; they occur also in both cerebro-spinal and sympathetic nerve fibres.

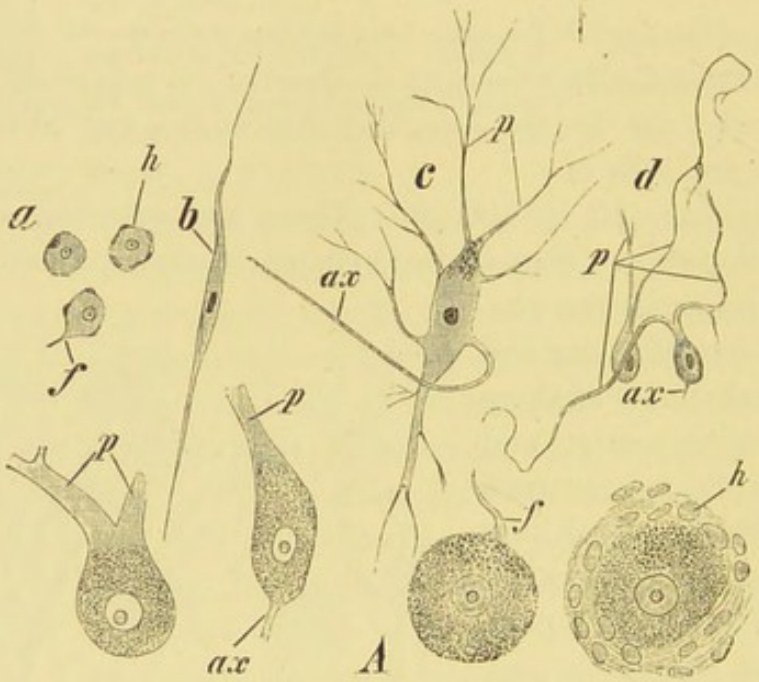


FIG. 174.—Various forms of ganglion cells. *a*, *A*, spherical unipolar ganglion cells from the Gasserian ganglion of the fifth cranial nerve of man. (Method No. 27, Appendix.) *a* × 80 d., *A* × 240 d. Only two cells show poles, *f*; the two other cells are surrounded by a covering containing a nucleus, *h*. *b*, spindle-shaped; *c*, multipolar ganglion cells from the spinal cord of an ox, × 80 d. (Method No. 28, Appendix.) *d*, multipolar ganglion cells (those of Purkinje) from the cortex of the cerebellum of man. (Method No. 29, Appendix.) *d* × 80 d. *p*, poles of protoplasm; *ax*, axis cylinder pole. The nuclei of *b*, *c*, and *d*, have, by the method employed in preparing them, lost their characteristic form.

A connection of the ganglion cells occurs in such a manner that a network of fibrils is formed by the protoplasmic poles of several ganglion cells.

Closely related to nerve cells are the nerve fibres. These may be here described, although their cellular nature is still the subject of controversy.

8. **Nerve Fibres** occur in two forms, designated as (*a*) medul-

lated, and (*b*) non-medullated nerve fibres. The two forms are not to be regarded as sharply separated locally or generically. It is quite a usual phenomenon for one and the same nerve fibre to be, in the beginning of its course, medullated, and towards the end of its course non-medullated.

(*a*) *Medullated Nerve Fibres* are apparently homogeneous, faintly shining fibres, from 1 to 20 μ in thickness. The most important part is a fine elastic cylindrical fibre traversing the axis of the fibres. This is called the *axis cylinder of Purkinje, or the band of Remak* (Fig. 175). Delicate longitudinal stripes, which are sometimes observable on it, are the expression of the fact that the axis cylinder may be composed of fine fibrils; a very noticeable transverse striping (Fig. 176), which becomes visible after treatment with a solution of nitrate of silver, has not yet been clearly explained. The axis cylinder is surrounded by the sheath of the marrow, or the *white substance of Schwann*. This consists of a fluid, strongly-refractive, fat-like substance called

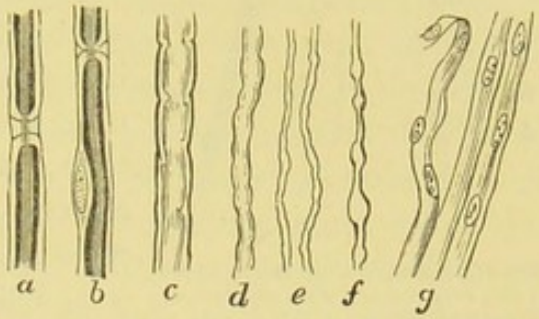


FIG. 175.—Nerves. *c*, ordinary-sized nerve fibre, showing axis cylinder surrounded by white substance; *d*, smaller nerve fibre, with white substance scarcely visible; *f*, varicose nerve fibre, from grey matter near surface of cerebrum; *a*, nerve fibre, stained by perosmic acid, showing one of the nodes of Ranvier, or complete interruption of the white substance; *b*, nerve fibre, showing nucleus and node of Ranvier (the axis cylinder is blackened by the action of perosmic acid); *g*, non-medullated nerve fibres from sympathetic, having no white substance, and nucleated at intervals; *e*, smallest fibre.

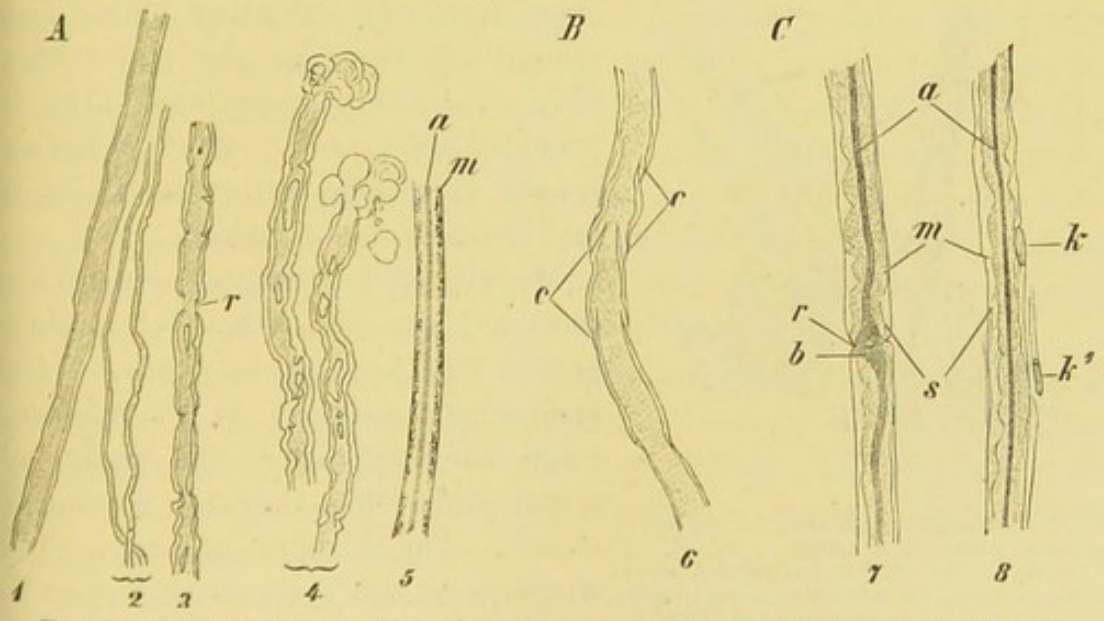


FIG. 176.—Medullated nerve fibres, from the sciatic of the frog, × 240 d. *1*, *2*, *3*, Fresh with solution of common salt. (Method No. 30, Appendix.) *3*, fibre with constrictions, *r*; *4*, nerve fibres under the influence of water. (Method No. 31, Appendix.) *5*, nerve fibre treated with absolute alcohol. (Method No. 32, Appendix.) *m*, coagulated marrow; *a*, axis cylinder. *B* and *C*, medullated nerve fibres of the rabbit, × 560 d. *6*, Fresh; *c*, cylindro-conical segments. *7*, *8*, Hardened. (Method No. 33, Appendix.) *a*, Axis cylinder; *b*, biconical swelling; *r*, constriction; *m*, white substance coagulated and lifted off from *s*, Schwann's sheath; *k'*, nucleus of the endoneurium.

myelin. Under favourable circumstances, we perceive that the white substance is not continuous, but is divided at somewhat irregular distances into cylindro-conical segments by means of oblique incisions or grooves (*Lantermann's Notches*, Fig. 176, *B*).

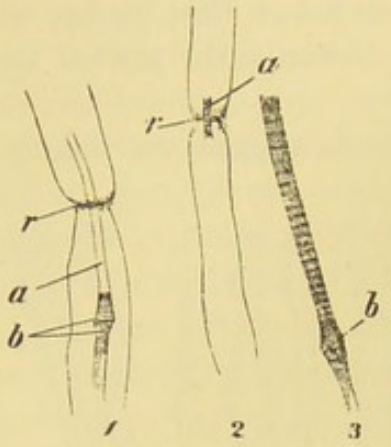


FIG. 177.—Medullated nerve fibres of the frog treated with solution of nitrate of silver, × 560 d. 1. *r*, nodes; *a*, axis cylinder blackened only to a slight extent; *b*, biconical turgescence caused by the axis cylinder being squeezed in the isolation process. 2. *a*, axis cylinder *in situ* blackened only to a slight extent. 3. axis cylinder with transverse striping. The axis cylinder is not visible in the case of 3. (Method No. 34, Appendix.)

The central band, which, during life, is quite homogeneous, experiences, after death and when different reagents have been added to it, a structural metamorphosis. At the beginning, the nerve fibre possesses a double contour; at a later period, the central band or marrow may be broken up into small globular masses. The sheath of the white substance is a delicate structureless cuticle, known as *Schwann's sheath* or *neurilemma*. On the inner surface of the sheath, prolate spheroidal nuclei may be seen surrounded by a small quantity of protoplasm. At regular distances, constrictions are observed where the white substance is wanting, so that the axis cylinder and Schwann's sheath come into contact. These constrictions are known as *nodes*, or the *nodes of Ranvier*. The axis cylinder shows a biconical swelling in the neighbourhood of the nodes. Treatment with solutions of nitrate of silver shows also cement substance on the nodes (Fig. 177, *r*). Every medullated fibre is provided with nodes, which, being arranged at regular intervals, divide it into interannular segments.

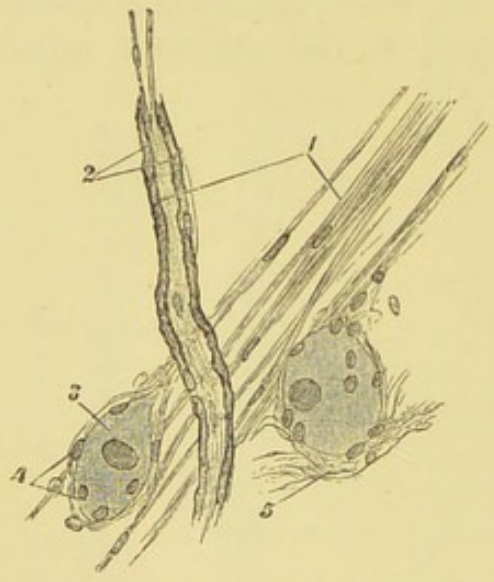


FIG. 178.—Teazed preparation of the sympathetic nerve of a rabbit, × 240 d. 1, Non-medullated; 2, thin medullated nerve fibres; 3, ganglion cells; the characteristic appearance of the large nuclei has been lost by the action of the osmic acid used in making the preparation; 4, nuclei of connective tissue fibres; 5, delicate connective tissue fibres. The protoplasm surrounding the nuclei of the pale nerve fibres is not visible with this magnifying power. (Method No. 35, Appendix.)

Medullated nerve fibres occur in the roots and branches of the cerebro-spinal nerves, but they are also present in the sympathetic nerves. At a subsequent stage, they appear in the brain and spinal cord, where they lose Schwann's sheath. The thickness of a nerve fibre warrants no conclusion with respect to its motor or sensory qualities; on the other hand, it is established that the fibres increase in thickness in proportion to the length of their course.

Division of the medullated nerves first takes place at their peripheral end. The medullated nerve fibres have a limited duration of life. They degenerate; the white substance and the axis cylinder gradually become granular, and the granular mass is rich in nuclei. Out of this granular mass, white substance and axis cylinders are again developed.

(*b*) The *non-medullated, pale, or grey nerve fibres*, sometimes also termed *Remak's nerve fibres*, are translucent, delicate longitudinally striped fibres of variable thickness. They consist of delicate fibrils and of nuclei, which, surrounded by a small quantity of protoplasm, lie at certain intervals on the upper surface of the fibres. The non-medullated fibres are found in the central nerve system, in the terminal extensions of the cerebro-spinal nerves, and especially in the sympathetic nerve system. They do not run near each other without joining neighbouring fibres, as the medullated nerve fibres do, but they divide and again unite so as to form elongated meshes by anastomosis.

Chap. IX.—THE PHYSIOLOGICAL PROPERTIES OF EPITHELIUM.

Epithelium consists, as seen in Chap. VIII., of one or more layers of actively-growing cells on one surface of a homogeneous membrane: numerous blood-vessels ramify on the other surface. The term *endothelium* has been applied to the lining membrane of the shut cavities of the body, such as the pleura, peritoneum, etc. Epithelial cells may be either spherical, irregularly cylindrical, polyhedral, or flattened in form. They may exist in one or more layers, and they appear to be cemented together by some kind of intermediate substance. When the cells are flattened, irregular in form, and exist in a single layer, as in the pleura and peritoneum, the intermediate substance is demonstrable by the action of certain reagents, especially a solution of nitrate of silver, which, when applied in certain conditions, blackens the intercellular matter. The following varieties of epithelium are met with in the body—

1. *Simple, flat, squamous, or tesselated epithelium*, in which there is scarcely more than a single layer of developed cells, such as is observed on the internal surfaces of the serous and synovial membranes, and on those of the heart, blood-vessels, and absorbent vessels. This includes the variety termed *endothelium*.

2. *Laminar or stratified epithelium*, consisting of a number of flattened cells, usually in various stages of alteration, such as exist in the epidermis and on the mucous membranes near the entrance of the alimentary and genito-urinary passages. Nails, hair, and horns are modifications of this texture.

3. *Columnar or prismatic epithelium*, consisting of a single layer of elongated nucleated cells; below these, however, are in general seen others of a more spherical shape, which are, in fact, the columnar particles in an earlier stage of growth. Such is the structure lining the whole alimentary canal, from the stomach downwards, and also part of the ducts of secreting glands.

4. *Spheroidal epithelium* may be regarded almost as belonging to the last mentioned form; it constitutes, in some places, a transition form between the columnar and tesselated varieties; it is best seen in the urethra, ureters, and pelvis of the kidney, and also in the ducts of the mamma, and of the cutaneous and some other glands.

5. *Ciliated epithelium* is generally of the columnar form, and consists of nucleated cells, upon the free surface or ends of which minute microscopic hair-like projections or *cilia* are placed, which, when covered with fluid, move with great rapidity. This kind of epithelium lines all the air passages, and the cavities and passages communicating with them, such as the nasal ducts, the conjunctiva of the eyelids, the sinuses opening into the nasal passages, the Eustachian tube and tympanum, part of the soft palate and pharynx; also the uterus and Fallopian tubes, and the ventricles of the brain.

Each of these forms of epithelium has a special function. Tesselated epithelium covers an extensive surface for protective or absorptive purposes; columnar epithelium has numerous cellular elements on a surface of limited extent, so as to permit great nutritive vitality; stratified epithelium, from the abundance of cells, admits of the rapid growth and shedding of these for functional purposes; and ciliated epithelium maintains vibratile movement.

Epithelium forms a continuous layer over the surface of the body, and lines all the internal passages and cavities. This fact is of physiological importance, inasmuch as it indicates that all substances entering or leaving the body must, in some way or other, pass through an epithelial layer.

1. *Physical Properties of Epithelium.*—Where epithelium is exposed to pressure and other external influences, it becomes more or less hard and firm, as may be seen in the nails, in the epidermis covering the palms of the hands and soles of the feet, in "corns," and in the callosities which appear on the hands of those performing much manual labour. The columnar epithelium of the intestinal canal is, on the contrary, soft and easily detached. The cohesion of epithelium is usually feeble, but such structures as the epidermis may sustain considerable distension without rupture, as may be seen over large abdominal tumours. Elasticity is very imperfect. An epithelial layer is a bad conductor of heat

and electricity. The hairy covering of many species of animals, and the feathery covering of birds, by diminishing the amount of heat lost by radiation from the surface, assist in maintaining the temperature of the body. Epidermis absorbs water with great rapidity, except where it is covered by a thin layer of oily matter.

2. *Vital Properties of Epithelium.*—The nutrition of an epithelial layer depends upon the transudation of nutrient fluid from the blood-vessels underneath the layer of cells. It is usually very active, except perhaps in stratified epithelium. Most epithelial cells are constantly engaged in the formation of particular proximate principles. This is especially true of spheroidal epithelium, in the cells of which various substances, such as ptyalin, pepsin, etc., are formed. In some epithelial cells, chemical transformations apparently take place. Thus, the superficial layers of the epidermis undergo changes which so alter the cells as to render them insusceptible to the action of acetic acid, while, in the deeper cells of the same layer, pigment may be formed. The exact mode of development of epithelial cells is still unknown, but there appears to be an incessant development of new cells from the surface of the membrane on which the deeper layer is situated. The new cells gradually pass outwards, and on reaching the superficial layer, last for a certain time and then drop off. This process of desquamation, as already stated, is preceded by chemical transformation. There is thus a constant elimination from the body of the materials which form epithelial cells.

Epithelial tissue is not sensitive, but in the structure of the terminal organs of sense, epithelial cells may be modified for special purposes, as will be illustrated in treating of these organs.

3. *Influence of Epithelium in Absorption and Elimination.*—The function of epithelium in the process of absorption, or the introduction of matter from the external medium into the fluids or tissues of the body, may be briefly stated as follows—

(1) *Absorption of Gases and Volatile Substances.*—The pulmonary surface in the ultimate air cells of the lung, which is covered by a thin layer of flattened epithelial cells, absorbs oxygen and volatile matters. The surface of the skin, and perhaps also the intestinal mucous surface, absorb small quantities of gases.

(2) *Absorption of Fluids and Soluble Substances.*—All epithelial surfaces may absorb water, or solutions of salts or other substances, but there are great differences in this respect. Thus, the pulmonary mucous membrane absorbs water freely; the epithelial lining of the bladder scarcely absorbs it at all; while the intestinal mucous membrane permits readily the passage of water and of soluble matters. In certain

circumstances, also, the epidermis covering the skin may permit the absorption of water.

(3) *Absorption of Fat.*—The cylindrical or columnar epithelium of the lining of the small intestine absorbs fat by a process the mechanism of which will be described in treating of intestinal absorption.

(4) *Influence of Epithelium in Elimination.*—The elimination of matters from the body may take place either by exhalation of watery vapour, of various gases, or of volatile substances, from epithelial surfaces, or by a true process of secretion, in which the spheroidal epithelial cells lining the ultimate pouches of glands constitute the active agents. The process of secretion is a mode of activity of glandular epithelium.

4. *Ciliated Epithelium.*—Epithelial cells, on which cilia are placed, are generally of the columnar, sometimes of the spheroidal, rarely of the flat kind. These bodies constitute minute hair-like processes, attached to, or forming a prolongation of, the wall of the cell at its free side, and when covered with fluid, are in constant vibratory motion.

Ciliated epithelial cells do not differ materially from other cells of the same form, excepting in the possession of the cilia; they usually contain distinct nuclei; and they appear to be abraded from the surfaces on which they are situated, and to be replaced by successive layers of new cells, formed from the subjacent texture, and subsequently acquiring the ciliated structure when they reach the surface.

The number of cilia attached to each cell is subject to considerable variety. In some instances, only one or two; in others, a crown or circle of numerous cilia occupies the free surface of the cells.

The individual cilia are, in the human subject, generally from 8 μ to 4 μ in length; in invertebrata, they are often considerably longer. They are also proportionately thicker and blunter in vertebrate than in invertebrate animals.

Ciliated epithelium occurs in the human body in the following situations, some of which have been already mentioned, p. 303—1st, on the mucous membrane of the air-passages, and the various sinuses and tubes communicating immediately with them, as in the nares (excepting close to their external openings); part of the soft palate, upper and lateral part of the pharynx, Eustachian tube, frontal, maxillary, sphenoidal, and ethmoidal sinuses; on the conjunctiva palpebrarum, lachrymal sacs and nasal ducts; on the mucous lining of the larynx, trachea, and bronchia, as far as their fine subdivisions, but not extending into the air-cells of the lungs: 2nd, in the adult female, the epithelium is ciliated in the lining membrane of the uterus, Fallopian tubes, and their fimbriated margins: and 3rd, in the membrane lining

the ventricles of the brain, the cells have been observed to be of a ciliated kind.

Ciliary Motion.

The movement of each cilium is most commonly of a folding or lashing kind. That of the whole set of cilia, covering a membranous surface, shows the passage of successive waves, somewhat in the same manner as different parts of the crop in a field of wheat are repeatedly bent in succession by the wind passing over it. When the ciliary motion is active, the movement is so rapid that the optical effect is the appearance of a stream of fluid flowing along the surface. This is the case especially when we look at a marginal line of cilia, as in the gills of a common mussel.

Four varieties of ciliary motion may be noticed—(1) the *hook-like*, in which each cilium makes the movement of a finger which is alternately bent and extended; (2) the *funnel-shaped*, in which the upper portion of the hair describes a circle in swinging, and the whole a cone, whose apex is formed by the firmly-attached base of the cilium; (3) the *oscillating*, in which the whole hair sways more like a pendulum, from side to side; and (4) the *undulating*, when the hair executes a movement like the lash of a whip moderately wielded, or that of the tail of a spermatozoon. Of all these forms of ciliary motion, the first appears to be by far the most frequent.

Ciliary movement is independent, so far as mere movement is concerned, of the circulatory and nervous systems; although, no doubt, these must affect its nutrition. Elevation of temperature up to about 40° C. increases the movement, when it ceases, probably from coagulation of the protoplasm in the cilia. Cold retards the movement. All substances having a chemical action on protoplasm, if applied in solutions of sufficient strength, stop ciliary action. Thus, water accelerates the movement at first, but soon stops it, probably by acting on the protoplasm. As a rule, fresh water stops ciliary motion in parts removed from inhabitants of salt water. Bile, acids, alkalies, and alcohol, if of sufficient strength, stop ciliary action; but, if in weak solutions, they may accelerate the movement. Ciliary movement requires the presence of oxygen, while the presence of carbonic acid seems to have a retarding influence. Cilia may be arrested by the action of chloroform or ether, and it is interesting to observe that they may recover from the influence of these substances. Electricity, whether applied as a continuous current, or from an induction coil, produces no appreciable effect.

Ciliary movement continues for a short time in small detached por-

tions of the texture, and for a longer period in the entire part, after the destruction of the brain and spinal marrow, or after the first invasion of apparent death. The time of this persistence varies from a few hours to several days in man and warm-blooded animals, and is even considerably longer in cold-blooded reptiles; it is much influenced by temperature, the nature of the fluids in contact with the texture, and other circumstances.

Various theories have been advanced to explain the action of cilia. The most probable is that it is due to the property of contractility inherent in protoplasm. As Engelmann suggests, we may suppose the contractile protoplasm to be chiefly on the concave side of a curved cilium, so that, on contraction, the cilium will be brought downwards, and on the contractile motion ceasing, the cilium may be erected by an elastic recoil of the substance forming its convex border. That ciliary movement is dependent on nutritional changes occurring in the cell with which the cilium is connected is proved by the fact that if the cilium has its connection with the cell severed, all movement ceases, and if a cilium is cut into two fragments, only the fragment moves that is still connected with the cell.

Chap. X.—THE INTERCELLULAR SUBSTANCE.

In the early embryonic period, the animal body consists entirely of cells, while later on, a smaller or larger quantity of unformed or formed intermediary substance is found between the cells. This intercellular substance is formed by the cells, and it may be regarded either as a secretion of the cells or as arising from a transformation of the peripheral layers of the protoplasm of the cell. In certain cases there may be a total metamorphosis of the cells. It is very difficult to come to an opinion as to the mode of formation of individual intercellular substances, and there are many points regarding the formation of intercellular matter that are still the subject of controversy.

When the intercellular matter exists in small quantities, it is called *cement substance*, as we find it existing between epithelial cells, connective tissue cells, smooth muscular fibres, etc., but when the intercellular substance appears in larger quantities, surpassing the mass of the cells, it is then termed *ground substance* or matrix. The ground substance is either amorphous, as in the jelly-like ground substance of the tissue of the umbilical cord, or it possesses a definite shape, when it may be termed *formed ground substance.*

There are the following varieties of tissue in which the ground substance or matrix assumes a definite form—

(1) *The Ground Substance of Connective Tissue.*—The elements of this substance are the connective tissue fibrils, threads of the utmost degree of fineness, which are bound so as to form bundles of various degrees of thickness (termed the connective tissue bundles), by means of a small amount of unformed cement substance. These bundles are soft, flexible, possessed of little elasticity, and they are specially characterized by their pale outlines, longitudinal striation, and their wavy course. The bundles on treatment with picric acid split into fibrils; on the addition of diluted acids, as, for example, acetic acid, they become completely transparent. Alkaline fluids destroy them, and when boiled, they yield gelatin.

FIG. 179.—Variously thick bundles of connective tissue from the intermuscular connective tissue of man, X 240 d. (Method No. 36, Appendix.)

When connective tissue comes into contact with an epithelial layer, it not infrequently assumes the form of a structureless membrane, termed a *basement membrane*, or *membrana propria*. In some cases, as in the salivary glands, the *membrana propria* consists, not of the structureless membrane, but of a membrane composed of a layer of flattened nucleated cells.

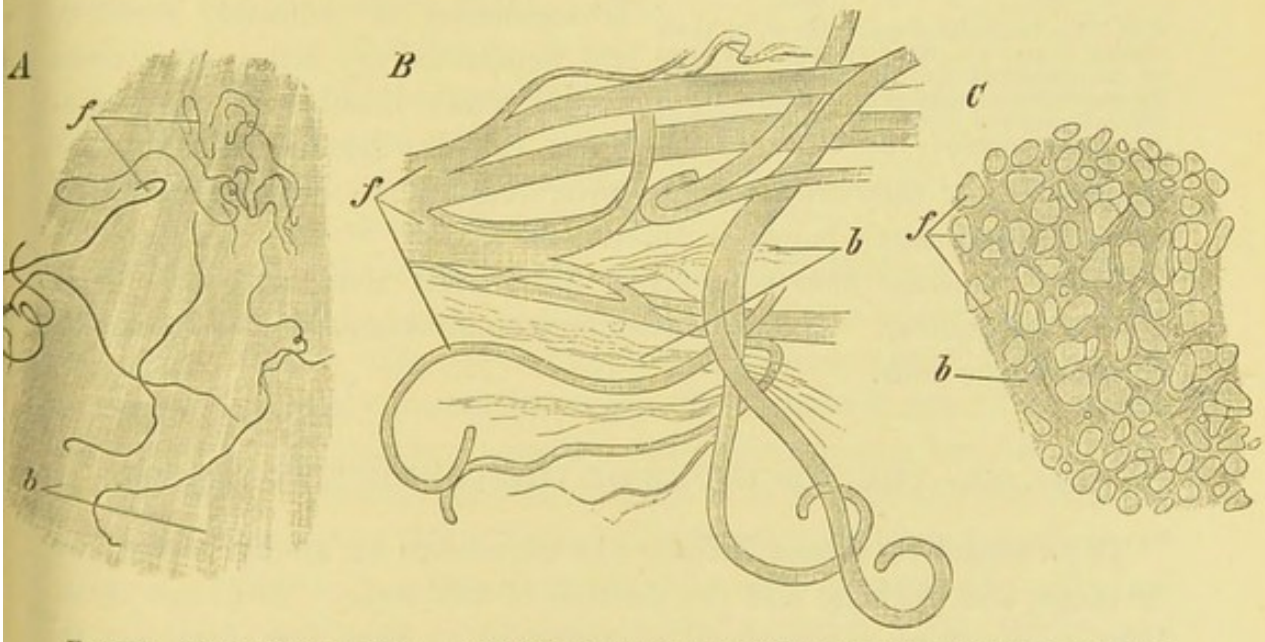


FIG. 180.—Elastic fibres, X 560 d. *A*, delicate elastic fibres; (*f*) from the intermuscular connective tissue of man; (*b*) bundles of connective tissue, swollen and gelatinous after addition of acetic acid. (Method No. 37, Appendix.) *B*, very thick elastic fibres; (*f*) from the ligamentum nuchæ of the ox; (*b*) bundles of connective tissue. (Method No. 38, Appendix.) *C*, from a transverse section of the ligamentum nuchæ of the ox. (*f*) elastic fibres; (*b*) bundles of connective tissue. (Method No. 39, Appendix.)

The *Matrix of Hyaline Cartilage* may also be regarded as formed of the same material as constitutes the matrix of fibrillated connective

tissue. The matrix of hyaline cartilage, by the usual methods of investigation, appears structureless and homogeneous, but by certain methods, such as artificial digestion, it may be caused to split up into bundles of fibres. The behaviour of the matrix of cartilage to polarized light also indicates a fibrillar structure. The matrix of cartilage is firm and highly elastic; on boiling it yields chondrin.

(2) *The Matrix of Bone* consists of fibrils impregnated with salts of lime, especially the basicphosphate of lime, giving the bone a great degree of firmness and hardness. The matrix of dentine of tooth resembles that of bone, but is still harder.

(3) *The Elastic Substance:* its structural form is the elastic fibre (Fig. 180) which is characterized by its sharp dark outlines, its strong power of refraction, and its marked resistance to acids and alkalies. The elastic fibres vary in thickness (up to 11 μ) and appear sometimes in the form of finer or coarser reticulations, which are sometimes narrow-meshed, and at other times, wide-meshed. Narrow-meshed reticulations woven out of thick elastic fibres constitute transitional forms passing into *elastic membranes* (Fig. 181), which, whether homogeneous or delicately striated, are penetrated by holes of various sizes. Such membranes are sometimes called *windowed* or *fenestrated membranes*, and they are no doubt produced by the blending or union of very broad elastic bands or fibres.

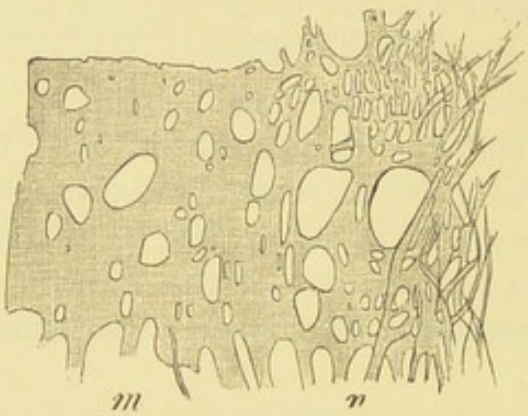


FIG. 181.—Reticulated network, *n*, of thick elastic fibres, becoming on the left hand *m*, a fenestrated membrane, or membrane having numerous perforations or holes. From the endocardium of man, × 560 d. (Method No. 40, Appendix.)

Lastly, *cuticular formations* are to be classed along with the intercellular substances. These are genuine secretions occurring on the upper surface of the cells.

CHAP. XI.—THE STRUCTURE OF THE CONNECTIVE TISSUES.

The connective tissues include (1) connective or areolar tissue, (2) cartilage, and (3) bone and the dentine of the teeth. Although these tissues appear on a superficial examination to have few characters in common, a study of their development and of their histological structure, as revealed by various reagents, indicates that they belong to the same group. They have a common origin and their structure is of the same type. Thus they all originate in the mesoblast. They consist essentially of cells of the same kind lying in a matrix modified in character accord

ing to the tissue, and they all have the common function of forming the skeleton or framework of the body, and the material by which all the parts of the body are bound or connected together and by which support is given to delicate structures in many organs. Further, their relationship is shown by the fact that in the construction of the bodies of some of the lower vertebrate forms, the connective tissues may replace each other, so that a portion of the same organ may be cartilaginous in one group, bony in a second, and formed of fibrous tissue in a third.

1. The Connective Tissues Proper.

These embrace (*a*) jelly-like or mucous connective tissue, (*b*) fibrillar tissue, and (*c*) reticulated connective tissue.

(*a*) *Gelatinous, Jelly-like, or Mucous Connective Tissue* consists of a large quantity of structureless intermediate mucoid substance, enclosing delicate bundles of connective tissue and also containing a few round or stellate cells. In the higher animals, it occurs only in the umbilical cord of very young embryos, but it is widely diffused in the lower animals[1] (Fig. 182).

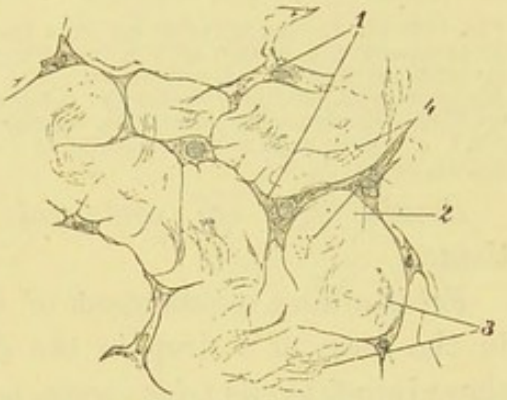


FIG. 182.—From a transverse section of the umbilical cord of a human embryo of the fourth month, × 240 d. 1. cells; 2. intermediary substance; 3. bundle of connective tissue almost obliquely cut; 4. bundle cut through transversely. (Method No. 41, Appendix.)

(*b*) *Fibrillar Connective Tissue* consists of a matrix formed of fibrils. It also contains cells and elastic fibres. The matrix of fibres has been already shown (p. 323). There exist between the bundles of connective tissue, fissure-like spaces, called the *connective tissue fissures*, or *lacunæ*, filled with a fluid or plasma. These lacunæ are connected with the lymphatic system, and they may be regarded as the beginning of this system in the loose connective tissue. The cells (Fig. 183, *A*) are irregularly polygonal, or stellated, strongly flattened, and are often bent or fractured in various ways. They fit into the narrow spaces between the bundles of connective tissue. Stellate connective tissue cells not infrequently embrace a bundle of connective tissue fibres. If we treat such a bundle with acetic acid, it swells up in places between the constrictions of the cells, and the bundle appears to have a fibre twisted round it. The processes of the cells were at one time taken for fibres, and they were called spiral fibres (Henle). (Fig. 183, *B*.) Other connective tissue cells are round,

[1] The vitreous humour of the eye is often erroneously classed under the head of mucous tissue. The reticulations in the vitreous humour are not filled with jelly-like matter but with fluid.

rich in protoplasm, coarse-grained, and of large size. (Waldeyer.) These are termed plasm cells, and they are found in the vicinity of small blood-vessels (Fig. 183, *C*). To the like category belong the fat cells, which are conspicuous for their facility in taking up colour when treated with the aniline dyes. All these cells are termed *fixed* or *stationary connective tissue cells*. They must be distinguished from the *wandering* or *migratory cells*, leucocytes, resembling the forms which likewise occur, although in smaller quantity, in fibrillar connective tissue.

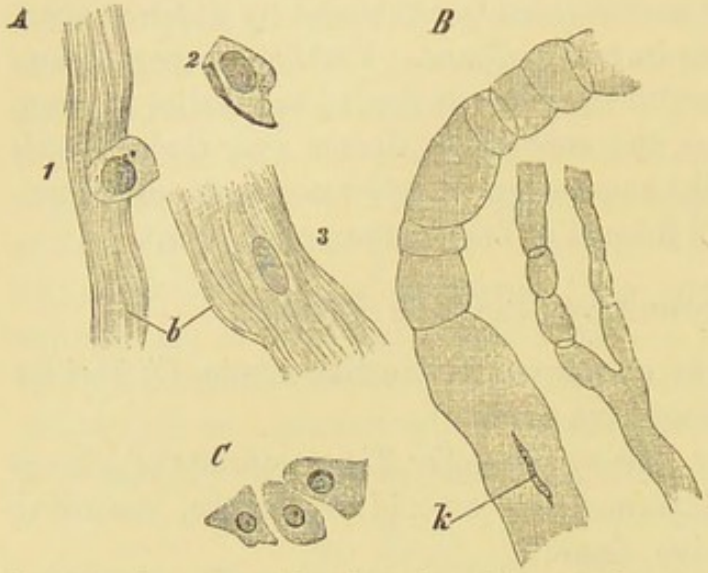


FIG. 183.—*A*. 1, connective tissue from intermuscular connective tissue; 2, broken cell; 3, cell, whose protoplasm is invisible; (*b*) bundle of connective tissue. (Method No. 42, Appendix.) *B*. bundle of connective tissue surrounded by a spiral of elongated cells. *k*, nucleus. (Method No. 43, Appendix.) *c*, plasma cells from the eyelid of a child. (Method No. 44, Appendix.)

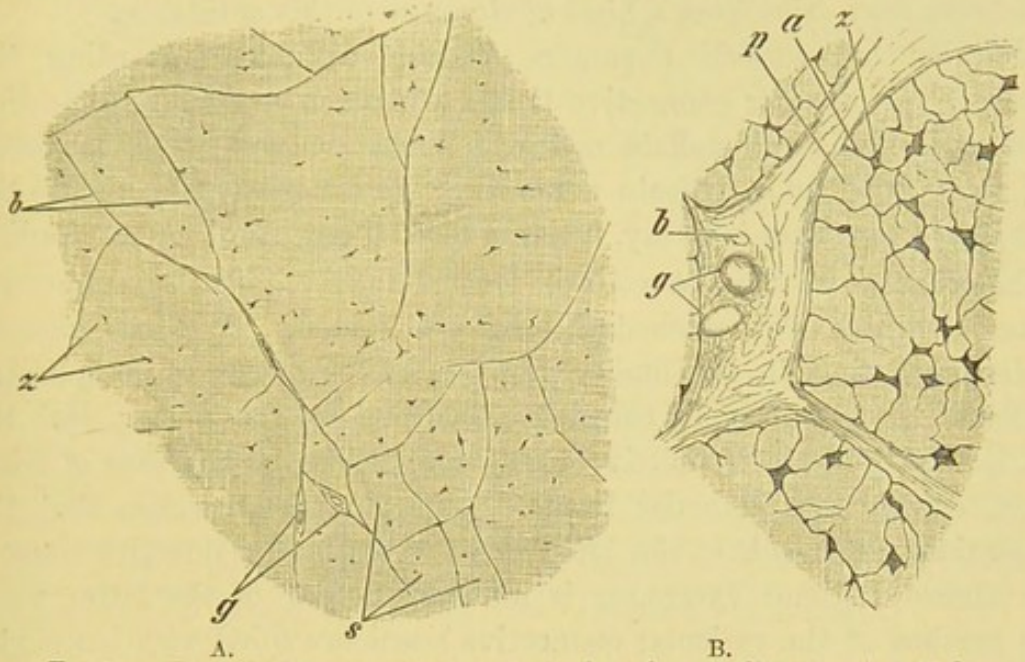
Elastic fibres are contained in almost all kinds of connective tissue.

Fat is often a constituent of fibrillar connective tissue. It appears in the form of a drop in the flat connective tissue cells, and changes these into fat cells (*vide* above, p. 305). The different elements of fibrillar connective tissue may be united without giving rise to structures of definite form, *formless connective tissue*, or they may produce a definite shape and the tissue may then be termed *formed connective tissue*. Formless connective tissue is conspicuous for the looser connection and more diversified interlacing of the bundles of connective tissue. It occurs between neighbouring organs, binding these together and filling out the intervals between them. Hence it may be termed *areolar* or *interstitial tissue*. The cells of this tissue not infrequently contain fat.

Formed connective tissue is characterized by a closer connection and a more regular course of its bundles. To this variety of connective tissue belong the true skin, the mucous membranes, serous membranes, the compact coverings of the nerve system, of the blood-vessels, of the eye, of many glands, and of the periosteum and perichondrium. It also includes the sinews or tendons, fasciæ, and fibrous septa or aponeuroses.

Tendons are characterized by the parallel course of the fibres forming them. These are firmly united. Tendons contain very few elastic fibres. A tendon is composed of a number of bundles of connective tissue held together by a small quantity of loose connective tissue.

Each of these secondary bundles of connective tissue is formed by a number of fibrils running in a straight line, and united by means of a small quantity of cement substance to the smaller primary bundles. Between the primary bundles are the cellular elements of tendon. These are flat connective tissue cells placed in a row, and, as they are curved like a hollow tile, they partially embrace the primary bundles, and unite with neighbouring cells by means of flat prolongations. These are termed *Ranvier's cells.*



A. B.

FIG. 184.—From the transverse section of a tendon of an adult man. A. × 80 d. *s*, bundles of tendon; *b*, loose connective tissue containing, at *g*, transverse sections of blood-vessels; *z*, cells. (Method No. 45, Appendix.) B. × 240 d. *p*, primary bundles embraced by prolongations *a*, of the cells, *z*; *b*, loose connective tissue; *g*, transverse sections of blood-vessels. (Method No. 46, Appendix.)

Elastic fibres are present in great quantity only in the loose connective tissue; in the bundles of connective tissue in tendon, they occur only sparingly in the form of delicate, wide-meshed reticulations. The blood-vessels in tendons are contained only in loose connective tissue, covering the bundles; and lymphatic vessels are principally found on the outer surface of tendons. The scanty nerve fibres are non-medullated, and these pass into end-organs resembling the motor-end-plates in muscular fibre.

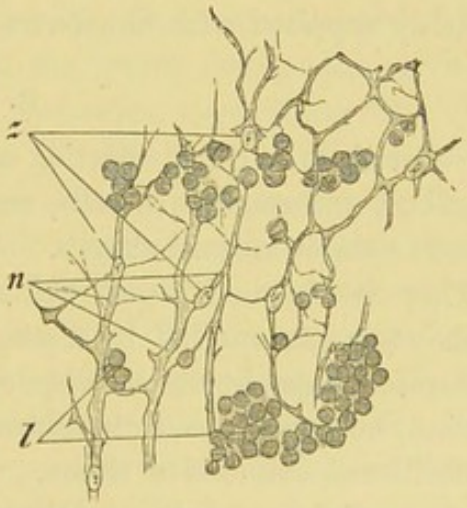


FIG. 185.—From a section of a human lymphatic gland which has been shaken in fluid, × 560 d. *n*, reticulated net-work; *z*, connective tissue cells; *l*, leucocytes. (Method No. 47, Appendix.)

The *fasciæ* are composed of bundles of connective tissue forming an irregular meshwork.

The *ligaments* are distinguished from tendons only by their more or less large supply of elastic fibres.

White fibrous tissue is developed from certain cells primarily derived from the mesoblast. These cells are at first spherical, but they become elongated and spindle-shaped, and each cell contains an oval nucleus. Each cell gives rise by longitudinal fibrillation to a bundle of connective tissue fibres, and the nucleus may disappear. It would appear that the primitive cells may also form a homogeneous substance around them, and that fibres may arise from a kind of cleavage of this substance.

Reticular or Adenoid Connective Tissue.—Opinions regarding the structure of reticular connective tissue are much divided. According to some, it consists of stellate *cells* which anastomose by their processes and thus build up a delicate net-work. This notion gave rise to the term, *kytogenous*, that is to say, a tissue formed out of cells.[1] According to the other view, the net-work is formed only of *fibres* of connective tissue to which flat nucleated cells are joined. We can succeed in demonstrating, in the higher vertebrates, by means of complicated methods, the contours of the flat cells lying on the fibres; but the fact is that fibrillar connective tissue may, even in the case of adult tissue, change into reticular tissue. The circumstance also that the adaptation of flat cells to the bundles of fibres is in connective tissue a rule almost without exception is much in favour of the latter view. The meshes of the reticular connective tissue are filled with leucocytes compactly pressed together. Reticular connective tissue having the meshes filled with leucocytes occurs principally in lymphatic glands. It is for this reason termed *adenoid*, that is, gland-like tissue. It is richly supplied with blood-vessels (Fig. 185).

2. Cartilage.

Cartilage is firm, elastic, easily cut, of a milk-white or yellowish colour, and consists of cells, and of a matrix or ground substance. The cells exhibit various shapes. They are usually roundish or flattened. They lie in cavities in the matrix which they completely fill, and they are surrounded by a strongly refracting, sometimes concentrically striated, capsule, the *cartilage capsule*. The matrix is either of the same kind, homogeneous, or interwoven with elastic fibres, or it is formed of a fibrillated connective tissue. In accordance with this general view, we distinguish (*a*) hyaline cartilage, (*b*) elastic or yellow fibro-cartilage, and (*c*) connective tissue or white fibro-cartilage.

(*a*) *Hyaline Cartilage* is of a light-bluish hue, like that of a stain of

[1] Mucous tissue, as it occurs in the jelly of the umbilical cord, might also be claimed as a kytogenous tissue.

milk in a glass. It is found in the cartilages of the respiratory apparatus, of the nose, of the ribs, of the joints, in the synchondroses, and in

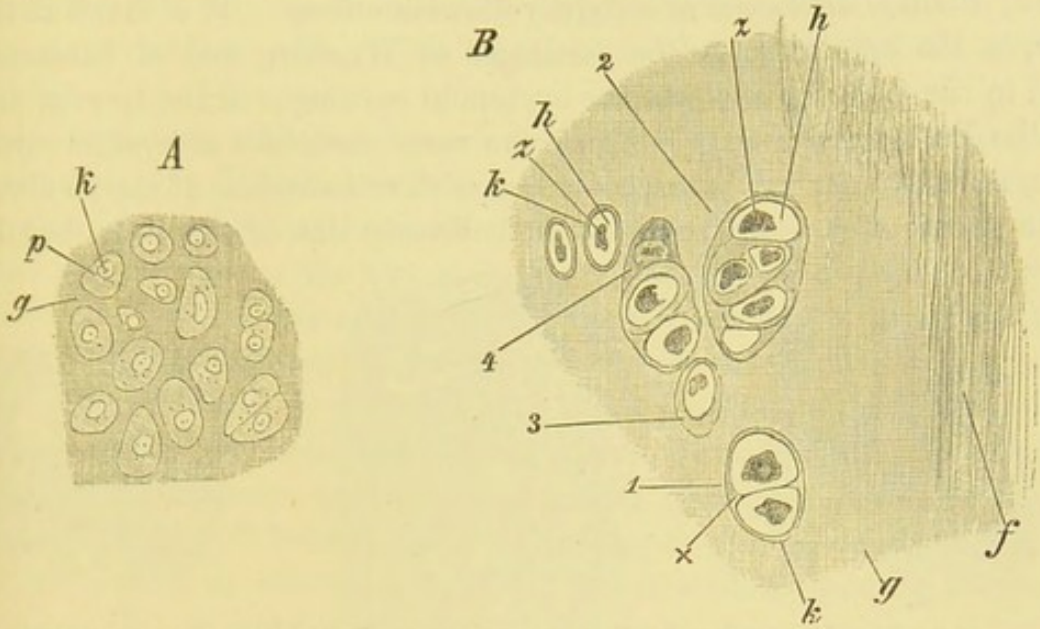


FIG. 186.—Hyaline cartilage. × 240 d. *A*, surface view of the *processus ensiformis* of the frog, fresh; *k*, nucleus; *p*, protoplasm of cartilage cell, completely filling the cavity in the cartilage; *g*, hyaline matrix. (Method No. 48, Appendix.) *B*, from a transverse section of the cartilage of a human rib examined in water several days after death. The protoplasm of the cartilage cells (*z*) has shrunk from the wall of the space (*h*) in the cartilage. The nucleus of the cartilage cell cannot be seen. *1*, two cells in one cartilage capsule (*k*); at the × begins the development of a partition wall; *2*, five cartilage cells embraced within a capsule—the lowest cell has fallen out, so that we perceive the empty cavity in the cartilage; *3*, cartilage capsule cut obliquely—on this account, it appears thicker on one side; *4*, cartilage capsule not cut at all—the cartilage cell shines through; *g*, hyaline matrix changed in *f*, into rigid fibres. (Method No. 49, Appendix.)

the embryo it forms the cartilaginous skeleton, which afterwards is exchanged for bone. It is characterized by its homogeneous matrix, but the matrix may undergo peculiar modifications. Thus, the matrix of cartilage of the ribs and of the cartilages of the larynx are changed, by molecular linear arrangement, into rigid fibres, which lend to the cartilage a brilliancy visible to the naked eye, somewhat resembling that of asbestos. Further, there are found, in the hyaline matrix, in the cartilage of advanced age, deposits of lime salts, which appear at the beginning in the form of small granules, and then as complete capsules, situated round the cartilage cells. The cells of hyaline cartilage very frequently show forms which have their cause in the processes of growth. Thus, we may find two cells in one cartilage capsule (Fig. 186, *k*). These have been developed by fission of the cartilage cell; in other cases, we may observe between two such cells a thin partition wall of hyaline substance already developed. Further, in some instances, a partition wall is not immediately formed. The two cells may divide again and again, and thus we have groups of four, eight, or more cartilage cells surrounded by a single capsule (Fig. 186, 2). Such phenomena have justified the description of a special mode of cell divi-

sion, the so called endogenous cell division. Cartilage cells of adults not infrequently contain small drops of fat.

(*b*) *Elastic Cartilage* is of a light yellowish colour. It is found in the ear, in the epiglottis, in the cartilages of Wrisberg and of Santorini, and in the *processus vocales* of the arytenoid cartilages of the larynx, and in the Eustachian tube. It shows the same structure as hyaline cartilage, only its matrix is interpenetrated with reticulations of elastic fibres. The elastic fibres are not produced directly out of the cells, but by

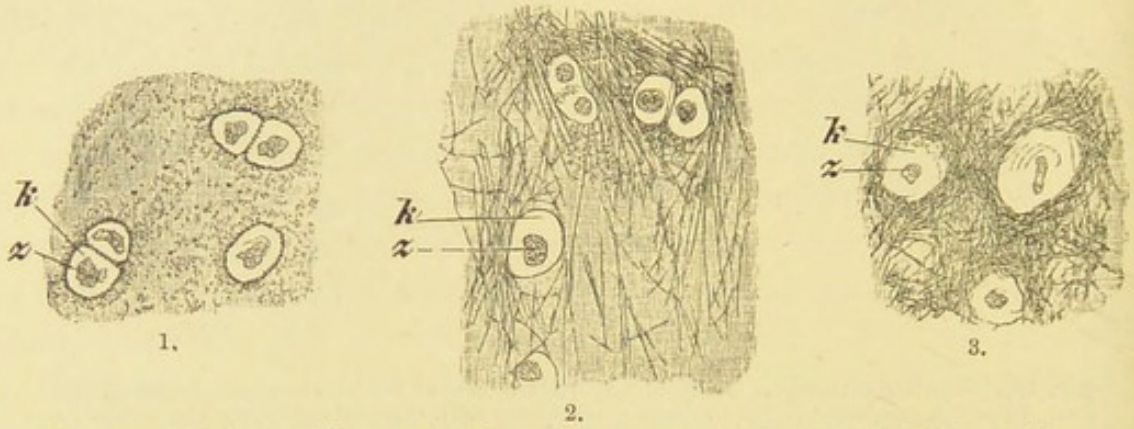


FIG. 187.—1, Elastic cartilage. × 240 d. *z*, cartilage cell (nucleus not visible); *k*, cartilage capsule. 1, from a section through the *processus vocalis* of the arytenoid cartilage of a woman of 30 years. Elastic substance is in the form of granules. 2 and 3, from a section through the epiglottis of a woman of 60. (2) More delicate reticulation. (3) Thicker reticulation. (Method No. 50, Appendix.)

means of a metamorphosis of the matrix, and they appear in the environment of the cartilage cells as granules (Fig. 187, 1), and at a later period of life they become fibres.

(*c*) *Connective Tissue or White Fibro-Cartilage* is found in the *ligamenta intervertebralia*, or intervertebral discs, in the uniting substance of the pubic and sacro-iliac symphyses, the interarticular plates of the sterno-clavicular, temporo-maxillary, knee, and wrist joints, the substance serving to deepen the borders of the acetabulum and the glenoid cavity of the shoulder joint, the tendinous sheaths, and the sesamoid bodies occurring in some of the tendons; but some of these, which are more fibrous than cartilaginous, are covered with a thin layer of true cartilage. The matrix of connective tissue cartilage is fibrillar connective tissue (Fig. 188, *g*), whose loose bundles run in all directions and form an irregular

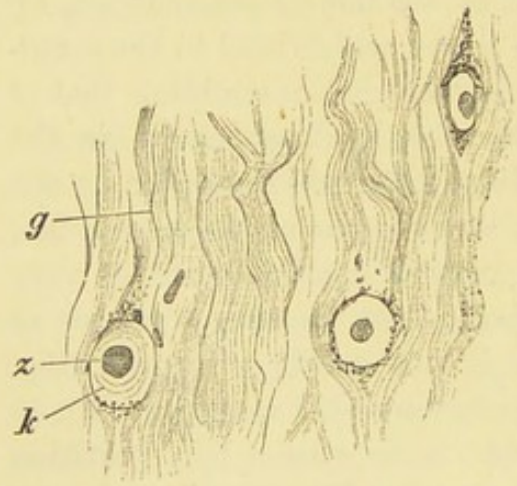


FIG. 188.—From a horizontal section of human *ligamenta intervertebralia*. × 240 d. *g*, connective tissue matrix; *z*, cartilage cell (nucleus indistinguishable); *k*, cartilage capsule surrounded by calcareous granules. (Method No. 51, Appendix.)

network. The few thick-walled cartilage cells existing in this variety lie in small groups at great intervals. In the centre of the *ligamenta intervertebralia*, there is found a soft jelly-like mass, which contains large groups of cartilage cells. This structure is the remains of the *chorda dorsalis*.

All cartilages, with the exception of those of the joints, have on their outer surface a fibrous membrane, the *perichondrium*, which consists of bundles of connective tissue running in different directions, mixed with some elastic fibres. At the place where cartilage and perichondrium come into contact, the one kind of tissue insensibly merges into the other. The perichondrium, therefore, adheres firmly to the cartilage. The perichondrium is supplied with vessels. The vessels in growing cartilage lie in canals or grooves. In the adult, cartilage is destitute of vessels. Nutrition is in this case effected through diffusion from the outer surface. Whether there exist proper canal-like passages, resembling those of the bones, in which the nourishing fluid circulates, is still doubtful, in spite of various contentions to the contrary.

Cartilage contains from 54 to 70 per cent. of water, a substance termed *chondrogen* (p. 79), which yields chondrin on prolonged boiling, a small quantity of fat (from 2 to 5 per cent.), and a variable amount of salts. The salts consist of phosphates of lime and of magnesia, chloride of sodium, carbonate of soda, and sulphates of soda and potash. The amount of salts has been found to vary from 2 to as much as 7·29 per cent. As life advances, the percentage of salts in cartilage increases. It is to be noted that cartilage contains only a very small proportion of salts of potash. By prolonged boiling, white fibro-cartilage yields gelatin, and yellow fibro-cartilage, elastin.

Cartilage is originally developed from a cellular blastema. In the first stage, the cells are set close together in a soft substance, which exists in small quantity. Subsequently, this substance increases in amount between the cells, and by its increase and encroachment upon the cells, it separates them to a greater and greater distance from each other. The farther growth of cartilage takes place, in part by the multiplication of the cells by fission, and in part by the formation of matrix around and between the cells. As above explained, changes in the structure of the matrix lead to the formation of the different varieties of cartilage.

3. Bone.

If we saw through a fresh tubular bone, we immediately perceive that its structure is not everywhere the same. The part near the periphery or surface is composed of a rigid, hard substance termed *substantia compacta*. There exist, round the medullary cavity of the bone, small and delicate *laminæ* or *trabeculæ*, which, running in all directions, form an irregular reticulated structure. This honeycomb-like substance is termed *substantia spongiosa*. The meshes of the substantia spongiosa and the central cavity are filled with a soft matter termed the *marrow*. The surface of bone is covered with a fibrous membrane termed the *periosteum*. The relation between compact and spongy substance is somewhat different in short bones, inasmuch as these consist chiefly of spongy substance, and the compact substance is confined solely to a narrow zone on the surface. Flat bones have sometimes thicker, sometimes thinner, layers of compact substance, whilst the internal portion of a flat bone is filled with spongy substance. The structure of the epiphyses of tubular bones resembles that of short bones; they consist chiefly of spongy substance.

The Microscopical Structure of Bone.

1. The *substantia spongiosa* is built up of small *laminæ* of bone, which consist of a matrix perforated by a network of canals. The matrix is formed of organic and inorganic parts, so that a high degree of hardness, rigidity, and elasticity is attained. It is homogeneous or slightly striated, and it contains numerous hollow spaces, from 15 to 27 μ long, termed *lacunæ* (Fig. 190, *h*), which are united by numerous processes, the

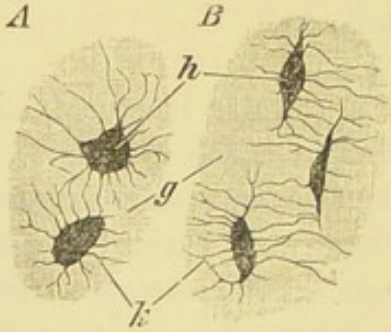


Fig. 189.—From a dry strip of the bone of an adult man, X 560 d. *h*, lacunæ; *A*, seen from the surface; *B*, from the side; *k*, canaliculi of bone; *g*, matrix of bone. (Method No. 52, Appendix.)

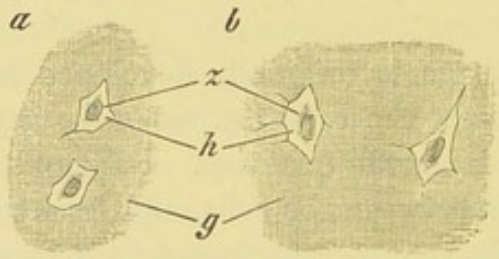


Fig. 190.—From a section, *a*, of the humerus of a human embryo of four months. *b*, of bone of an adult, X 560 d. Bone corpuscles, *z*, lying, *h*, in the lacunæ; the canaliculi are visible in some places; *g*, matrix. (Method No. 53, Appendix.)

bone canals or *canaliculi*. These canals not only communicate with each other, but also open freely on the surface of the small laminæ of the bone.

In this manner, a system of small canals penetrates the entire matrix. In the lacunæ there lie nucleated cells of an oval form. Whether the processes of the cells passing into the canaliculi anastomose is still doubtful. The small laminæ of the substantia spongiosa contain no vessels.

2. The *substantia compacta* is built up in a somewhat more complex manner. It contains, in addition to the above-mentioned system of narrow canals, a system of much coarser canals, 22 to 110 μ broad, which divide dichotomously and form a wide-meshed net-work. These coarser canals contain blood-vessels, and they are termed *Haversian canals.* The direction of their course in the long bones, in the ribs, in the clavicle, and in the inferior maxilla, is parallel to the long axis of the bone. In the short bones, as, for instance, the vertebræ, the direction is mainly perpendicular. In flat bones, on the other hand, the Haversian canals run parallel to the surface of the bones, not infrequently in radiated lines streaming from one point. An example of this arrangement is seen in the parietal bones. The Haversian canals open on the outer (Fig. 191, ×), as well as on the inner (Fig. 191, × ×) surface, and they are directed towards the substantia spongiosa. The matrix of the compact substance is stratified into lamellæ. Three systems of lamellæ (Fig. 192) may be distinguished: (1) A system of lamellæ forming a ring round the Haversian canals. When these are cut transversely, they appear like a number (8 to 15) of rings placed concentrically round the Haversian canal. Such concentric lamellæ are called the *Haversian* or *special lamellæ.* (2) The intersections of the Haversian systems of lamellæ impinge partially on each other, but they are also partially separated by layers of the bone, which are stratified in another direction. We term these lamellæ which run more irregularly between the Haversian systems of lamellæ, the *interstitial* or *intercalary lamellæ.* (3) These are united by a third system of lamellæ which run parallel with the outer surface of the bone, and these are termed the *external systems of lamellæ.* Lamellæ are also sometimes found on the inner surface; and such are termed *internal lamellæ.* The lacunæ have definite positions in the substantia compacta. In the Haversian lamellar systems, they are placed with their longitudinal axis parallel to the longitudinal axis of the Haversian canals, and bent towards the surface, so that in transverse sections they appear concentrically curved to the transverse section of the Haversian canal (Fig. 192). In the interstitial lamellæ the lacunæ are irregular, but in the external lamellæ they run with their surfaces parallel to the surfaces of the lamellæ.

3. The *marrow of the bones* exists in the axial cavities of the long

bones, occupies the meshes of the spongy substance, and also occurs in the larger Haversian canals. As it is either of a red or yellow colour,

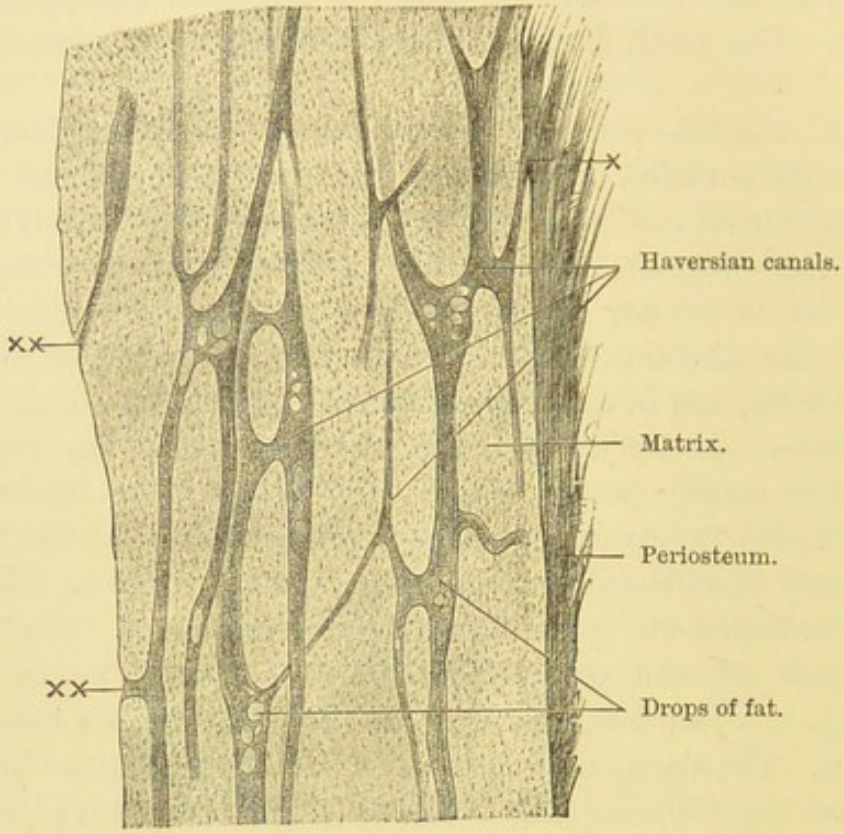


FIG. 191.—Portion of a longitudinal section through a metacarpal bone of a man, × 30 d. In the preparation drops of fat are seen in the Haversian canals. At ×, ×, ×, the Haversian canals open on to the surface. (Method No. 54, Appendix.)

we distinguish *red* from *yellow* marrow. The yellow marrow is richer in fat than red marrow; otherwise the elements in both varieties are identical. The red marrow is found in the spongy substance of short and flat bones, in the epiphyses of the long bones, and in the central cavity of long bones of small animals. The yellow marrow fills up the medullary cavity of the long bones.

In old and sick persons the marrow may be of a mucous character, and reddish-yellow. It is then termed *gelatinous* bone marrow, and it owes its character simply to the small amount of fat in it.

The elements of marrow are (1) a small amount of fibrillar connective tissue; (2) fat cells; (3) leucocytes (which are here termed bone marrow cells); and (4) giant cells (*myeloplaxes*). The latter are large cells of very irregular shape consisting of protoplasm and of one or more nuclei. There are giant cells with clear nuclei and giant cells with nuclei glittering and intensely brilliant in colour. The shape of the nuclei is very various, round, irregular, ribbon-shaped, or ring-shaped (Fig. 193, 2 *r*) or it may show a network (Fig. 75, p. 210). Out of giant cells containing one nucleus, cells containing many nuclei may be developed by means of

the fission of the single nucleus (Fig. 193, 3 *r*), or along with a corresponding fission of part of the protoplasm. If both nucleus and protoplasm

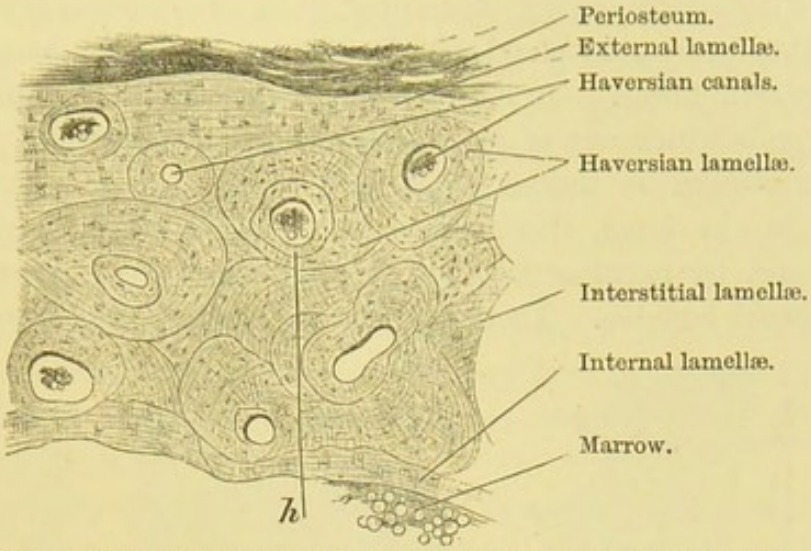


FIG. 192.—Portion of a transverse section of a metacarpal bone of a man, X 50 d. In the Haversian canals there is still some marrow (fat cells). *h*, Haversian spaces. (Method No. 55, Appendix.)

divide, cells with one nucleus are produced. It has also been supposed that in the formation of giant cells containing many nuclei, there may have been the coalescence of several cells of simpler construction. Finally, in red marrow, we may find (5) cells containing a nucleus, with yellow-coloured protoplasm, resembling red blood corpuscles. They are regarded as mother cells (*hæmatoblasts*) of the red blood corpuscles.

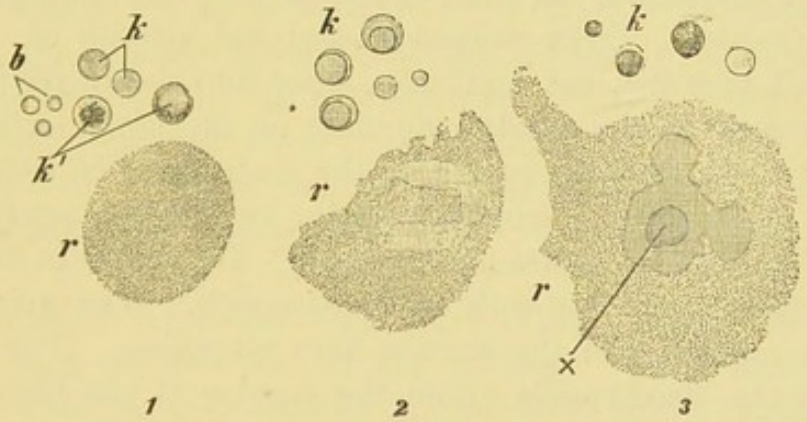


FIG. 193.—Elements of bone marrow freshly isolated from the vertebræ of a calf, X 560 d. 1, in a solution of common salt; 2, coloured with picrocarmine; 3, after the addition of acidulated glycerine. *k*, marrow cells; *k'*, two bone marrow cells, containing deposits of pigment granules—the right seen on the side, the left on the surface; *b*, coloured blood corpuscles (without nucleus); *r*, giant cells. The right shows sideways two nuclei in process of fission and one exactly similar on the surface ×. (Method No. 56, Appendix.)

Yellowish pigment granules occurring in different cells are regarded as the remains of red blood corpuscles that have undergone disintegration.

4. The *Periosteum* is a membrane consisting of strong connective

tissue fibres. We can distinguish two layers. The outer layer is characterized by its richness in blood-vessels, and it is intimately connected with neighbouring structures, such as tendons, fascia, etc.; the inner layer is poor in blood-vessels, but, on the other hand, it is very rich in elastic fibres. On its inner surface, we find a layer of punctated cubical cells which are of importance in the development of the bone. The periosteum is united sometimes more firmly, sometimes more loosely to the bone, the connection being established by the blood-vessels and by peculiar bundles of connective tissue penetrating into the external lamellæ. These bundles are termed the *fibres of Sharpey.*

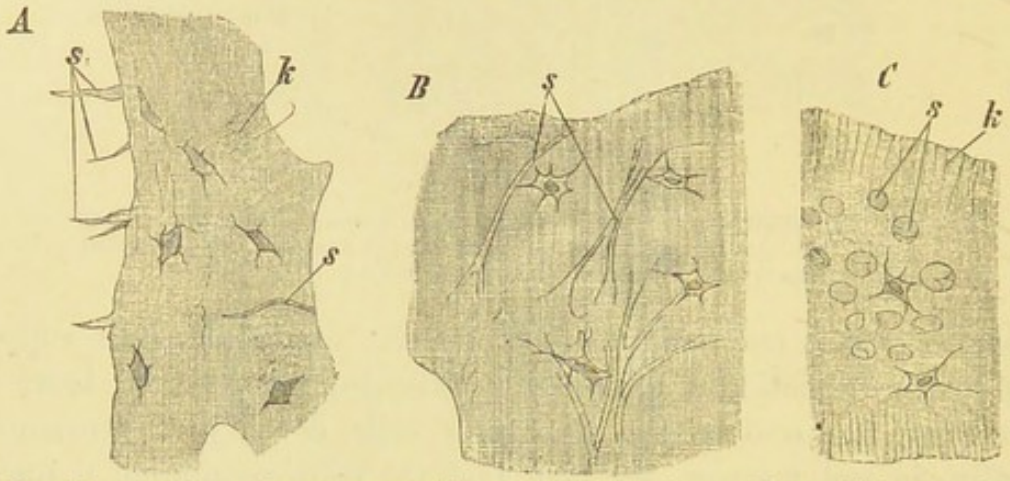


FIG. 194.—Portions of transverse sections of humerus of a new-born child, × 240 d. *s*, Sharpey's fibres. *A*, From a teazed preparation, seen, *B*, lengthways; *C*, transversely. *k*, canaliculi of bone. (Method No. 57, Appendix.)

5. The *blood-vessels* of the bone, of the marrow, and of the periosteum, are closely related. From numerous veins and arteries of the periosteum, small branches, not capillaries, enter into the Haversian canals, and the vessels in the canals may unite on the inner surface of the medullary cavity with the vessels of the marrow. The latter receive blood by means of the arteriæ nutritiæ, or nutrient arteries, which branch off through the substantia compacta. The vessels in the marrow unite to form a rich network of blood-vessels. The veins issuing out of the capillaries of the marrow have no valves. It is very probable that the blood-vessels within the marrow of the bones may, in some places, have no definite walls.

6. The *nerves* are situated partly in the periosteum, where they sometimes terminate in *Vater's corpuscles* (a form of touch corpuscle), and they may be found in the Haversian canals and in the marrow. They are both medullated and non-medullated.

DEVELOPMENT OF BONES OR OSSIFICATION.

The bones are formations appearing late in development. There is a time in embryonic life in which muscles, nerves, vessels, brain, and the

spinal marrow are already well formed, but there is yet not a trace of bone. The skeleton of the body is at that time formed of hyaline cartilage. With the exception of some portions of the skull and of the face, all the osseous parts of the skeleton are first represented by cartilage. We thus find, for example, in the upper extremities, the humerus, radius, ulna, carpus, and the parts of the skeleton of the hand composed of portions of cartilage, which, unlike the bones appearing later, are not hollow, but solid throughout. Gradually the bony skeleton comes to occupy the place of the cartilaginous skeleton. All the bones which were, in the embryonic period, formed by means of cartilage, are called cartilaginous or primary bones. The other bones, which have no cartilaginous predecessors, are termed secondary or connective tissue bones.

To the primary bones belong all the bones of the trunk, of the extremities, the greater portion of the base of the skull, the occipital bone, with the exception of the upper flat portion of it, the sphenoid bone, the petrous portion of the temporal bone, the small bones of the ear, the ethmoid bone, and the vomer.

To the secondary bones belong the bones forming the lateral parts of the skull, the roof of the skull, and almost all the bones of the face.

A.—Development of the Primary Bones.

Two processes are to be observed here—(1) The formation of bone substance *in* cartilage already existing, termed *enchondrial* or *endochondrial ossification*, and (2) the formation of bone in the immediate neighbourhood—that is to say, *on* cartilage. The latter process is termed *periosteal*, or better *perichondrial ossification*. Both begin almost simultaneously, the perichondrial often somewhat earlier than the other.

1. *Enchondrial Ossification.*—The first changes consist in the cells enlarging in a definite part of the cartilage. They then divide in such a way that several cells lie in one cavity of the cartilage. The matrix becomes finely granular and dim by the deposition of calcareous (or earthy) salts (Fig. 195, *V*). Such places where the cartilage is thus changed can soon be observed by the naked eye, and they are termed *centres of ossification*, or better, *centres of calcification*. The portions of the cartilage more remote from a centre of calcification increase in thickness and length, while at the centre of calcification no further growth of cells takes place. A portion of the bony skeleton is developed at that spot (Fig. 195). Meanwhile there has appeared on the upper surface of the centres of calcification a tissue termed *osteogenous tissue*, rich in young

cells and vessels. This penetrates into the cartilage, and causes the calcified matrix to break down. The cartilage cells become free and mingle with the cells of the osteogenic tissue, and thus a small cavity

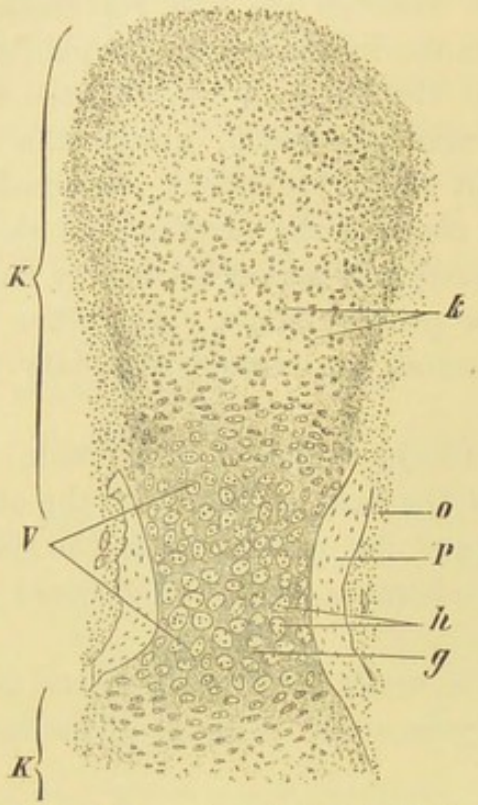


FIG. 195.—From a dorsoplantar longitudinal section of the great toe of a human embryo of four months. Two thirds of the first phalanx are indicated, × 50 d. *V*, centre of ossification; *h*, cavities of the cartilage enlarged, containing several cartilage cells. The cells cannot be recognized here under a feeble magnifying power, but only their point-shaped nuclei. *g*, calcified matrix of cartilage; *K*, hyaline cartilage; *k*, growing cartilage, we perceive the cartilage cells arranged in groups of three to four cells; each group is produced by the repeated division of one cartilage cell; *o*, osteogenous, or bone forming tissue; *P*, perichondrial bone. (Method No 58, Appendix.)

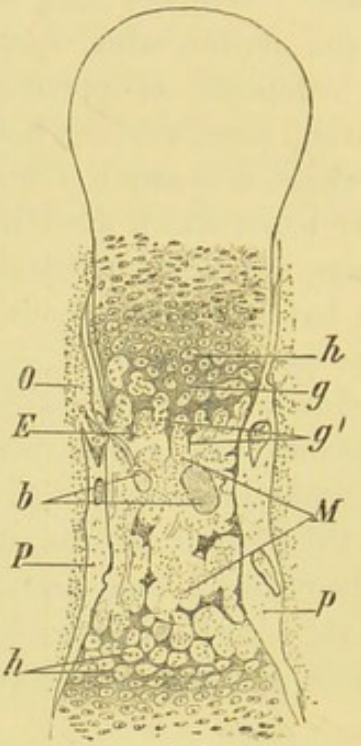


FIG. 196.—From a dorsopalmar longitudinal section of a finger of a four months' human embryo. Two thirds of the second phalanx are shown, × 50 d. *M*, primordial medullary space containing, *b*, cartilaginous marrow and blood-vessels; *h*, enlarged cartilage cavities; *g*, calcified matrix of cartilage, in the form of indented processes, *g'*, projecting into the medullary space; *O*, osteogenous tissue; *P*, perichondrial bone; *E*, enchondrial bone is formed only in the form of fine leaflets. (See under higher power in Fig. 197.) (Method No. 59, Appendix.)

is formed at the centre of calcification. This cavity is termed the *primordial medullary space.*

The portion of cartilage next the centre of calcification undergoes changes similar to those above described—that is to say, there are changes in the matrix and in the cartilage cells. There is gradually an ever-advancing increase of the medullary space, while new parts of the cartilage melt down. Entire groups of cartilage cells thereby become exposed, while the calcified cartilage substance, situated between these, is still preserved in the form of indented processes, projecting into the medullary space (Fig. 196, *g*). The medullary space is now a cavity communicating with many surrounding diverticula or pouches, and all are filled with blood-vessels and cells, termed the *cartilage marrow cells.*

These cells play an important part in bone development. They either become the marrow cells of bone, preserving their form, or they become *fat cells*, or—and this the most important—they become bone-forming cells or osteoblasts. *Osteoblasts* are cells which are deposited after the manner of unstratified epithelium on the walls of medullary spaces, and there they develop the matrix of bone (Fig. 197).

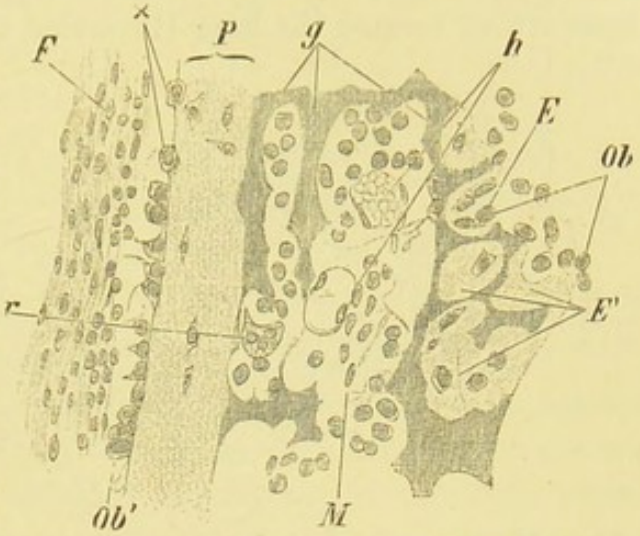


FIG. 197.—From a longitudinal section of the first phalanx of the finger of a human embryo of four months, × 240 d. *M*, inlets or recesses of the primordial medullary space, filled with cartilage cells, and *h*, blood-vessels; *r*, giant cell; *g*, calcified matrix; *E*, young enchondrial bone substance seen from the side; *E'*, seen on the broad surface. Here we already perceive indented bone cavities with bone cells. *Ob*, Osteoblasts still irregularly scattered about; *P*, perichondrial bone; *Ob'*, osteoblasts by this time arranged in a layer; the two uppermost osteoblasts x are already surrounded to the extent of one half with bone matrix. *F*, periosteum. (Method No. 60, Appendix.)

At the beginning, all osteoblasts rest on the matrix of the bone; but at a later date they come to be embedded in the matrix itself, and they thus become *bone corpuscles* or *bone cells*, lying in the lacunæ (Fig. 190, p. 332). The medullary space is now lined by the activity of the osteoblasts, with a thin layer of bone, which gradually becomes thicker, and the indented laminæ of calcified cartilage (Fig. 197, *g*), are quickly surrounded with circular layers of young bone (Fig. 197, *E*). Thus, the earlier solid portion of cartilage is by degrees converted into spongy bones, whose pouches or irregularly-formed honeycomb-like meshes still contain the remains of calcified matrix (Fig. 197, *E*, *g*).

2. *Perichondrial Ossification.*—This mode of ossification is effected also by means of osteoblasts, which form osteogenic tissue on the outer surface of the centres of calcification (Fig. 196, *O*). By the activity of osteoblasts, strata of bone substance are also formed on the outer surface of the cartilage (Fig. 195, *P*), but the masses of bone thus formed from perichondrium are distinguished from those formed in cartilage in having no remains of calcified matrix of cartilage, the formation of bone in perichondrium being effected only on the circumference of bones, not in the interior of cartilage. The perichondrial layer of the bones does not consist of a stratum of uniform thickness, but in many places it is very much thicker than at others (Fig. 198, *h*, *h*). Depressions are also formed, in which blood-vessels lie surrounded by osteoblasts. At the beginning, the grooves or depressions are only channels open towards

the periphery; but with the gradually increasing thickening of the perichondrial strata of bone the channels are closed up (*h'*), so as now to become canals containing vessels. These are the Haversian canals. By the activity of the osteoblasts enclosed in the Haversian canals, new bone strata, termed the later Haversian lamellæ, are formed.

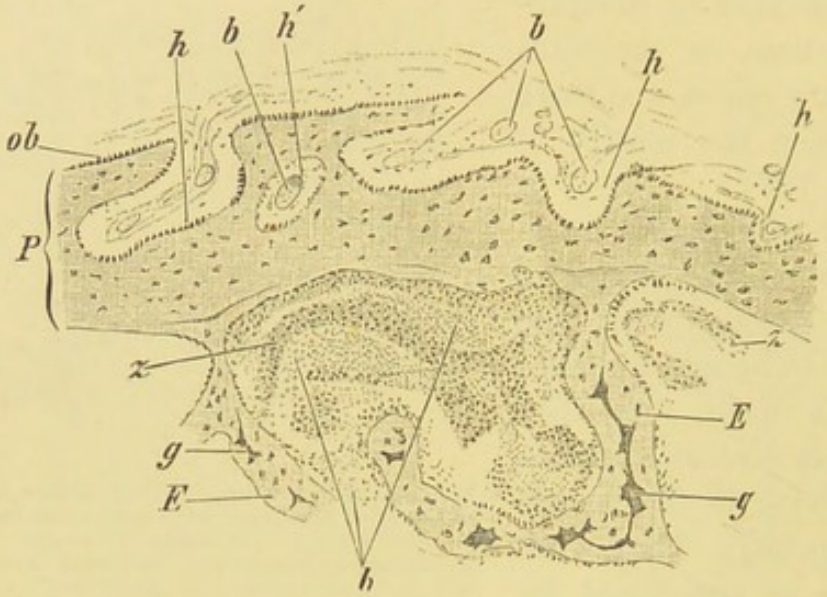


FIG. 198.—Portion of a transverse section through the diaphysis of the humerus of a human embryo of four months, × 80 d. *P*, periosteal trabeculæ having osteoblasts, *ob*, on their margins; *h*, *h*, *h*, small Haversian canals shut in by bone formation; *h'*, small Haversian canal closed; *E*, enchondrial trabeculæ of bone likewise covered with osteoblasts and containing the remains of calcified cartilage matrix, *g*; *z*, marrow cells; *b*, blood vessels. (Method No. 61, Appendix.)

Thus a bone has been formed out of a cartilage by the dissolution and removal of the cartilage and the supplying of its place by bone (*enchondrial ossification*) and by the deposition of new masses of bone on the outside (*perichondrial ossification*). The essence of the processes above described consists in a dissolution of the original cartilaginous portion of the skeleton and in a rebuilding of it by the development of bone substance. We term this mode of bone formation the *neoplastic type* of ossification as distinguished from a mode seldom occurring (as, for example, in the ossification of the angle of the lower jaw) in which the cartilage is not destroyed but is simply turned into bone, the matrix of the cartilage becoming the matrix of the bone and the cartilage cells becoming bone cells. This mode is termed the *metaplastic type* of ossification (Fig. 199).

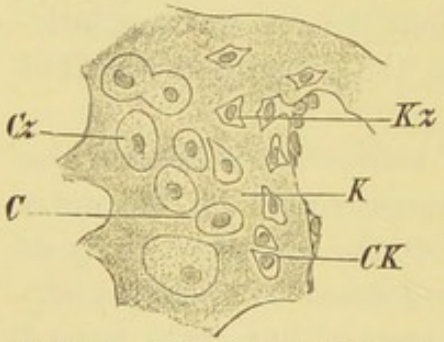


FIG. 199.—From a transverse section of the lower jaw of a newly-born dog, × 240 d., *Metaplastic type*. *C*, matrix of cartilage becoming, *K*, bone matrix, directly; *Cz*, cartilage cells; *Kz*, bone cells; *CK*, transition from cartilage cells to bone cells. (Method No. 62, Appendix.)

B.—Development of the Secondary Bones.

In this case, the basis on which ossification is effected is not cartilage but connective tissue. Individual connective tissue fibres become calcified; on these, osteoblasts (Fig. 200), proceeding from embryonic cells, are deposited; bone is then formed in the manner above described.

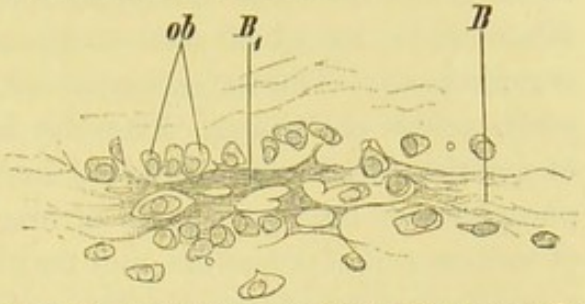


Fig. 200.—From a surface section of the bone on the crown of the head of a human embryo, × 240 d. *B*, bundles of connective tissue calcified at B_1; *ob*, osteoblasts. (Method No. 63, Appendix.)

In the preceding descriptions we have noticed only the processes occurring in the first development of a bone. The further growth of a bone, as, for example, the growth of the tubular bones, now takes place in such a manner that longitudinal growth is carried on by extension of the primordial marrow space and enchondrial ossification on the basis of the evergrowing cartilage. The growth in thickness is carried on by the deposition of new strata of bone by the periosteum.

Flat bones formed in connective tissue grow by means of the formation of new masses or layers of bone on the margins (superficial growth), and on the flat surfaces (growth in thickness). The growth of all bones is, however, probably effected not only by means of the deposition of new strata of bone or *appositional* growth, but also by means of the expansion of the bone substance already formed or by *interstitial* growth. It must also be noticed, in the last place, that the bone substance, after it has once been formed, does not remain in a completed state, but very soon again passes through a process of dissolution. This dissolution occurs not only in the formation of the cavities of the tubular bones and in the typical surfaces of absorption, but also in such places as those on which new bone substance is once more formed at a subsequent period.

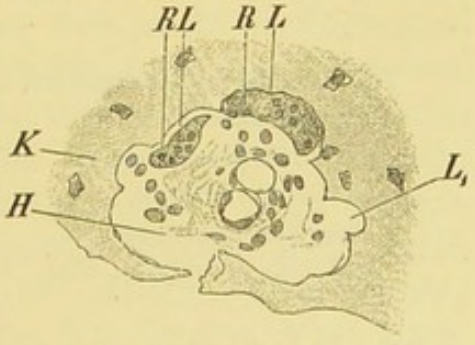


Fig. 201.—From a transverse section of the humerus of a newly-born kitten, × 240 d. Small Haversian canal, *H*, containing two vessels and marrow cells; *K*, bone; *L*, Howship's lacunæ, in which, *R*, giant cells (*osteoclasts*) lie; L_1, empty lacuna. (Method No. 64, Appendix.)

Wherever reabsorption of bone substance occurs, we find giant cells placed in fossæ or depressions of the bone termed *Howship's lacunæ*. The giant cells are termed *osteoclasts*.

Chemical Composition of Bone.

We may determine the chemical composition of a bone either by burning the bone and analyzing the ash, or by macerating the bone in dilute acids, by which the earthy matters are dissolved, leaving the organic basis. The latter consists of a substance named *collagen*, which yields *gelatin* on boiling. Even dry bones yield from 30 to 40 per cent. of gelatin. The salts contained in bone consist chiefly of the tri-basic phosphate of lime, phosphate of magnesia, carbonate of lime, chlorides of sodium and of potassium, and fluoride of calcium. Analysis also yields a small quantity of albumin, fat, and alkaline sulphates, which are probably derived from the blood-vessels and nerves of bone. It is probable that the earthy matter is simply infiltrated into the organic substance of bone, and that it is not in a state of actual chemical combination with it.

The compact tissue of bone contains from 3 to 7 per cent. of water, while the amount in spongy tissue may vary from 12 to 30 per cent. The amount of earthy matter, as determined by the quantity of ash, varies considerably, as shown by the fact that Rees obtained the following results—Earthy matter from temporal bone, 63·5 per cent.; from scapula, 54·51 per cent.; from the bones of a fœtus of seven months, 59·62 per cent.; of an infant of nine months, 56·43 per cent.; of a child of five years of age, 67·8 per cent.; of a man of twenty-five years of age, 68·97 per cent.; of a woman of sixty-two years of age, 69·82 per cent.; and of a woman of seventy-two years of age, 66·81 per cent.

The following analyses of bone show its composition in 1000 parts—

	Femur of man aged 30 years.	Femur.	
		Compact tissue.	Spongy tissue.
Organic matter,	310·3	314·7	358·2
Mineral matter,	689·7	685·3	641·8
	1000·0	1000·0	1000·0
The mineral matter consisted of—			
Phosphate of lime, Fluoride of calcium,	596·3	582·3	428·2
Carbonate of lime,	73·3	83·5	193·7
Phosphate of magnesia,	13·2	10·3	10·0
Chloride of sodium, etc.,	6·9	9·2	9·9
	689·7	685·3	641·8
The organic matter consisted of—			
Fat,	13·3	314·7	358·2
Collagen,	297·0		
	310·3	314·7	358·2

The long bones of the skeleton are somewhat richer in mineral matter than the others. Phosphate of lime, as the above analysis shows, exists in larger amount in compact than in spongy bone. The bones of herbivora are richer in carbonate of lime than those of carnivora. Salts become more abundant as age advances, so that the bones become more brittle. It has been found possible, by giving to an animal, food containing salts of alumina or strontia, to replace part of the lime in bone by one or other of these substances, without altering the structure and general properties of bone.

Chap. XII.—THE PHYSICAL AND VITAL PROPERTIES OF THE CONNECTIVE TISSUES.

1. Physical Properties.

1. *Specific Weight or Density.*—This varies within wide limits, of which the extremes are afforded by adipose tissue and bone. The lightness of adipose tissue, which is largely employed in the body as a protective substance for delicate organs, is of importance, as it diminishes the total weight of the body, and, consequently, the muscular force required to move it.

2. *Consistence.*—This varies from a diffluent or semifluid state as in jelly-like matter of the umbilical cord, to considerable hardness as in bone. The consistence depends upon the amount of water contained in the substance. Thus, the jelly-like tissue contains about ninety-eight, and bone about three per cent., of water.

3. *Cohesion.*—This depends upon the adhesion of the molecules forming the tissue, or the union side by side of the fibres composing it. The amount and direction of the cohesion may be indicated by the manner in which the tissue yields to force. Thus, costal cartilage will break more easily in the transverse than in the longitudinal direction, while in such structures as tendon, it is more easy to separate the fibres from each other than to tear them across. The forces which act upon connective tissues, and which are resisted by their cohesion, are traction or pulling, pressure, flexion, and torsion. The amount of cohesion may be determined by ascertaining the weight that will cause rupture. Thus, if we take the unit of square surface as a square millimetre, the weight in kilogrammes required to cause a rupture of the tissue has been found to be as follows—Bone, 7·7 ; tendon, 6·9 ; arteries, 0·16 ; veins, 0·12. Bone and tendon present great resistance to traction. Thus the tendon of the plantaris muscle of man will support a weight of about 15 kilogrammes, without breaking. By resistance to traction, the tendons and ligaments accomplish a certain amount of mechanical work ; sometimes the membranes formed by the connective tissues afford support to

groups of muscles, or assist in giving strength to the walls of cavities. Resistance to pressure is manifested by the flat bones, the intervertebral discs, and the cartilage covering articular surfaces. It thus assists in the maintenance of posture, and in walking. Resistances to pressure and torsion are only exerted in certain circumstances. For example, when the hand supports a heavy weight, the arm being horizontal, the bone tends to become bent; in inspiration, the costal cartilages and ribs undergo torsion, which ceases during expiration, when the cartilages and bones return to their previous condition. (Beaunis.) The connective tissues also give cohesion to the various organs.

The structure of the connective tissues is always related to the mechanical uses for which they are adapted. As regards cohesion, we find it to be determined with reference to the direction of the forces which tend to rupture the tissue. When the forces act as pulling forces, the cohesion is greatest in the longitudinal direction, and the structures are composed of fibrils; but, if forces act on the tissue in several directions, as in fibrous membranes, aponeuroses, etc., the structure is an irregular network of fibres running in many different directions. On the other hand, if resistance to pressure is to be attained, as in the neck of the femur or in the os calcis, we find an arrangement of osseous lamellæ so placed as to give the greatest amount of strength with the smallest amount of material. The amount of the connective tissues in an organ affects the cohesion of its parts, and thus some organs are firmer and stronger than others. Contrast, for example, in this respect, the friable structure of the liver with the tough, fibrous structure of the lung.

4. *Elasticity.*—This property includes (1) the change of form of an elastic body under the action of some force; and (2) the return of the body to its original form when this force ceases to act.

The amount of elasticity is determined by ascertaining the force necessary to change the form of the object to which the force is applied, and the perfection of the elastic action is noted by the quickness and accuracy with which the object returns to its former condition after the mechanical force ceases to act. Thus, when a strong force is required to change the form of an elastic substance, its elasticity is great; and, on the other hand, when a feeble force is sufficient to overcome the elastic force, the elasticity is said to be small. Again, if, when the mechanical force acting on the elastic substance ceases to act, the substance returns exactly to its former condition, its elasticity is said to be perfect, while it will be more or less imperfect, according to the extent to which the substance fails to attain its former dimensions. Certain substances may be strongly but imperfectly elastic, as a silver wire; others may be feebly but perfectly elastic, like an india-rubber band; while a third class are both highly and perfectly elastic, like a steel or glass rod.

It is evident that elasticity may be called into action in different ways according to the direction of the force acting on the elastic body. Thus, it may be evinced after pulling or traction, after squeezing or compression, after bending or

flexion, and after twisting or torsion. With most substances, and within limits, the lengthening of an elastic body is proportional to the tractile or pulling force. Above these limits, the amount of lengthening is not directly proportional to successive increments of the tractile force, but it becomes less and less with each increment. The amount of elasticity of torsion is determined by measuring the angle of torsion.

The elasticities of different bodies are stated in figures that express the *modulus of elasticity*. The modulus for longitudinal extension or compression is termed *Young's modulus*, which may be defined as numerically equal to the pull applied per unit cross sectional area divided by the elongation per unit length produced by that pull. Let P be the pull, a the cross sectional area, and let l be the length of the fibre and e the elongation produced ; then—

$$M = \frac{P/a}{e/l} = \frac{Pl}{ea}.$$

For example, it was found that a weight of 12700 grammes, when hung on a steel wire 503 cm. long and ·0045 sq. cm. in cross sectional area, elongated it by 7 mm. (·7 cm.) Hence, for this wire,

$$M = \frac{12700/·0045}{·7/503} = 2065 \times 10^6 \text{ grammes weight per square centimetre.}$$

Wertheim gives the moduluses of the following substances in grammes weight per square centimetre :—Bone, $2304·1 \times 10^6$; tendon, $163·41 \times 10^6$; nerves, $18·89 \times 10^6$; living muscle at rest, $·95 \times 10^6$; veins, $·87 \times 10^5$; arteries, $·052 \times 10^6$. The latter figure may be taken as the modulus of elastic tissue, which abounds in the larger arteries. From these figures, we may calculate the elongation produced by any pull applied (the pull being within the elastic limits) thus : Let P be the pull applied—

$$e = \frac{P}{M} \times \frac{l}{a},$$

where l and a are the length and cross section of the body stretched.

The connective tissues may be divided, with reference to their elasticity, into two groups—(1) Yellow elastic tissue, which is feebly but perfectly elastic, that is to say, it changes its form under the influence of feeble force, and returns exactly to its original form ; (2) the second group includes connective tissue properly so-called, namely, tendons and ligaments, which are highly but imperfectly elastic, that is to say, they change form only under the action of powerful forces, and do not return completely to their original state.

The elasticity of connective tissues plays an important part in the body—(1) It is a permanent force which resists other permanent forces, such as gravity or muscular action. Thus the elasticity of the inter-vertebral discs, and of the *ligamenta subflava*, assists in maintaining the erect position of the vertebral column, and in expiration the elasticity of the costal cartilages and of the ribs restores the original form of the thorax when the inspiratory muscles have ceased to act. (2) Elasticity transforms an intermittent into a continuous movement; thus, the elasticity of the arterial walls converts the intermittent flow of the blood in the arteries into a continuous current in the capillaries, by coming into play in the intervals between cardiac beats. (3) It tends to maintain the form of organs against temporary forces that may act on them—traction, pressure, or torsion. (4) Elasticity economizes

muscular work by coming into play in the intervals between successive shocks. Suppose an intermittent force acts on a heavy body by traction, it has been found by experiment that the introduction of an elastic medium between the heavy body and the point at which the force is applied will enable the same work to be done with a smaller expenditure of force than if the force acted through a rigid or non-elastic structure. (Marey.)[1]

2. Vital Properties.

(A) Vital Properties of the Fibrillar Connective Tissues.

1. *Nutrition.*—The nutrition of the connective tissues is feeble—that is to say, metabolism is not very active in them, except during the process of growth. Ordinary areolar tissue is richly supplied with vessels. Waste products are removed chiefly by the lymphatics, which are abundant.

2. *Sensibility.*—Connective tissues consisting of fibres are not very sensitive. There are nerve-terminations in tendon (p. 327), but no special terminations exist in ordinary areolar tissue. When connective tissue is cut, no doubt pain may be caused by the severance of sensory nerve-fibres passing through it.

(B) Vital Properties of Cartilage and Bone.

No blood-vessels enter hyaline cartilage, and it therefore derives nourishment from the vessels of adjoining textures, more especially from bone, by a process of imbibition. Where cartilage exists in large masses, canals may be formed in its substance for the conveyance of blood-vessels, but cartilage never contains a plexus of capillaries. Cartilage is devoid of sensibility.

The nutrition of bone, even in the adult, is active, as is shown by the morphological changes (softening, fatty degeneration, etc.), which it may undergo in the course of disease. The bones are well supplied with blood-vessels, derived partly from periosteal vessels, and partly from vessels in the medullary canal. Fine vessels run through all parts of the compact tissue in the Haversian canals. From these, nutritious fluid permeates the substance of the bone, by the canaliculi uniting the masses of protoplasm (bone corpuscles) in the lacunæ.

Bone in the healthy condition is feebly sensitive; but when inflamed, especially near the periosteal surface, it becomes acutely painful. The marrow and periosteum of bone contain numerous nervous filaments, which occasionally manifest extreme sensibility, as during the process of inflammation.

[1] Marey. Du moyen d'économiser le travail moteur de l'homme et des animaux. Rôle de l'élasticité dans les appareils moteurs des êtres vivants. *Physiologie Expérimentale*, 1876.

Cartilages form smooth surfaces in the joints, and thus allow the various parts of the skeleton to move on each other with a minimum of friction, while, by their consistence and elasticity, they break the force of concussion. Certain of the permanent cartilages also enter into the formation of the external ear, the nose, the eyelids, the Eustachian tube, the larynx, and the windpipe, maintaining the form of these organs, and giving attachment to muscles and ligaments.

Bones, in the adult, form the framework of the skeleton. Certain bones, as those of the extremities, are the passive organs of locomotion, whilst others, when clothed with the soft tissues, form the cavities of the body, and serve as protective structures for delicate organs.

Chap. XIII.—PHENOMENA OF FILTRATION AND OF OSMOSIS IN RELATION TO TISSUES.

At this stage the student should make himself familiar with the phenomena of filtration and of osmosis, as these processes play an important part in the nutrition of the tissues and they are well illustrated by the passage of fluids through the connective tissues.[1]

1. Filtration through Organic Membranes.

If we pour a fluid into a glass cylinder, one end of which is covered by an organic membrane, and if the membrane imbibes the fluid, or a portion of it, filtration occurs. The rapidity of filtration is in direct proportion to the pressure exerted by the fluid upon the membrane, and it increases very rapidly with a rise of temperature. Solutions of crystalloids pass almost without change, and the density of the fluid left behind is the same as that which has passed through the membrane. In the case of colloids, however, the proportion of the dissolved substance is always less in the filtered fluid than in that which remains behind; or, in other words, a membrane permits the passage of a larger relative quantity of water than of colloids.

When colloids exist in a fluid, they exert an influence upon the filtration of the salts dissolved in the same fluid; the greater the quantity of colloidal material in the fluid on the filter, the smaller will be the proportion of the dissolved salts that passes through the membrane; or, in other words, the presence of colloidal matter hinders the filtration of crystalloids. The filtration of fluids flowing in vessels is affected in the same manner by pressure and temperature; but certain

[1] The following has been prepared largely with the aid of Wundt's elaborate account in his *Physiologie Humaine*, p. 55.

phenomena which occur can only be explained by assuming special properties in some organic membranes.

2. Diffusion through Organic Membranes.

When an organized membrane is interposed between two fluids, both of which may be imbibed by the membrane, and which are miscible, an exchange of the two fluids takes place through the membrane, and this exchange goes on until the two fluids have the same composition. There may be, however, an unequal exchange between the two fluids, so far as volume of water is concerned, so that for one part of the first fluid which passes to the second, a smaller quantity will pass from the second to the first. In comparing the diffusive properties of different fluids, it is necessary to make the experiment at the same temperature, and with one of the fluids constant in composition. Distilled water is therefore employed, in comparative experiments, as the standard fluid. Suppose a saline solution is placed in a glass tube, closed at the bottom by an organic membrane, and the apparatus is immersed in a shallow vessel nearly filled with water, some of the salt will be found after a time to have passed into the water, and some of the water will have passed through the membrane into the tube. Such an arrangement is termed an *endosmometer*, of which there are many forms. One of simple construction is shown in Fig. 202. It will also be found that there is a constant relation between the weight of the water which has passed in the one direction, and the weight of the salt which has passed in the other. The quantity of water which passes is always a multiple, or, in some cases, a fraction of the quantity of the substance in solution which diffuses. The weight of the quantity of water necessary to replace by diffusion a unit of weight of the dissolved body (1 gramme), is called the *endosmotic equivalent* of the body. This equivalent depends (1) on the chemical nature of the body; and (2) on the degree of concentration of its solution. The following table, given by Jolly, shows the equivalents of certain substances—

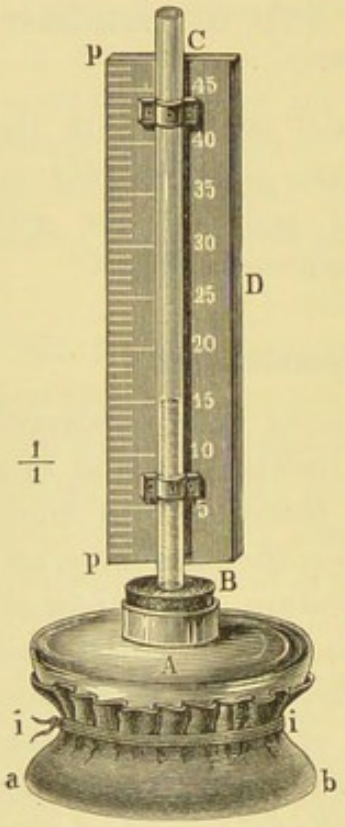


Fig. 202.—Endosmometer. *A*, glass cylinder constructed so that an organic membrane (piece of bladder), *a*, *b*, can be tied over its lower end by the ligature *i*, *i*. A tube *C* is passed through a cork *B*, fitted tightly into the upper and constricted end of the cylinder. *D*, a scale attached to *C*, divided into millimetres on the border *p*, *p*.

Name of Substance.	Endosmotic Equivalent.
Chloride of sodium,	4·0
Sulphate of soda,	11·0
Sulphate of potash,	12·0
Sulphate of magnesia,	11·5
Sulphate of copper,	9·5
Sulphuric acid,	0·3
Caustic potash,	200·0
Alcohol,	4·3
Sugar,	7·2

That is to say, suppose the endosmometer to be filled with solutions of the substances in the above list and to be placed in distilled water, 4 grammes of water would pass through the membrane into the endosmometer for 1 gramme of chloride of sodium, 11 grammes of water for 1 gramme of sulphate of soda, and so on. Thus, by this method of comparison it can be shown that the endosmotic equivalent of chlorides is small, of nitrates greater, of bases very great, of acids small, and of acid salts much smaller than of neutral salts.

As Jolly, in these experiments, took no account of the degree of concentration of the fluids, and as he always employed dried membranes, these figures do not show the quantities of these substances which would pass through organic membranes in the living state. Hofmann has shown also that the amount of water of hydration or of crystallization, even in the same salt, influences to a remarkable extent the endosmotic equivalent.

When more water passes towards the saline solution than the amount of the latter which enters the water, the diffusion is said to be *positive*, and *negative* when the reverse is the case. With alkalies positive endosmosis is strong; with acids, negative endosmosis is the rule, while salts are positive and range between the two extremes. When endosmosis is positive, the equivalent increases with the degree of concentration; when, on the contrary, it is negative, the equivalent diminishes as the concentration increases. Thus, in the diffusion of sulphuric acid with water, more acid will pass into the water as the acid becomes concentrated; but, on the contrary, in the diffusion of potash with water, more water will pass into the potash as the latter is concentrated. According to Ludwig, sulphate of soda is an exception amongst bodies showing a positive endosmosis, as its equivalent diminishes by concentration.

Diffusion occurs with a *constant rapidity* so long as the solution has the *same concentration*, and as the *temperature* remains the same.

(*a*) *Effects of the Degree of Concentration.*—The rapidity, however, does not depend only upon the endosmotic equivalent, but also upon the solubility of the substance and its chemical composition. It increases with concentration. When saline solutions diffuse into water, the rapidity with which the salt passes towards the water, as well as that with which the water passes to the salt, increases with the degree of concentration, but not at the same rate. The rapidity with which the water passes to the salt becomes greater, whilst the rapidity with which the salt

passes to the water remains nearly proportional to the degree of concentration. Thus it follows that the more a solution approaches saturation, the greater is the quantity of water which passes in the same time.

(*b*) *Effects of Temperature.*—As the temperature increases, the rapidity of the diffusion also increases, and the rapidity of diffusion increases more rapidly as the temperature rises. This will be seen in the following table, given by Eckhard, showing the rapidity with which common salt passed through the fresh pericardium of an ox in the same time with an increasing temperature :—

Temperature in Degrees C.	Quantity of Common Salt which passed.
8·0,	0·303
9·6,	0·364
13·8,	0·396
18·3,	0·474
22·5,	0·549
26·0,	0·628

(*c*) *Effects of a Mixture of Saline Matters.*—When a solution diffuses through a membrane not only with water, but with a solution of the same or of a different substance, that is, where two solutions of the same substance or of different substances, are on opposite sides of the membrane, the diffusion depends partly on the degree of concentration of the two solutions, and partly upon the chemical properties of the two bodies dissolved. Suppose two solutions of the same substance, but of unequal degrees of concentration ; the more concentrated solution will diminish, while the more dilute will increase in density. In this case, the endosmotic equivalent has a constant value. On the other hand, the rapidity of diffusion will be in the inverse ratio of the difference of concentration of the two fluids present ; that is to say, as the initial difference of concentration of the two fluids diminishes, the rapidity of diffusion will also become less.

(*d*) *Nature of Substance.*—All colloidal substances in solution pass with great difficulty through organized membranes. Such bodies attract water, so that, when their solution diffuses with this fluid, there is a positive current of water. The endosmotic equivalent of these substances is very high, but on the other hand the rapidity of their diffusion is low. Albumin in solution has a stronger endosmotic affinity for saline solutions than for water, and the current of albumin increases very rapidly with concentration of the saline solution. When a mixture of colloidal bodies in a fluid along with some crystalloids is permitted to diffuse into water, none of the colloidal matters passes through the membrane. Thus from a solution of gum, albumin, and sugar, none of the first two will pass, but the sugar will pass through with great ease. There is thus a kind of mechanical separation of the substances. To this general rule, however, there is one exception, namely, when by the diffusion of a substance mixed with colloidal matters, there is produced on the other side of the membrane a liquid toward which the colloidal matter has a great tendency to diffuse. Thus when a mixture of albumin with common salt is exposed to diffusion into water, the salt alone at first passes through the membrane, but when the water on the other side of the membrane contains a certain amount of salt, the albumin then diffuses with considerable intensity. Von Wittich and Funke have studied, as regards diffusion, the differences of solutions of various albuminoids, and they have found that of these bodies, peptones possess the property of diffusibility to the greatest extent.

(*e*) *Effects of Electric Currents.*—A continuous electric current passed through a fluid, in a diffusion apparatus, affects diffusion; the quantity of fluid situated on the side of the negative pole increases, whilst that on the side of the positive pole diminishes. The mass of fluid moves in the direction of the positive current, and the quantity of fluid carried away is always greater as the fluid is easy to move and as the galvanic current is more intense, and the amount is independent of the nature of the surface and of the thickness of the porous plate.

When water is allowed to diffuse with a saline solution, and a galvanic current is directed through the two liquids from the water to the salt, more water will pass to the saline solution than would have passed if the current had not been there. If now the direction of the current be changed, so as to pass from the salt to the water, more salt will then pass towards the water, or, in other words, the osmotic action has been inverted by the galvanic current.

Albumin is found in the body, combined with alkalies, as albuminate of soda or of potash, and it behaves in these compounds as a feeble acid. Suppose that albumin and saline matters are submitted to diffusion with water, and that at the same time a current is passed through the solutions, it will be found (1) if the positive current be directed from the solution of albumin towards the water, salts will pass from the side of the water, and albumin will remain near the positive pole; (2) if the current pass from the water to the albuminous solution, water will pass to the albuminous solution, and albumin will pass through the membrane into the water, and will be deposited near the positive pole. The albumin then behaves as acids do, having passed from the negative towards the positive pole. These interesting facts, ascertained by Von Wittich, indicate the possibility of the physical phenomena of nutrition being affected by a continuous galvanic current, and they suggest many researches of therapeutical importance.

(*f*) *Nature of Organic Membrane.*—The nature of the membrane affects osmotic action. Dry membranes have always a higher endosmotic equivalent than those which are fresh or wet. In comparing organic membranes of different structure, it is found that the dimensions of their pores have an important influence, just as is known to be the case in diffusion through plates of clay. Buchheim has shown that for a membrane with large pores, the endosmotic equivalent of a salt is smaller as the affinity of this salt for water is greater, while, on the other hand, for very dense membranes, the equivalents are proportional to the affinity of the salts for water. The following table by Harzer shows the variations of endosmotic equivalents for different membranes—

	Ox Bladder.	Pig's Bladder.	Ox Pericardium.	Collodion Membrane.
Chloride of sodium, - - -	6·460	4·335	4·000	10·200
Chloride of potassium, - -	5·601	...	3·891	13·632
Sulphate of soda, - - - -	18·764	12·231	8·915	6·097
Sulphate of potash, - - -	13·908	11·700	8·181	4·147

Of all of these membranes, that formed of collodion is the most dense; the bladders of the ox and the pig are both less, and the pericardium of the ox occupies a mean position. The sulphates have a much stronger affinity for water than the alkaline chlorides.

All the phenomena of osmosis may be attributed to the following causes: (1) to a force of attraction of two fluids, the one for the other;

(2) to a relative attraction that the substance forming the membrane exercises upon the two liquids in diffusion—a force which determines the mode in which liquids pass, and the rapidity with which they pass, through small porous canals; (3) to the narrowness of the pores through which the liquids pass; and (4) to the diminution of adhesion of the liquid to the wall of the porous canals, by reason of elevation of temperature. Brücke was the first to attribute the phenomena of diffusion to an attraction between the walls of canals and water.

The importance of applying these facts regarding filtration and osmosis to the phenomena of the nutrition of living tissues is becoming more and more recognized. In the present state of our knowledge, however, it is impossible to follow all the stages of the process, and we can only make general statements.

The connective tissues are surrounded on all sides by fluids such as blood, serous transudations, and lymph. These fluids may be regarded as saline solutions of albuminous matters, or as solutions containing both crystalloids and colloids. The fluids are imbibed by the connective tissues just as they would be taken up by porous substances, by a process which may be called *capillary imbibition*. But when the fluids reach the ultimate tissues in the form of protoplasmic masses, or of protoplasm more or less modified, we have to deal with a homogeneous structure containing no pores. Here an interchange occurs between the fluid and the tissues by a kind of *molecular imbibition*, a process somewhat similar to that by which a membrane, as above stated, allows osmotic phenomena to occur. Molecular imbibition possesses the two following characters: (1) When a tissue imbibes a fluid, it usually increases in volume, but this increase does not always correspond to the quantity of the fluid imbibed, and in some cases actual diminution in size of the mass of tissue may occur after imbibition; and (2) tissues imbibe more distilled water than water containing saline substances, and consequently the fluid which is imbibed by a membrane will be less concentrated than the fluid in which the membrane is immersed. This probably explains why serous effusions are in general less concentrated than the plasma of the blood. (Beaunis.)

The passage into the tissues of part of the plasma of the blood through the thin walls of the capillaries, under the influence of blood pressure, is an example of filtration. The greater the amount of pressure exerted in the vessels, the greater will be the amount of fluid forced through their walls. A similar phenomenon is seen in the separation of water and saline matters from the blood in the Malpighian bodies of the kidney, where the blood passes under considerable pressure through a complicated arrangement of minute vessels. Colloidal

matters, such as albumin, in these circumstances pass with difficulty, and only under strong pressure, whereas, on the other hand, crystalloids pass more readily, and under feeble pressure. Any circumstances, therefore, which increase beyond a certain limit the pressure in the vessels, may be attended by the appearance of albumin in the urine.

It is to be especially noted that in the living body we rarely find the conditions of osmotic phenomena so simple as to consist of a fluid on each side of a membrane, and each under the same pressure. Almost invariably one of the fluids is under a greater pressure than the other, and thus the interchange that takes place must be due partly to filtration and partly to osmotic action. In addition it is possible that there may be some kind of attractive influence exerted by the living tissues themselves, and thus the process by which they obtain fluid pabulum becomes more complicated. A special influence of this kind, to be remembered in studying secretion, by which certain matters are removed from the blood, is the *selective activity* of epithelial cells, of which examples will be given in treating of secretion.

3. Absorption of Gases by Moist-Organized Membranes, and by Fluids.

Diffusion between gases and liquids is not modified in its essential points by the interposition of a wet organic membrane. The *coefficient of absorption* of a gas is the volume dissolved by one volume of water at 0° C. and 760 mm. pressure. The quantity of gas exchanged between two gases on opposite sides of a membrane, one of them being dissolved in a fluid, depends partly on the coefficient of absorption of the gas and partly on the pressure of the gases on opposite sides of the membrane. A gas which possesses a large coefficient of absorption, such as carbonic acid, is absorbed by a wet membrane in greater quantity than oxygen, hydrogen, or nitrogen. The amount of absorption diminishes with elevation of temperature and with diminution of pressure. Absorption of gas by a liquid depends upon the pressure of the gas on the surface of the liquid; the greater the pressure the greater is the amount of gas absorbed by the fluid. When a state of equilibrium between the tension of the gas in the fluid and the pressure of the external gas is attained, absorption ceases. If the pressure of the external gas diminishes, and the tension of the gas in the fluid increases, a portion of the gas dissolved in the fluid will pass into the external gas until a state of equilibrium of pressure is re-established. Again, if a mixture of gases be exposed to a liquid, each gas is dissolved independently of the others, in proportion to its partial pressure. There

is thus a state of continual gaseous exchange established between fluids of the body in which gases are dissolved and the external atmosphere. This process, as we shall hereafter see, is the essential phenomenon in respiration, as it occurs in the ultimate air cells of the lung. It is highly probable that the same physical explanation may be given of the interchange of gases which constantly occurs between the gases dissolved in the blood and those set free in living tissues, as one of the ultimate chemical products which are the result of their vital activity.[1]

[1] Consult, regarding the laws of absorption and of diffusion of gases, Wundt's *Physique Médicale*, p. 201.

SECTION IV.

THE CONTRACTILE TISSUES.

The phenomenon of contractility is exhibited by various cells, such as the colourless corpuscles of the blood, connective tissue corpuscles, the corpuscles of lymph, and the corpuscles of pus. These amœboid cells, when examined with sufficiently high power, manifest a slow circulation of the granules lying in the protoplasm, and also slow changes of form, such as have been already described in treating of that substance. They have been seen to take up into their substance small particles of pigment, such as carmine, indigo, and aniline, and even globules of milk. Contractile bodies may wander through the interstices of the tissues, a phenomenon termed the *migration of cells*. Contractility, however, is more especially manifested by muscular fibre, which is arranged to build up the chief portion of the organs termed the *muscles*, and which also exists in the coats of the hollow viscera and of the vessels.

Our knowledge of the physiological properties of muscles has been largely derived from the study of the living muscles of the frog. These retain their vitality long after the death of the animal. The physiological student should make himself familiar with the general anatomy of the muscles of the hinder limb of the frog, as shown in Fig. 203.

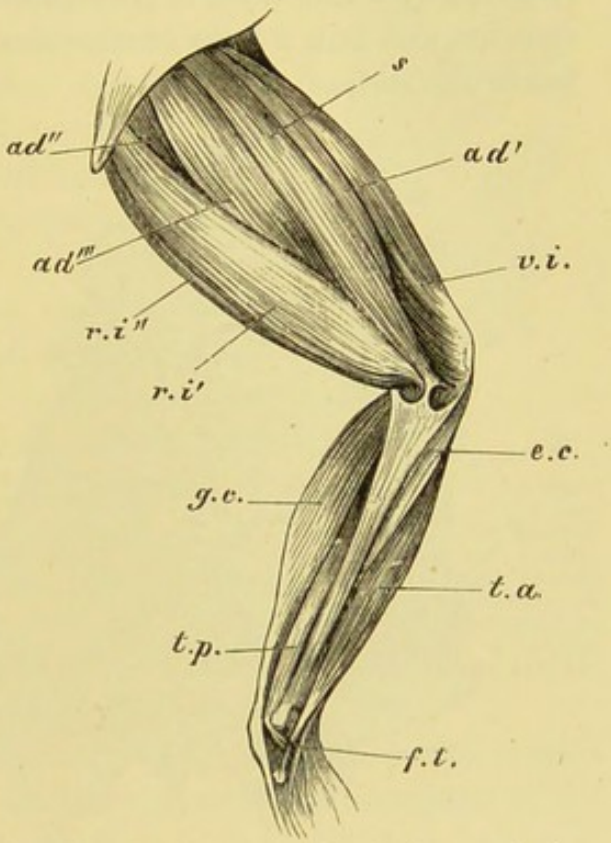


FIG. 203.—Muscles seen on the anterior aspect of a frog's hinder limb. *s*, sartorius; *ad'*, adductor longus; *v.i.*, vastus internus; *e.c.*, extensor cruris; *t.a.*, tibialis anticus; *f.t.*, flexor tarsi; *t.p.*, tibialis posticus; *g.c.*, gastrocnemius; *r.i'.*, rectus internus major or gracilis, or adductor magnus; *r.i''.*, rectus internus minor or cutaneus; *ad'''*, adductor magnus; *ad''* adductor brevis.

Chap. I.—THE SPECIAL STRUCTURE OF MUSCLES AND THEIR RELATIONS TO NERVES.

In the lower forms of invertebrate beings, the contractile elements consist of filiform processes of cells situated in the endoderm or in the ectoderm. Such cells, first described by Kleinenberg, are termed *myo-epithelial cells* (Figs. 204 and 205). The epithelial portion of the cell

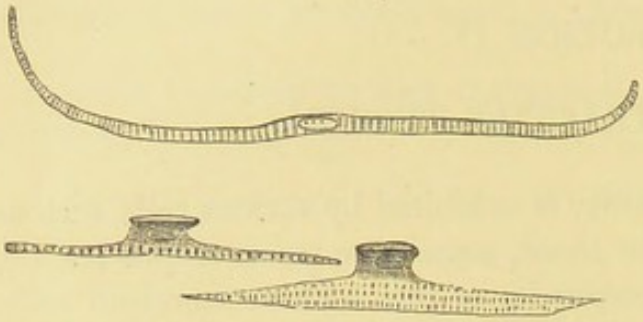


Fig. 204.—Muscle cells from Lizzia Köllikeri. The elongated upper cell is from the circular fibres of the subumbrella; the two lower cells are myo-epithelial cells from the base of a tentacle.

Fig. 205.—Myo-epithelial cells of Hydra. *m*, *m*, muscular fibres.

may become so much reduced in size as to leave only the nucleus surrounded by a thin layer of protoplasm, but the elongated process or fibre remains, and thus a layer of muscular tissue may be formed immediately below the surface.

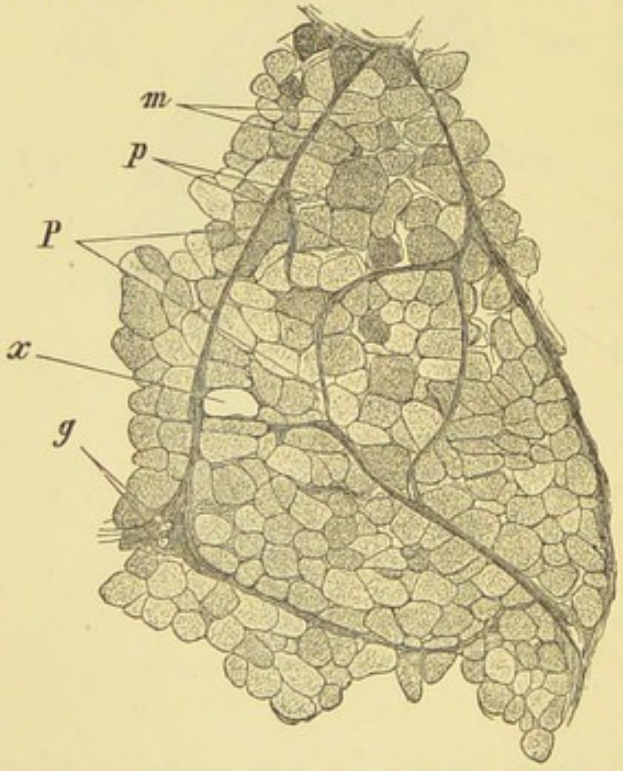


Fig. 206.—Portion of a transverse section of a muscle of the thigh (adductor) of a rabbit, × 60 d. *P*, perimysium internum, containing at *g* two blood-vessels cut across. *m*, muscle fibres; they have in many places separated from each other so that we can perceive *p*, the perimysium of individual muscle fibres. At *x* a transverse section of a muscle fibre has fallen out. (Method No. 65, Appendix.)

According to F. M. Balfour, the muscular elements of the higher groups, including mammals, also belong to the myo-epithelial type. Embryonic muscle cells are at first epithelial cells; they become spindle-shaped; part of the protoplasm is differentiated into striated muscular fibres, and the undifferentiated part seen around the nucleus is the epithelial element of the cells. By further division, the number of fibrils in each cell increases, so that a primitive bundle is formed, which is surrounded by a sheath or sarcolemma. The chief part of the muscular system of the trunk is formed from the mesoblast, which, as

already shown, is derived primarily from the epiblast, and thus the epithelial origin of muscle becomes apparent.

The structure of muscular tissue has already been described in treating of cells, but before considering the functions of the tissue, we shall give an account of the mode of union of the fibres so as to form *muscles*, the mode of connection of the fibres with tendons, the arrangement of the blood-vessels, and the manner in which the nerves terminate in muscle.

(1) *Formation of a Muscle.*—The muscular fibres run side by side, and they are held together by sheaths of loose connective tissue. Neighbouring muscular fibres never come into direct contact, but each individual fibre is surrounded by its own sheath, the sarcolemma. A bundle of such fibres may be enclosed by a sheath of connective tissue, termed the *perimysium internum*, and a number of such bundles constitutes a muscle, which is surrounded by a thicker layer or sheath of connective tissue, the *perimysium externum*. The internal perimysium communicates with the external at many places, and thus a transverse section of a muscle shows the appearance depicted in Fig. 206. The perimysium consists of fibrillar connective tissue and elastic fibres. It occasionally contains fat cells, and it is the bearer of the nerves, blood-vessels, and lymphatics. The perimysium internum has ramifying upon it only capillaries and the delicate terminations of nerves.

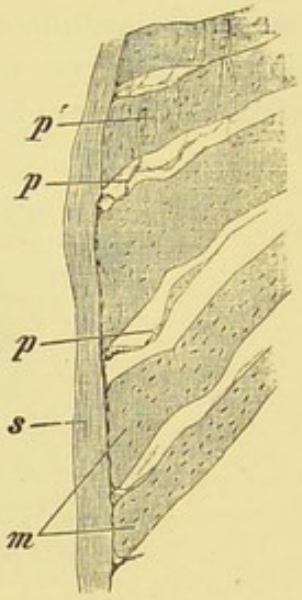


FIG. 207.—Portion of a longitudinal section of the gastrocnemius muscle of a frog, × 50 d. *s*, tendon; *p*, perimysium of a single muscle fibre, seen from the side; at *p'*, seen from the surface as transverse lines; *m*, muscle fibres. (Method No. 66, Appendix).

(2) *Connection of Muscular Fibres with Tendon.*—The perimysium of individual bundles of fibres becomes continuous with the connective tissue fibres forming the tendon, and the sarcolemma ends at the termination of the striated muscular fibre (Fig. 207).

(3) *Blood and Lymph Vessels.*—The blood-vessels of striated muscles are very numerous. The capillaries are amongst the most delicate in the human body, and they form a netting of elongated rectangular meshes (Fig. 171, p. 312). The capillaries are always outside the sarcolemma. The lymphatics, also numerous, run alongside of the ramifications of the smaller blood-vessels.

(4) *Involuntary or Non-Striated Muscles.*—Involuntary or non-striated muscle fibres are bound very firmly together by a structureless cement substance. Septa, or partitions between bundles, composed of connective tissue, occur only at very great intervals (Fig. 208). The

union is either such as to form parallel fibrous membranes, as in the muscular layers of the intestinal canal, or a complicated network of fibres, as in the wall of the urinary bladder or uterus. The larger blood-vessels run in the septa of connective tissue; the capillaries, on the contrary, penetrate between the individual fibres and form an elongated capillary network. The lymphatics, pursuing a similar course, are present in considerable numbers.

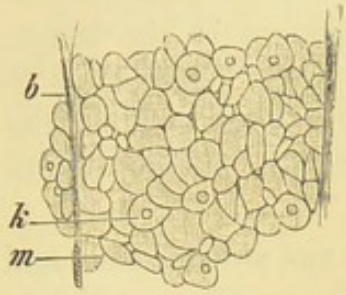


FIG. 208.—Portion of a transverse section of the circular muscular layer in the human intestine, × 560 d. *m*, smooth muscle fibres the nuclei of which are transversely divided at *k*. *b*, septum of connective tissue. (Method No. 67, Appendix.)

(5) *Termination of Nerves in Muscle.*—The nerves, ramifying over, or among the fasciculi, of a muscle, divide and subdivide until numerous minute branches are formed. These form a plexus, the *intermuscular nerve-plexus.* From these small branches, very minute branches arise, each consisting of one single medullated nerve-fibre, and these end in individual muscular fibres. A nerve, when it enters a muscle, does not contain as many fibres as there are fibres in the muscle. In the muscle, the axis cylinder

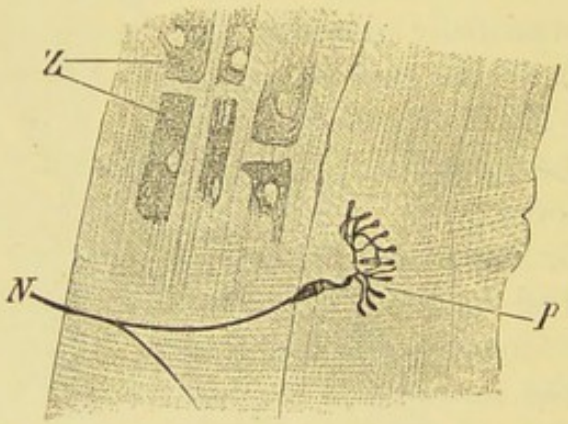


FIG. 209.—Motor nerve-ending from the muscular fibre of the intercostal muscle of a hedgehog, × 240 d. The transverse striation of both fibres is not everywhere visible. On the left muscle fibre there are flat connective tissue cells, *Z* showing clear nuclei. *N*, medullated nerve fibres subdividing; the marrow is not recognizable by the method of making the preparation; *P*, end plates. (Method No. 68, Appendix.)

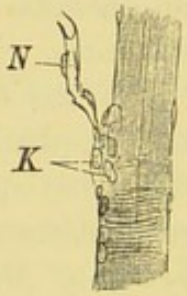


FIG. 210.—Motor nerve-ending of a muscular fibre in one of the muscles of the eyeball of a rabbit, × 240 d. *N*, medullated nerve fibre; *K*, nuclei of the disc. The transverse striation of the fibre is seen only in the lower half of the figure. (Method No. 69, Appendix.)

divides again and again until there are as many nerve fibres as muscular fibres. When the ultimate nerve fibre approaches the muscular fibre, the nerve fibre loses the white substance of Schwann, and the axis cylinder of the nerve fibre pierces the sarcolemma and terminates in the muscular fibre, in what is termed a *motor end plate.* These end plates vary in form and general appearance. Sometimes they seem

to consist of very minute fibres, formed by the splitting up of the axis cylinder, anastomosing so as to form a network, but usually they take the form of irregularly shaped granular masses or discs, containing numerous vesicular nuclei.

The end plates in the muscle of mammalia range in diameter from 39 to 60 μ, while they also vary in thickness. The nuclei, from 4 to 9 μ in breadth, often contain several nucleoli. It is important to note the intimate union existing between the nervous and muscular tissues. Indeed the one blends into the other. Kühne has pointed out that the axis cylinder spreads out into a pale slightly granular substance having truncated processes, and that the central part of the axis cylinder forms a kind of neural eminence or swelling within this expansion. (See Figs. 211 and 212.) It is not improbable that the

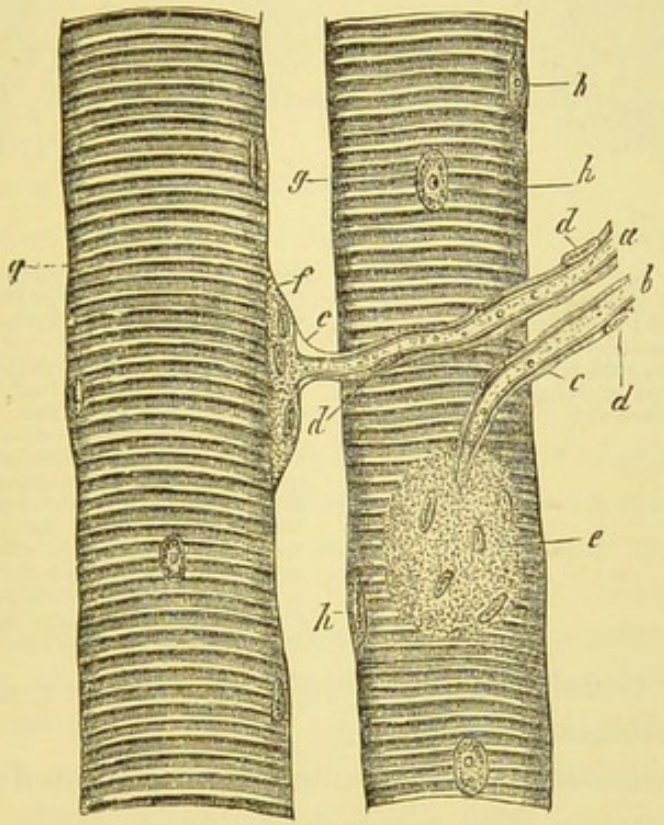


FIG. 211.—Two muscular fibres from the psoas of a guinea-pig, showing the terminations of the nerves. *a*, *b*, the primitive fibres with their transition into the terminal plates, *e*, *f*; *c*, neurilemma with nuclei, *d*, *d*, continuous with the sarcolemma, *g*, *g*; *h*, *h*, nuclei in muscle.

finest fibrils may end in the anisotropous portion of the muscular fibre. In many fishes and amphibians no such end plates exist, and in their stead we find the axis cylinder, after it has perforated the sarcolemma, dividing into delicate fibrils which course longitudinally within the muscular fibre, showing isolated nuclei here and there, and ultimately blending with the contractile substance of the fibre.

The blending of the neural and muscular structures, as shown in certain invertebrates, is also well illustrated by Carnoy in Fig. 213.

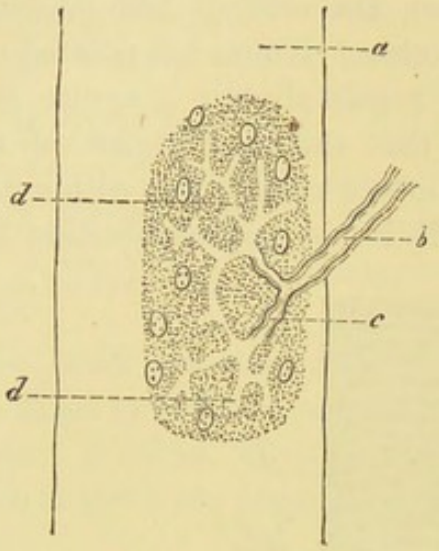


FIG. 212.—A muscle fibre, *a*, from a lizard; *b*, nerve fibre; *c*, dichotomous division in the end plate, with transition into the true end-organ or plate of Kühne, *d*, *d*.

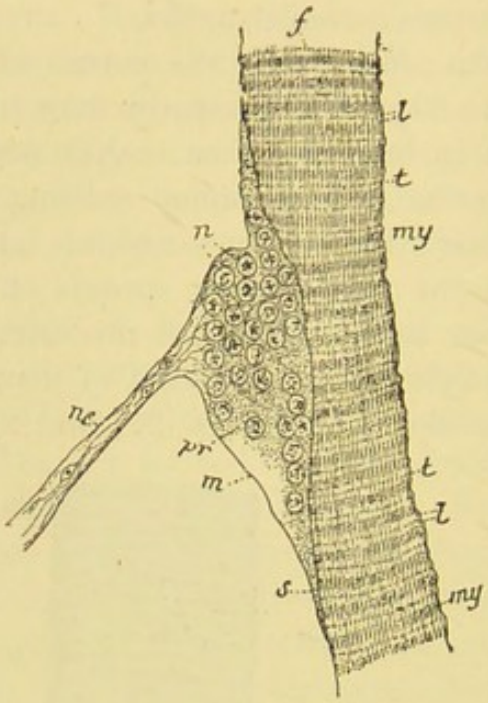


FIG. 213.—Fusion of a nerve with a muscular cell in a chrysalis of a fly, showing the so-called *plaque* or eminence of Doyère. *ne*, nerve, showing small polar cells; *pr*, protoplasmic mass, formed by the fusion of the reticulum or network in the muscular fibre with the part of the nerve coming into contact with it, showing numerous nuclei, *n*; *m*, *s*, neurilemma fused with sarcolemma; *t*, striæ formed by the transverse fibres of the muscular network; *f*, longitudinal striations; *my*, myosin in the meshes of the network; *l*, lighter coloured portions of the fibre formed of a reticulum, the meshes of which do not contain myosin.

Muscles also contain sensory nerve fibres, but their mode of origin has not been satisfactorily made out.

The nerve terminations in involuntary or non-striated muscle consist of delicate networks, from which more delicate fibrils arise, and the latter have been satisfactorily traced into the interior of the fusiform cells forming this variety of muscular tissue.

CHAP. II.—THE CHEMICAL CONSTITUTION OF MUSCULAR FIBRE.

Muscular fibre is composed of two parts—(1) *muscle-plasma*, which may be separated by pressure from muscle removed from a recently killed cold-blooded animal, as a neutral or slightly alkaline gelatinous fluid; and (2) *an insoluble residue*, consisting probably of sarcolemma, nuclei, fat, and matters derived from the vessels, nerves, and lymphatics

mixed with the muscular tissue. The sarcolemma is unaffected by acetic acid, but it is dissolved slowly when heated with dilute solutions of the acids or alkalies. Hence it is believed to be of the nature of elastin. Nothing is known regarding the chemical composition of the disdiaclasts as distinguished from the fundamental matter of the fibre. The *muscle-plasma* may be obtained by compression at a low temperature (7° to 10° C.) of muscles deprived of blood. The plasma is yielded by the isotropous portion of the fibre. It is an alkaline, colourless, or faintly yellow fluid or syrup containing various albuminates. When obtained pure, it speedily coagulates and separates into two portions—*muscle-clot* or *myosin* and *muscle-serum*. Coagulation is accelerated by heat, distilled water, weak acids, ammonia, etc., and it is retarded by cold. Myosin is an albuminous body, soluble in 5 to 10 per cent. solutions of common salt or in weak hydrochloric acid. Acids, such as ·1 per cent. of hydrochloric acid, convert it into an allied substance named *syntonin*, which is not soluble in common salt nor in a solution of sulphate of magnesia.

Muscle-serum contains the following substances—

(1) *Albuminates*, such as albuminate of potash, which is precipitated on heating from 20° to 45° C., and by neutralizing the alkaline fluid. This proteid is not myosin, inasmuch as it is not soluble in weak solutions of common salt. There exists also an alkaline potassium albuminate precipitated from muscle-serum when it is rendered acid. Serum albumin is precipitated when the fluid is rendered acid and heated up to 75° C. (Kühne. For Halliburton's results, see Appendix II.)

(2) Traces of *ferments*, namely, pepsin, ptyalin, and a special muscle ferment.

(3) *Colouring matter*, either hæmoglobin or myo-hæmatin. (See p. 138.) The colouring matter is said to exist in the isotropous portion of the fibre.

(4) Various *nitrogenous matters*, all of which have been discovered in extract of meat, namely, lecithin, creatin, creatinin, xanthin, hypoxanthin, taurin, urea, and inosinic and uric acids.

When muscular fibre is allowed to macerate in cold water, the water becomes red, and it dissolves some of the constituents of muscle. If such an extract is boiled, all soluble proteids are coagulated, and if the fluid is then filtered, a liquid is obtained holding in solution the non-nitrogenous matters and also the soluble nitrogenous matters above enumerated. A watery extract thus freed from proteids, and then evaporated till a thick viscid mass is obtained, is *extract of meat*. The following are the average percentage amounts of some of the nitrogenous substances obtained from muscle :—Creatin, in frog's muscle, ·263 ; carnin, in Liebig's extract, 1·00 ; uric acid (Haycraft), ·01 ; and urea, in rabbit's muscles (Picard), 3·00 (?). Traces of creatinin, sarcin, xanthin, inosinic acid, and taurin have also been detected, but no statement can be made as to the quantity usually present.

(5) Various *non-nitrogenous matters*, such as fats, the two lactic acids (ethylidene-lactic acid or para- or sarco-lactic acid and ethylene-lactic acid), inosite or muscle sugar, glycogen or animal starch, dextrin, and glucose. The amount of glycogen has been found to vary from ·43 per cent. in the muscles of a frog to from ·47 to ·95 in those of a rabbit. Inosite has been found only to the small amount of ·003 per cent. Small quantities of volatile acids, such as formic, acetic, and butyric acids, have also been found. In dead muscle, or in muscle which has been excited to frequent contractions in short intervals of time, lactic acid, or the variety called para- or sarco-lactic acid, has always been found. It has been supposed to originate from the sugar or glycogen present in the muscle.

(6) The principal *salts* are the phosphates of the alkalies and alkaline earths, chloride of potassium, and a small quantity of chloride of sodium and of sulphates of the alkalies. The ash of muscle amounts to 1 or 1·5 per cent. of the total weight.

(7) *Water*, to the extent of from 74 to 80 per cent.

(8) The following *gases* have been separated from 100 parts of muscle —Carbonic acid, 14·40; nitrogen, 4·90; and oxygen, ·09—total, 19·39. It is probable that much of the carbonic acid may have arisen from decomposition.

Von Bibra gives the following rough quantitative analysis of muscle from different species of animals in 1000 parts—

	Man.	Ox.	Bird.	Fish.	Frog.
Water, - - -	745·5	776·0	717·6	797·8	804·3
Solids, - - -	255·5	224·0	282·4	202·2	195·7
Albumin, - - -	19·3	19·9	26·8	23·5	18·6
Gelatinous matter, -	20·7	19·8	12·3	19·8	24·8
Alcoholic extract, -	37·1	30·0	41·2	34·7	34·6
Fat, - - - -	23·0	—	25·3	11·1	1·0
Vessels, etc., - -	155·4	154·3	176·8	113·1	116·7

Fresh beef, on calcination, has yielded 1·46 to 1·63 per cent. of ash, in 100 parts of which there are 35·94 of potassium, traces of sodium, 3·31 of magnesium, 1·73 of calcium, 4·86 of chlorine, ·98 of iron, 34·36 of phosphoric acid, 3·37 of sulphuric acid, 2·07 of silicic acid, and 8·02 of carbonic acid.

Glycogen or animal starch abounds in the muscles of the embryo and of the fœtus, and the muscles of young animals contain more water than those of animals advanced in age. The amount of fat also increases with age.

Living muscle in a state of rest, as already mentioned, is slightly alkaline from containing phosphate of potash, K_2HPO_4. After a period

of activity it becomes acid, partly by the formation of free lactic acid from carbo-hydrates, and, according to some authorities, partly from the presence of the acid phosphate of potash, KH_2PO_4.

Chap. III.—ELECTRICAL APPARATUS EMPLOYED IN THE STUDY OF MUSCLE.

In studying the phenomena of muscular contraction, we require to stimulate the living muscle or the nerve supplying it. The agent most commonly employed as a stimulus, because it is the most effective and the most convenient, is electricity. This agent may be obtained by various appliances, and it may be employed in different ways. The student should, at this stage, acquire a practical acquaintance with some of these methods, so that he may be able to use electrical apparatus intelligently, both in the study of physiology and in the treatment of disease.

1. General Statement and Definitions.

When a body is electrified or charged with electricity the measure of its electrification is called its electrical *potential*. Thus, if a plate of metal such as zinc or copper is immersed in a solution of sulphuric acid, there is a difference of electrical potential between the metal and the liquid. This difference of potential is not the same for zinc as it is for copper, and hence when a plate of zinc and a plate of copper are both immersed in the same liquid, without touching each other, there is a difference of potential between the copper and the zinc. Again, when a piece of zinc is in contact with a piece of copper, there is a difference of potential between the zinc and the copper. If, now, we suppose the plates immersed in the liquid to be put in contact outside by a wire, say of copper, as in Fig. 214, there will be a difference of potential between the zinc and the liquid, then a smaller difference of opposite sign between the liquid and the copper, and finally a difference between the copper and the zinc. The algebraic sum of these differences is not zero, and there results, in consequence of the tendency of electricity to flow from points of high to points of low potential, a *current* of electricity which, by a common convention, is

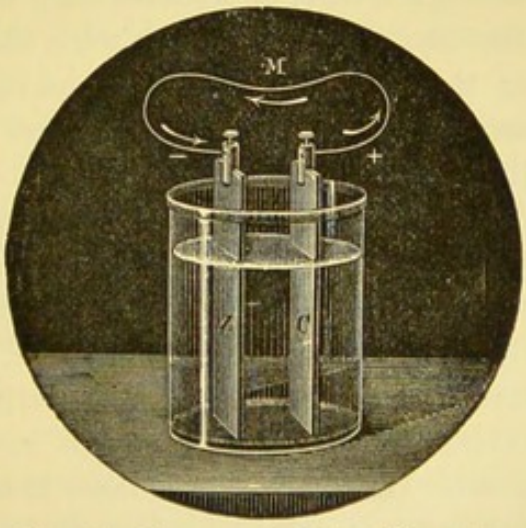


Fig. 214.—Diagram of a Voltaic Element.

said to flow from the copper to the zinc through the external circuit or wire M, and from zinc to copper through the liquid. This flow of electricity would reduce the whole circuit to the same potential were it not for the fact that the passage of electricity through the liquid decomposes it, and by so doing generates electricity, which serves to keep the current flowing. The energy required to keep the current flowing through the circuit is supplied by the chemical action, the result of which is the production of sulphate of zinc at the expense of the zinc plate and the liberation of a corresponding amount of hydrogen from the sulphuric acid ($H_2SO_4 + Zn = ZnSO_4 + H_2$). The hydrogen is set free at the copper plate. The arrangement just described is called a *voltaic element;* the copper and zinc plates being commonly termed the *plates*, and the liquid the *electrolyte*. The copper plate is called the *positive pole*, and the zinc plate the *negative pole*, and the wires forming the external circuit, or joining the two poles of the cell to any piece of apparatus, are called the *electrodes*.

The algebraic sum of the contact differences of potential above referred to is called the *potential* of the cell, but when the circuit is closed and a current caused to flow, it is more commonly called the electromotive force (e. m. f.) of the circuit, *i.e.* the electrical force or pressure which causes the current to flow. The electromotive force in the circuit of a voltaic cell producing a current is the same as the difference of potential between the plates when no current is flowing. It is not the same as the difference of potential between the poles of the cell when the current is flowing, in consequence of another property of the solids and liquid forming the circuit, namely, their electrical resistance. When, for example, a current of electricity is made to flow through a wire, it is found that, with the same difference of potential between the ends of the wire, the strength of the current is inversely as the length of the wire, and directly as its cross section. Thus, the same e. m. f. will cause a stronger current to flow in a thick wire than in a thin one of the same length, or in a short wire than in a long one of the same thickness. This property which the wire possesses of opposing the electric current is called its *electrical resistance*. In virtue of this resisting property, which is possessed in a greater or less degree by all substances, a certain e. m. f. (*a*) is required to cause the current to flow through each part of the circuit of the cell, and hence only a part (*b*) is available for the external wire circuit. This part (*a*) is greater and greater the longer and thinner the wire, M, and it is equal to the whole potential of the cell when the resistance of the external part is infinitely great compared with that of the liquid, that is, when no current is flowing.

The relation between the current strength, C, the e. m. f., E, and the resistance, R, was first correctly given by Ohm, and is known as *Ohm's law.* Algebraically, it may be put

$$C = \frac{E}{R}.$$

As stated above, the resistance of any conductor is directly proportional to its length, l, and inversely proportional to the area of its cross section, a. If we call the specific resistance S, that is the resistance of unit length of a wire of the conductor, the cross sectional area of which is unity, we may write

$$R = \frac{Sl}{a}.$$

A battery is an arrangement or apparatus for producing electricity. In its simplest form it is a *cell* or *element;* several elements constitute a *battery.* Elements are put up in series or for tension when the positive of the first is united to the negative of the second, and the positive of the second to the negative of the third, and so on, as shown in Fig. 215.

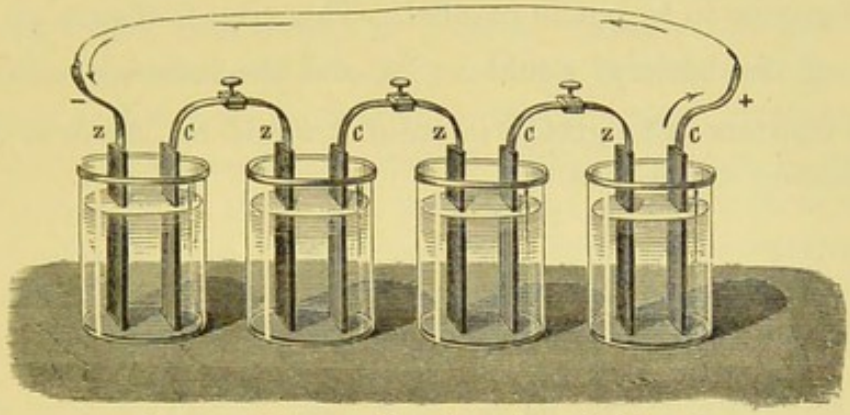


FIG. 215.—Battery of four elements united in series.

On the other hand, elements are united for *quantity* or *surface* when all the positive poles are joined together and all the negative poles are also joined.

The e. m. f. of a battery depends on the kind of chemical operations occurring in it, and to a small extent on the temperature at which they occur. A battery has always a certain amount of internal resistance, b, depending on its size and form and the liquids used. If we increase the size of the plates and bring them close together, the resistance is diminished. The e. m. f. of a battery and its internal resistance, r, are its *constants.* It is important to notice (a) that n elements in series behave like one element, of which the e. m. f. $= n\,e$, and the internal resistance $= n\,b$; and (b) that n elements, put up parallel or for quantity, act like one single element of the same electromotive force, e, and of which the internal resistance $= \frac{b}{n}$.

If a battery becomes weak, this is due to *polarization*, caused by an accumulation of hydrogen or other substance that may be deposited on the positive plate. This not only increases the resistance but also produces an electromotive force opposing the electromotive force of the cell, and thus polarization diminishes the e. m. f. of the cell. Many of the arrangements in different elements are intended to remove the hydrogen or other film and thus get rid of polarization.

If r be put for the external resistance of a galvanic circuit, then the total resistance R in the circuit will be $r + n\,b$, and the total e. m. f. $n\,e = E$. Hence the current flowing would, by Ohm's law, be

$$\mathrm{C} = \frac{n\,e}{r + n\,b}.$$

Now it is clear from this equation that if b be at all high compared with r, very little advantage will be gained by using a large number of cells in series. Let us consider what would be the effect of grouping the n cells, partly in series and partly in parallel. Divide the n cells into x groups and suppose the n/x cells of each group to be put in series, and the x groups to be put in parallel. The e. m. f. of each group, and therefore of the battery, would be $\frac{n}{x}e$, and the internal resistance $\frac{n}{x}b$. The total resistance of x groups in parallel would be $\frac{n}{x}b/x$ or nb/x^2. The current flowing would then be

$$\mathrm{C} = \frac{\frac{n}{x}e}{r + n\,b/x^2} = \frac{n\,e}{r\,x + n\,b/x}.$$

The right hand side of this equation may be written

$$\frac{e}{\frac{r\,x}{n} + \frac{b}{x}} \quad \text{or} \quad \frac{e}{2\sqrt{\frac{r\,b}{n}} + \left(\sqrt{\frac{r\,x}{n}} - \sqrt{\frac{b}{x}}\right)^2}.$$

Now this quantity, and therefore C, is clearly greatest when the second term of the denominator is zero, as the first term is constant. Hence the best result will be obtained when the cells are so arranged that

$$\frac{r\,x}{n} = \frac{b}{x}, \text{ or when } x^2 = \frac{n\,b}{r}.$$

This gives the proper number to take for x, and although it may not be always possible to divide the number of cells in the battery by this number, the nearest number to x which will divide n without a remainder should be taken. It is interesting to notice that the equation

$$x^2 = \frac{n\,b}{r},$$

may be written

$$r = \frac{n\,b}{x^2},$$

which shows that when the battery is arranged for best effect the internal resistance of the battery is equal to the external resistance in the

circuit. It must be kept in mind, however, that there is no advantage but a disadvantage in increasing the resistance of a given battery in order to make it equal to the external resistance. This rule can only be applied to such large batteries of high resistance cells that the resistance of the cells in series is great compared with the external resistance. An example may make the meaning of the above investigation clearer. Suppose we have a battery of 20 cells, the internal resistance of each of which is 1 ohm, and wish to pass as strong a current as possible through a resistance of 5 ohms. We have here $n = 20$, $b = 1$, and $r = 5$, and hence—

$$x^2 = \frac{nb}{r} = \frac{20 \times 1}{5} = 4, \text{ or } x = 2.$$

That is to say, the best result will be obtained by dividing the battery into two batteries of 10 cells each, and joining these two batteries parallel to each other—the terminal zinc of the one to the terminal zinc of the other, and the terminal copper of the one to the terminal copper of the other. The resulting battery is then equivalent to a battery of 10 cells, each having an internal resistance of half an ohm. The number of cells in the battery is thus halved, and the internal resistance of each cell is also halved, so that the total resistance of the battery is only one quarter of what it would be if the 20 cells were joined in series, while the e. m. f. is half what it would be in that case. This is an advantage in the case supposed, because the external resistance is only 5 ohms, while the internal resistance of the 20 cells in series would be 20 ohms. The current through the resistance, with all the cells in series, would be $c = \frac{20e}{25}$ or $\frac{4}{5}e$, and the currents through the resistance, with the battery arranged as 10 double cells, would be $c = \frac{10e}{10}$ or e, that is, one fifth stronger. Had there been 30 cells in the battery, it might have been arranged either as 10 triple cells or as 15 double cells with about equal advantage, because, in this case, $x^2 = \frac{30}{5} = 6$ or $x = \sqrt{6}$, which is about equally distant from 2 and from 3, but slightly nearer 2. The next number of cells, which gives an exact integer for x when $b = 1$ and $r = 5$, is 45, which gives $x^2 = 9$ or $x = 3$. Hence, for such a case, it would be best to arrange the 45 cells as 15 triple cells, and so on for other cases.

Electrical Measurements.

The system of electrical measurements now in use is founded on the centimetre as the unit of length, the gramme as the unit of mass, and the mean solar second

as the unit of time. This system is commonly referred to as the C. G. S. system. The electromagnetic units need only be referred to in this work; they are based on the forces exerted between two magnetic poles. Beginning with the unit of force or the dyne, which has already been defined (p. 10), the following description will indicate the relation between the different units—

1. *Unit Quantity of Magnetism*, or the unit magnetic pole, is that quantity of magnetism which, when placed at a distance of one centimetre from an equal quantity of magnetism, repels it with the force of one dyne.

2. *Unit Magnetic Field* is such a magnetic field that when unit quantity of magnetism is placed in it, it is acted on by a force of one dyne. The unit magnetic field is thus the field produced by unit quantity of magnetism at a distance of one centimetre.

3. *Unit current of electricity* is such a current that when made to flow round a circle of one centimetre radius, it produces a magnetic field of as many units intensity at the centre of the circle as there are centimetres in the length of the circumference of the circle. The unit current flowing in a circle of one centimetre radius would therefore act on unit quantity of magnetism placed at the centre of the circle with a force of 2π dynes.

4. *Unit quantity of electricity* is the quantity conveyed by unit current in one second.

5. *Unit difference of potential* is the difference of potential between the ends of a conductor of one centimetre length, when it is held with its length at right angles to the direction of magnetic force in a magnetic field, and kept moving uniformly with a velocity of one centimetre per second in a direction at right angles to its own length and the direction of the magnetic force in the field.

6. *Unit electromotive force* is produced in a closed circuit, if one centimetre of its length is held in the manner and moved in the direction and with the velocity described in the last definition.

7. *Unit resistance* is the resistance which, when cted on by unit electromotive force, allows unit current to flow.

8. *Unit capacity* is the capacity of a body which requires unit quantity of electricity to raise its potential by units.

In practice, the magnitudes of the units above defined were found inconvenient and certain multiples and sub-multiples have been adopted. The units of any importance for physiological purposes are the unit of current, the Ampère; the unit quantity of electricity, the Coulomb; the unit electromotive force, the Volt; the unit resistance, the Ohm; and the unit capacity, the Farad or Microfarad.

The *Ampère* is equal to one tenth of the c. g. s. unit of current, or approximately the current of an ordinary Daniell through an Ohm; the *Coulomb* to one tenth the c. g. s. unit of quantity, or the quantity of electricity conveyed by an Ampère in one second; the *Volt* to 100,000,000 times the c. g. s. unit of e. m. f., or approximately the e. m. f. of a Daniell's cell; the *Ohm* to 1,000,000,000 times the c. g. s. unit of resistance, or the resistance of a column of pure mercury at 0° C. 1 mm. square and 1,050 mm. in length; the *Farad* to $\frac{1}{1000000000}$ of the c. g. s. unit of capacity; and the *Microfarad*, which is the common practical unit, is the millionth part of a Farad.[1]

[1] The terms Ampère, Coulomb, Volt, etc., are often written without use of capital letters, thus ampère, coulomb, volt, etc.

2. Voltaic Elements.

Many varieties of voltaic elements may be employed for physiological purposes. The following are the most common—

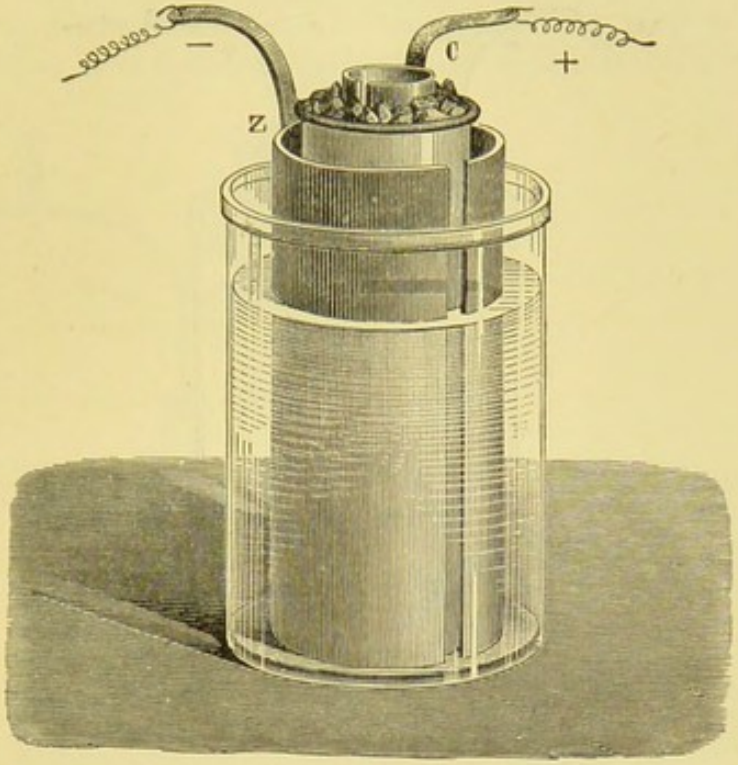


Fig. 216.—Daniell's Element.

(1) *Daniell.* There are various forms of this convenient element. The one shown in Fig. 216 consists of a glass jar containing a solution of sulphuric acid, 1 to 7 of water; in this is placed a roll of amalgamated zinc, Z; within the roll there is a porous earthenware jar containing a rolled piece of copper, C, immersed in a saturated solution of sulphate of copper. Sulphate of zinc is formed by the action of the sulphuric acid on the zinc and hydrogen is set free. The hydrogen passing through the porous vessel reduces part of the sulphate of copper, depositing copper on the copper plate, and the sulphuric acid thus liberated passes into the solution of the acid in the outer jar, and thus makes up for the loss of the acid caused by the continuous formation of sulphate of zinc. Thus, the current is very constant so long as an ample supply of crystals of sulphate of copper is placed in the inner compartment and so long as the zinc plate in the outer vessel lasts. The e. m. f. is = 1·072 volt. The positive electrode is connected with the copper and the negative electrode with the zinc. This element is useful when it is desirable to have a tolerably constant current.

(2) *Bunsen.* This element consists of a vessel containing dilute sulphuric acid, 1 to 7 of water, in which is a roll of amalgamated zinc; in this is a porous earthenware vessel containing a rectangular block of carbon in strong fuming nitric acid (Fig. 217). The hydrogen set free in the outer compartment by the action of the sulphuric acid on the zinc is absorbed by the nitric acid, and thus polarization is to a large extent prevented. Its e. m. f. is 1·9 volt. The positive electrode is con-

nected with the carbon and the negative with the zinc. This element is inconvenient for ordinary physiological purposes on account of the acid fumes given off when it is in action.

(3) *Grove.* This element, of which a small and convenient form is shown in Fig. 218, is similar to Bunsen's element, except that a strip of platinum, P, takes the place

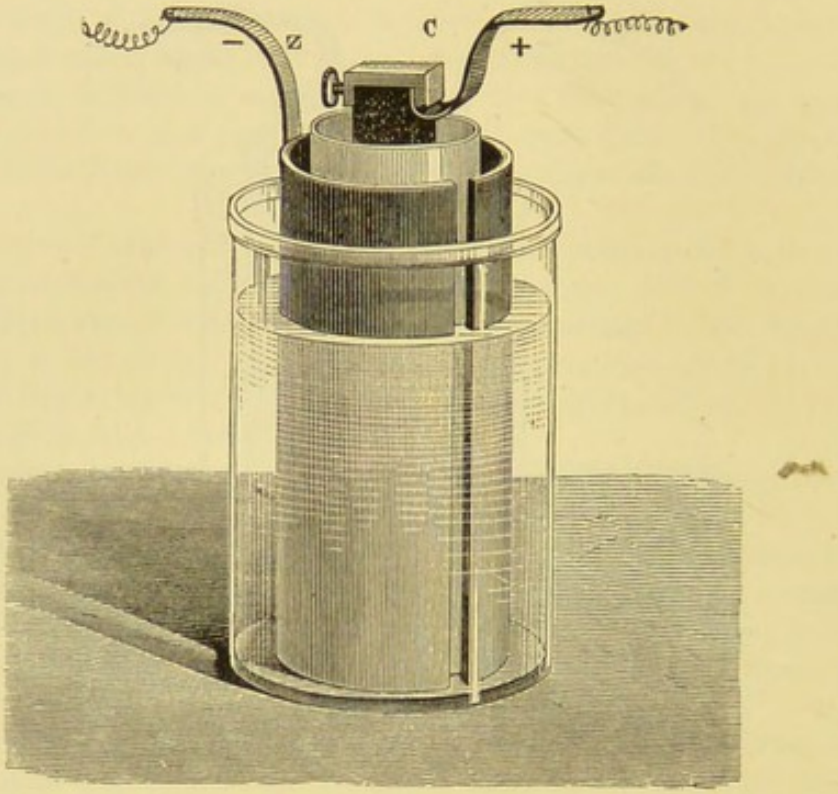


Fig. 217.—Bunsen's Element.

of the carbon in the fuming nitric acid. Its e. m. f. is 1·96 volt. The positive electrode, K, is connected with the platinum and the negative electrode with the zinc, Z.

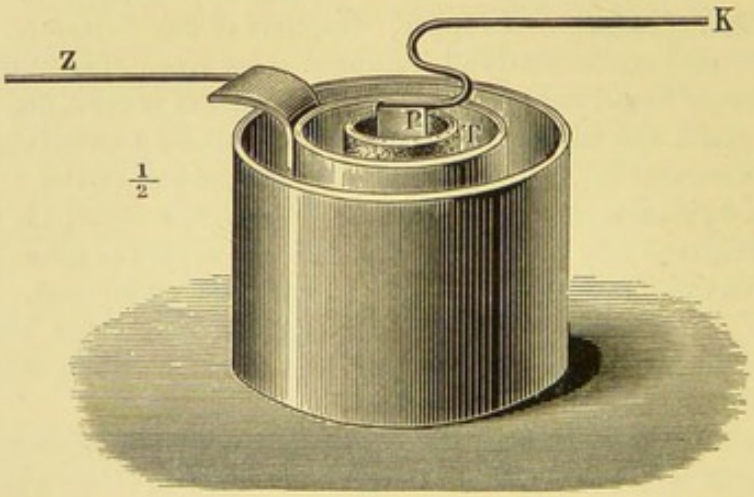


Fig. 218.—Grove's Element.

(4) *Leclanché.* This element, of which there are various forms, consists of a glass vessel or jar, containing a saturated solution of sal-ammoniac in water, in

which is immersed a roll of amalgamated zinc, Z (Fig. 219). In this is placed a jar T, containing a plate of carbon, K, surrounded with small pieces of carbon mixed with black oxide of manganese. Its e. m. f. is 1·48 volt. The positive electrode, B, is connected with the carbon and the negative electrode with the zinc, C. The Leclanché is a very convenient element, united in series, for medical purposes.

(5) *Gaiffe.* This is a small and convenient form of element much used in the construction of batteries employed by physicians. It consists of zinc (not amalgamated) in a 5 per cent. solution of chloride of zinc, and of silver surrounded

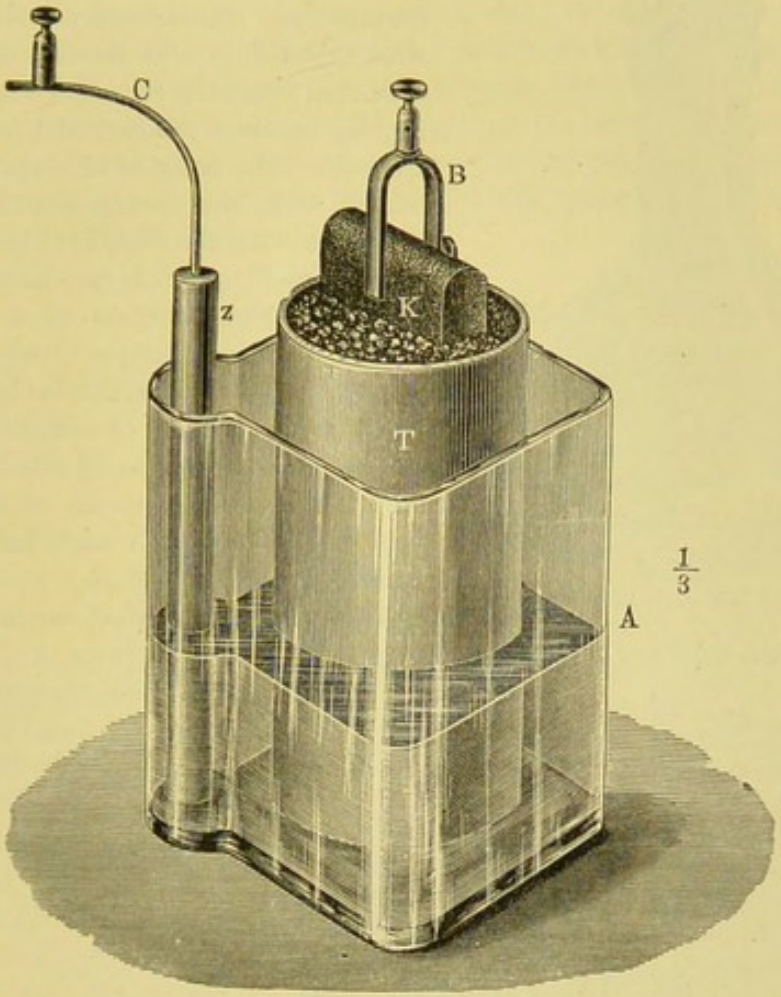


Fig. 219.—Leclanché's Element.

by chloride of silver. Its e. m. f. = 1·02 volt. The positive electrode is connected with the silver and the negative electrode with the zinc.

(6) *Smee.* Smee's element consists of two plates of amalgamated zinc, having a plate of platinized silver between them, the whole being immersed in a solution of sulphuric acid, 1 to 7 of water. The positive electrode is connected with the platinized silver and the negative electrode with the zinc. The e. m. f. is ·47 volt.

(7) *Marie-Davy.* This is an element much used in the construction of medical batteries. It consists of amalgamated zinc in slightly acidulated water and of carbon in a paste of mercurous sulphate. Its e. m. f. is 1·52 volt. The positive electrode is connected with the carbon and the negative electrode with the zinc.

(8) *Grenet* or *Bichromate.* This is an element of great convenience. It is formed of one plate of amalgamated zinc placed between two plates of carbon, and the whole is immersed in a fluid having the following composition—Water, 100 parts; sulphuric acid, 25 parts; and bichromate of potash, 10 parts. The zinc plate attached to the rod, B, may be pulled up out of the fluid when the element is not required, so that the fluid does not require to be changed frequently. Immediately after changing with fresh fluid this element may give an e. m. f. of 2·03 volts for a few seconds, but it rapidly becomes weaker, and falls to about 1·8 volt. The positive electrode, E, is connected with the carbon and the negative electrode with the zinc, D (Fig. 220).

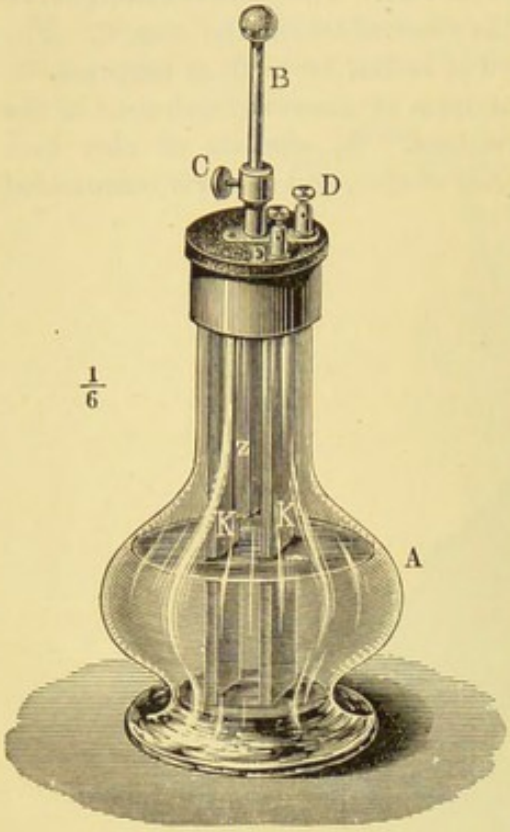


Fig. 220.—Grenet's Element.

(9) *Noë-Dörffel Thermo-Electric Battery or Pile.* This consists of a number of thermo-electric junctions concentrically arranged. One of the metals is German silver, the other is an alloy of antimony and zinc, the exact composition of which is known only to the manufacturers of the element. One of the metals of each junction is expanded to form a cylinder, so as to secure a uniform temperature at one junction. The other junction is heated by placing the apparatus over the flame of a spirit lamp. The elements are arranged in series to form a battery, as shown in the figure. Each element has an e. m. f. of $\frac{1}{16}$ volt, and a battery of 20 elements has therefore an e. m. f. of 1·25 volt. It is remarkable that such an arrangement transforms into electricity less than one per cent. of the heat energy given out by the source of the heat used in generating the current. For physiological purposes this thermo-electric battery is very convenient, as it is readily put in working order and there is no risk of injury to sensitive structures like nerves from acid fumes (Fig. 221). The positive electrode is connected with the alloy of the antimony and zinc, and the negative with the German silver.

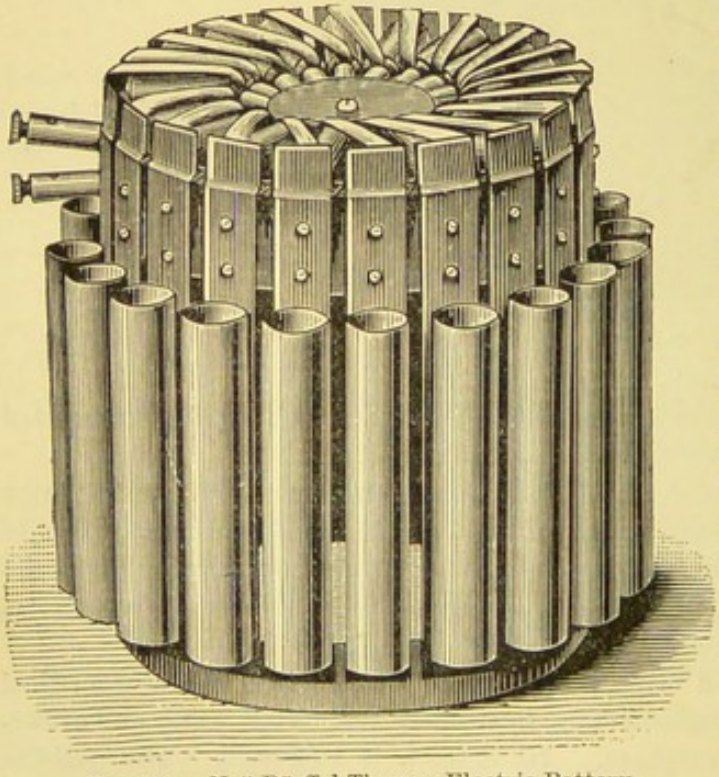


Fig. 221.—Noë-Dörffel Thermo-Electric Battery.

(10) *Storage Cell.* If there is a dynamo machine available, the physiologist may abandon the use of all kinds of batteries (except, perhaps, a Grenet and a Noë-Dörffel thermal pile) and employ a small accumulator, which may be charged from time to time.[1]

The late Professor Fleeming Jenkin thus summarized the probable sources of weakness in a battery—"When a battery does not give the expected results, one of the following defects is to be looked for: (1) Exhausted solutions—for example, in a Daniell battery, the sulphate of copper worked out, leaving the solution colourless, or nearly so; (2) bad contacts between the electrodes and the wires, oxidized or badly screwed-up binding screws, etc.; (3) empty or partially empty cells; (4) filaments of metallic deposit causing short circuiting between the battery plates; (5) creeping or deposits of salts forming short circuits either between the plates or from cell to cell. Shaking the cells increases their e. m. f. temporarily by disengaging the gases adherent to the plates. Floating filaments and broken plates give rise to false contacts, which cause the current given by a battery to vary suddenly when it is shaken."

3. Induction Coils.

The form of induction coil most commonly employed by physiologists is the *sledge inductorium* of Du Bois-Reymond, seen in Fig. 222, provided with *Neef's Interruptor*, represented diagrammatically in Fig. 223.

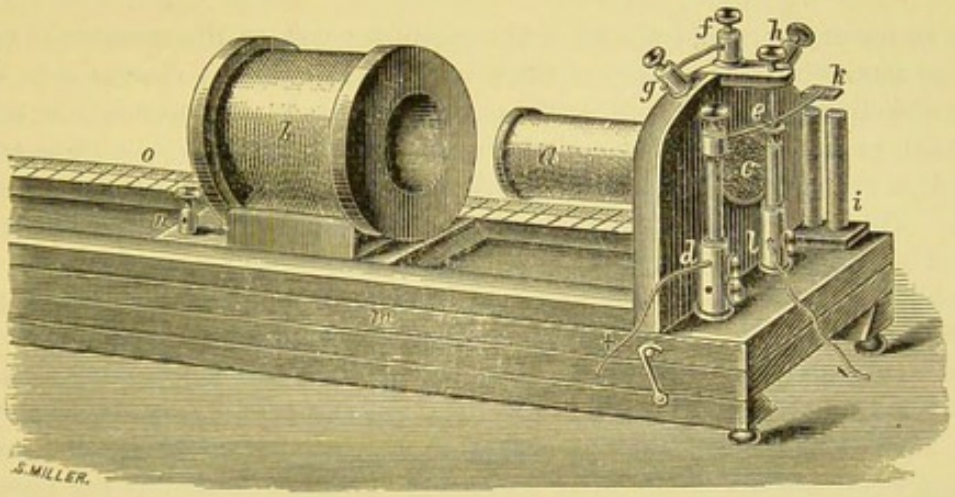


FIG. 222.—Inductorium of Du Bois-Reymond: *a*, primary coil; *b*, secondary coil; *c*, bunch of wires in centre of primary coil, for increasing intensity of induction current; *d*, binding screw, for attachment of wire from galvanic element. The current passes up the pillar *d*, along steel spring to *e*, from there to the screw the point of which touches the back of the spring at *e*. From *f* through wire of primary coil to *i*, along the two pillars of soft iron *i*, which it renders magnetic and thus draws down the head of the spring *k*. This interrupts the current at *e*, by breaking the contact of the spring with the screw point. When the current is thus interrupted, the spring flies up by its elasticity and again establishes the circuit at *e*. Thus the current is opened and closed automatically, and each time it is opened and closed there is an induction shock from the secondary coil *b*. The intensity of the induced current becomes weaker as we withdraw *b* from *a* along the graduated board *o*. The automatic apparatus was applied by Neef. The binding screw *g* is connected by a wire to *f*; the binding screw *h* by a wire to *i*. When the wires from the battery are connected with *g* and *h*, Neef's arrangement is out of the circuit, and an opening or closing shock may then be obtained by opening or closing a key (Fig. 224) interposed in the circuit. The interruptor was originally invented by Philipp Wagner.

[1] As to the structure and uses of accumulators, see Hospitalier's *Electricians' Pocket Book*, p. 210.

The descriptions appended to the figures explain the mechanism of the instrument. Neef's interruptor works automatically, and thus a rapid series of shocks is transmitted to the nerve or muscle.

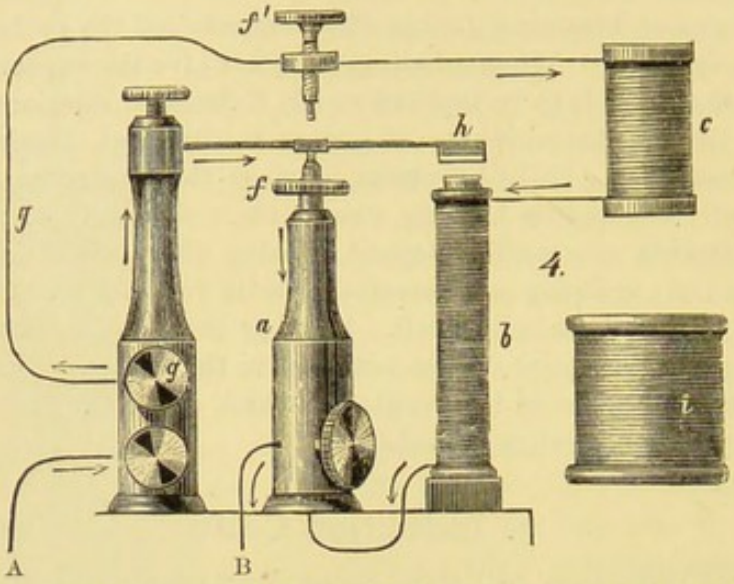


FIG. 223.—Diagrammatic view of the arrangement of Neef's interruptor at the end of the inductorium, seen in Fig. 222. The arrows indicate the direction of the current. A, wire connected with the + pole of battery; and B, with the − pole. *c*, primary coil; and *i*, secondary coil.

The secondary current induced in the secondary coil at the moment of opening is in the same direction as that of the primary, while that of closing is in the opposite direction. Thus, the direction of the currents in the secondary is reversed with each momentary opening and closing of the primary. With such an arrangement, it is found that the opening shock acts more powerfully than the closing shock. This is due to the fact that, at the moment of the closing shock, an extra current is induced in the primary coil by the action of each coil of wire on all the other coils. The extra current so induced in the primary, being in the opposite direction to the chief current in the primary, weakens the latter, so that the induced current occurring in the secondary coil at the moment of *closing* the primary is thus weakened. When the circuit of the primary coil is, on the other hand, opened, no extra current occurs in the primary, and its effect on the secondary coil is therefore greater, because the primary current at the moment of *opening* has not been weakened. Thus, the opening shock is considerably stronger than the closing shock.

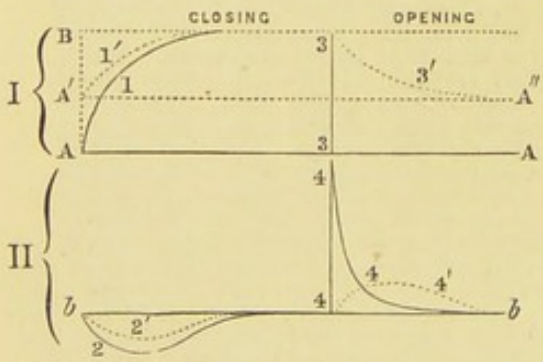


FIG. 224.—Diagram showing the effects of the extra currents on induction currents.

The relation of the opening and closing shocks is clearly shown by the diagram in Fig. 224, in which I refers to the current in the primary, and II to the current in the secondary coil, and A A is the basement line of the upper, and *b b* the basement line of the lower curves. On *closing* the primary circuit, the current in the primary coil in place of rising to its maximum, indicated by the dotted vertical line A B, attains this maximum slowly as shown by the curved line 1. This is owing to the production of the

extra current in the reverse direction. When the primary circuit is formed, there is a secondary induced current, represented by curve 2, placed below *b b*, because it is in the opposite direction to I. On *opening* the primary circuit, its current suddenly ceases, as shown by the vertical line 3 3, and is unaffected by any extra current. When the primary circuit is opened, an induced current is generated in the secondary coil, and this induced current at once reaches its maximum, as shown by the vertical line 4 4, and then falls off gradually, as shown by the curve 4 4′.

To equalize the two currents, Von Helmholtz invented the arrangement shown in Fig. 223. A loop of wire, *g*, is carried from the pillar *g* (the first pillar on the left) in the direction of the arrow to the screw *f′*, and *f′* is screwed up so that it does not come into contact with the back of the spring *h*, but *f* is screwed up so that with each vibration of *h*, the under surface of the spring touches the screw point *f*. The current from the + pole enters at A, and at first passes through the loop wire *g* to *f′*, thence to the primary coil *c*, and thence to the electromagnet *b*. When the soft iron core of the latter is magnetized, the hammer *h* is pulled down, and the under surface of the centre of the spring touches the top of the middle pillar at the screw *f*. This short circuits the primary, and the current then returns to the battery by passing down the pillar *a*, and out by the wire B to the − pole. But the closure of the short circuit so weakens the current flowing through the electromagnet *b*. that the hammer is released and the short circuit being broken at *f*, the current must pass by the long circuit, and thus the current in the primary coil *c* is never opened, but the opening shock in the secondary coil *i* is due to the weakening of the current in the primary at the moment of short circuiting. By this method, an extra current is induced in the primary at opening (or rather at the moment of weakening the current by short circuiting), and as this is in the reverse direction to the chief current in the primary, the opening shock is reduced in strength, and becomes nearly equal to the shock from the secondary coil at the moment of closing. The weakening of the primary circuit thus produced is shown in Fig. 224 A′ A″, in its diminished distance from B. When the short circuit is broken, as already described, the strength of the primary is at once increased, as shown by the curved dotted line, 1′. This increased strength of the primary produces an induced current in the secondary, as shown by the dotted curve 2′, below *b* II. The weakening of the primary by the re-establishment of the short circuit is pictured by the dotted curve 3′. This in turn produces an induced current in the secondary coil, represented by the curved line 4′. Thus, with the original arrangement, the strength of the closing induction shock is represented by the curve 2, and that of opening by the curve 4 4 4, while these values, with the arrangement of Von Helmholtz, are shown by the curves 2′ and 4′, and it will be seen that these curves are very similar in appearance.

When the arrangement of Von Helmholtz is employed, there is an extra current in the primary coil, both at the moment of opening and of closing the primary current, and the extra current may be employed as a stimulus by connecting the positive and negative poles of the battery with the pillars *g* and *a*, and the wires for carrying off the extra current to the tissues are connected to *f″* and *f*.

The primary coil should consist of at least 432 windings, having a resistance of 1·27 ohm, and the secondary should have 14,680 windings, with a resistance of 1362 ohms at 16° C. Professor Bow-

ditch has also improved the inductorium by having the secondary coil mounted on a rotating disk, so that it may be placed with its axis forming an angle with that of the primary. Thus, the strength of the secondary current may be modified according to the amount of angular deviation, and this may be read off from a graduated arc at the side of the base of the secondary coil. In using the inductorium for physiological purposes, the observer should always state (1) the battery employed, so as to indicate the e. m. f. ; (2) the number of windings and the resistance of the primary coil ; (3) the number of windings and the resistance of the secondary ; (4) the distance of the secondary from the primary ; (5) whether or not Von Helmholtz's loop wire was used ; and (6) the amount of angular deviation of the primary and secondary coils.

4. Accessory Appliances.

For the purpose of opening and closing currents, various instruments may be employed. One of the most convenient is termed a *key*, seen in Fig. 225. It consists of a rectangular wooden frame, by which the key may be screwed to the table. On the top is a square block of vulcanite, *a*, bearing two rectangular bars of brass, *b* and *c*, which may be joined by the handle, *d*, carving a horizontal piece of brass. The key is *closed* when the arm is horizontal, as in the figure. On moving the handle, *d*, backwards and to the right (see Figure), the brass arm is raised, and of course the contac' between *b* and *c* is broken. The key is then said to have been *opened*. Wires are attached by binding screws to *b* and *c*. If the key is interposed in the course of the wire leading from *one* of the electrodes of a battery or inductorium, the circuit will be broken when the key is opened, and closed, or formed when the key is closed ; but if the positive and negative poles are connected respectively with *b* and *c* by the two inner binding screws, while the two outer binding screws have wires passing from them to a nerve or muscle, or to a galvanometer, or other electrical apparatus, it is clear that when the key is closed the battery current will be *short circuited*,

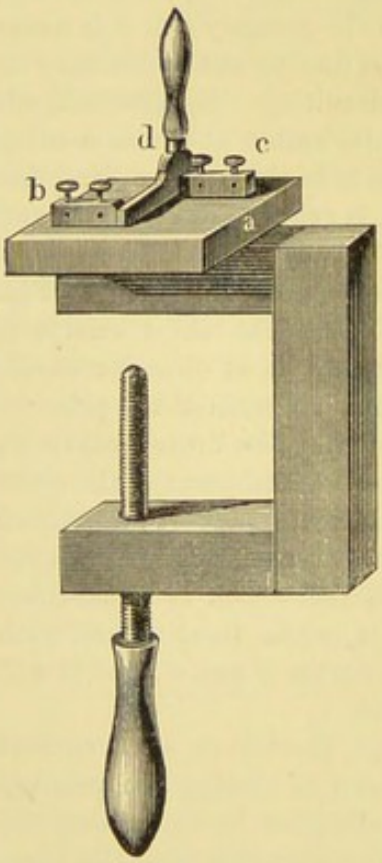


FIG. 225.—Du Bois-Reymond's friction key.

and that when the key is open, the current will then pass onwards. Thus, the key may be used (1) for opening and closing a current, and (2) for short circuiting.

For rapid opening and closing of a current, an arrangement like *Neef's Interruptor* may be employed, or a *vibrating metallic spring* may be interposed in the circuit, so arranged that, as it vibrates, a needle at the end of the spring will make and break contact by dipping into a small vessel containing mercury.[1] By varying the length of the spring, its vibration period may be increased or diminished. An ordinary *metronome*, fitted up as represented in Fig. 226, is also useful for making

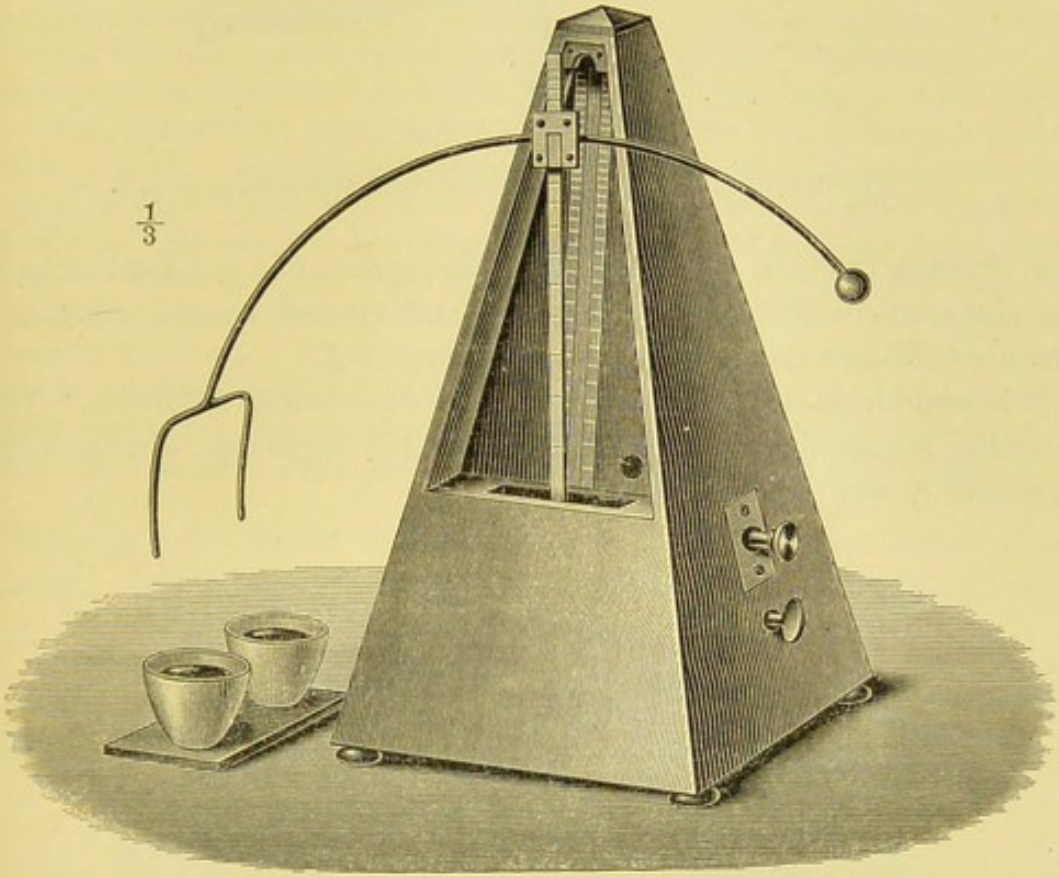


Fig. 226.—Metronome, arranged for making and breaking an electrical current.

and breaking currents. As the pendulum swings backwards and forwards, it makes and breaks contact by dipping into the small vessels containing mercury. These vessels are interposed in the circuit by wires dipping into the mercury, and it is clear that when the fork-shaped wire of the metronome also dips into the mercury the circuit will be formed. By moving the slider of the metronome up or down, the number of interruptions may be regulated.

[1] A beautiful and most convenient spring of this description is made by the Cambridge Scientific Instrument Company, and is very useful for many purposes.

When tolerably strong currents are used, interruptors consisting of a point dipping into and out of mercury are very troublesome on account of oxidation of the surface of the mercury. This difficulty is removed by the use of the ingenious arrangement of Hugo Kronecker, shown in Fig. 227. By allowing a stream of water, or, still better, of alcohol, to

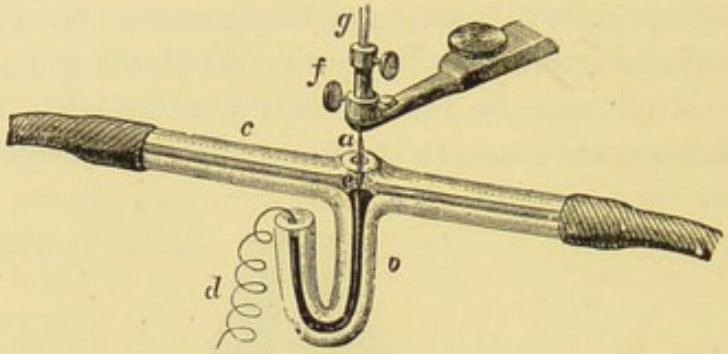


Fig. 227.—Kronecker's arrangement for securing clean mercurial contact.

flow through the tube *c*, the surface of the mercury in *o* is kept clean at the place where the current is closed and opened by the vibrator *g*, which establishes the current passing from *g* to *d*.

It is sometimes necessary to reverse the direction of a current. This is readily accomplished by the use of a simple apparatus, termed *Pohl's Commutator*, seen in Fig. 228.

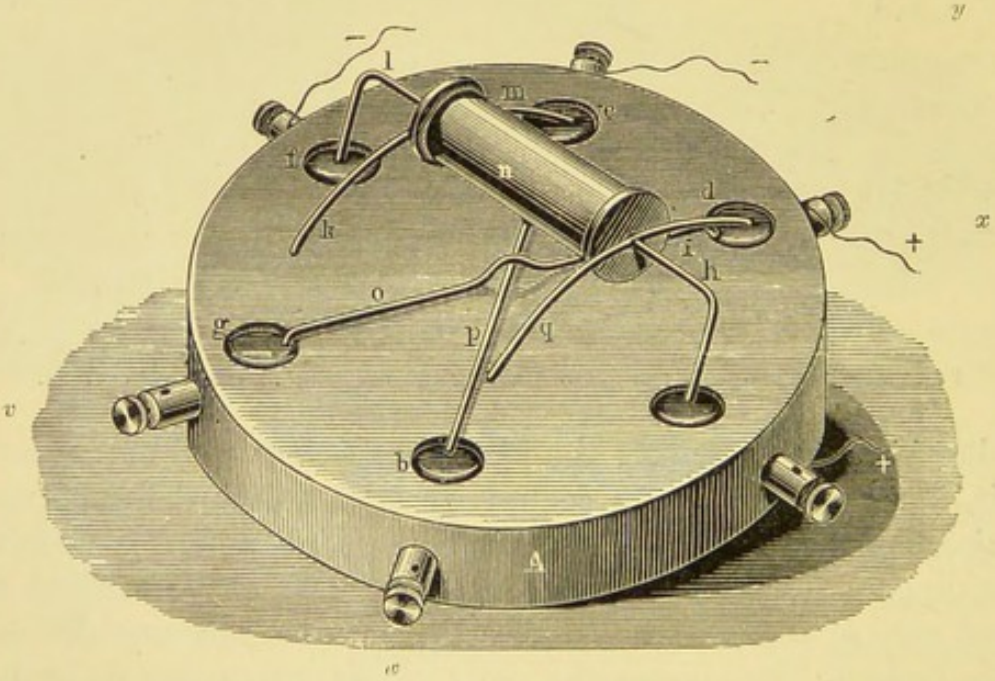


Fig. 228.—Pohl's Commutator. For description, see text.

It is a disk of wood *A*, having six little pits or depressions at regular distances, to each of which a binding screw is attached. From the holes *f* and *h* two wires

proceed, which are attached at their outer ends to the ends of the binding screws, but loosely, so that they may move from side to side. These wires pass into a piece of glass or vulcanite *n*, but without their inner ends touching. Thus, a bridge is formed; but if a current entered the end of the bridge by the binding screw marked + to the right, it could not pass across to the binding screw having the wire − attached on the left. Two curved wires *k m* and *q i* are soldered at their centres to the wires *f* and *h*, but with the ends free. As the bridge *n* can rock backwards and forwards, the ends *i m* may dip into the troughs of mercury *d c*, or the other ends *k q* may dip into the troughs *g b*. By this arrangement, when the wires dip into *c d*, as in the Figure, suppose a current entering at + on the right, it would pass along *h i* into *d*, and out by the curved wire *x*, round the circuit, back to the curved wire *y*, into *m*, thence by 1 into *f*, and thence back to battery by wire − on the left. On the other hand, if *k q* dipped into *g b*, the current would go out from *b* and return to *g*. Thus the current, by simply reversing the bridge, could be sent to a circuit *x y*, or to one represented by *w v*. Now suppose the cross bars *o p* inserted so that *p* joins the troughs *c b*, and *o* joins *d g*; then, if a current enters *h*, it will pass by *i* into *d*, and then out by *x* to circuit, back to *y*, then into *m*, then by 1 into *f*, and back by wire − to battery. If, however, the bridge be reversed so that *k q* dip into *g b*, when the current enters by *h*, it will not pass by *i* into *d*, because the circuit is broken there; but it will go by *q* into *b*, then by cross wire *p* over to *c*, then out by the wire *y*, round the circuit to *x* into *d*, then by cross wire *o* to *g*, then by *k* up to 1, and then back to battery by wire −. Thus, by the use of the cross wires *o p*, if the current enters by the binding screw + on the right, *x* will be + pole if the bridge is in the position seen in the Figure, and *y* will be the + pole if the bridge is reversed so that *k q* dip into *g b*. The cross wires thus *reverse* the direction of the current.

The most convenient arrangement for stimulating a nerve is an apparatus, termed the *Polarizable Electrodes* of Du Bois-Reymond, seen

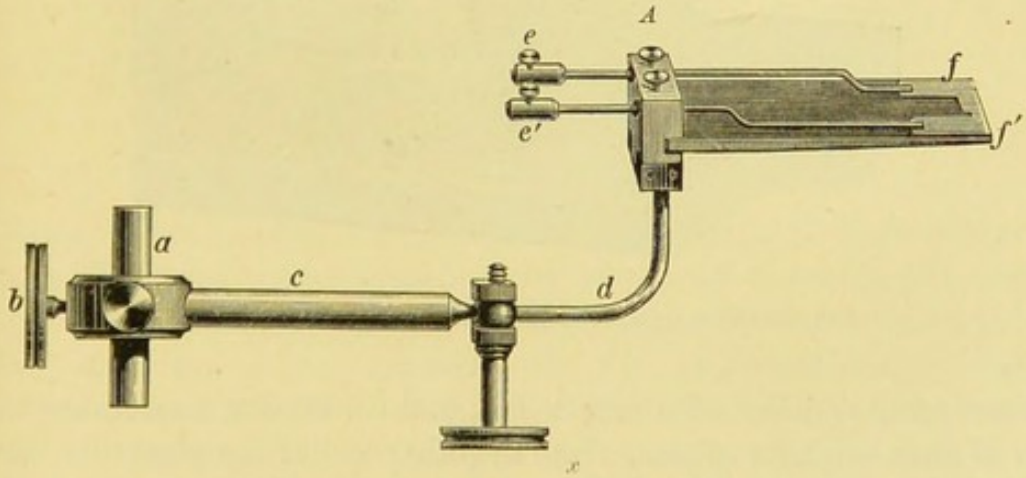


FIG. 229.—Polarizable Electrodes of Du Bois-Reymond. *a*, upright brass rod; *b*, screw for tightening the rod *c* on *a*; above screw *x* is a ball and socket universal joint; *d* is a curved brass rod bearing *A*, a piece of vulcanite, through which two wires pass, having binding screws *e e′* at one end, and rectangular platinum points *f f′* at the other.

in Fig. 229. The nerve is laid across the platinum points *f f′*. Even with such electrodes, irritable nerves might be stimulated by

currents produced by polarization caused by the action of the animal fluids on the metallic substances. In certain refined experiments the nerves are stretched across *Non-polarizable Electrodes*, shown in Fig. 230. This form may be used not merely for stimulating nerves, but, and more especially, for conveying currents from nerve and muscle into the circuit of a galvanometer. (See the Chapter on the Electrical Phenomena of Muscle.) The electrodes here shown are those used by Professor Burdon-Sanderson, but for the purpose of stimulating a nerve they may be of much smaller size. They consist of, *a*, a brass stand carrying a horizontal bar of vulcanite *b*, which in turn supports the electrode *c*. Each electrode is a curved glass tube *d*, closed at one end by a smal' piece of sculptor's clay *e*, moistened with saliva or with a

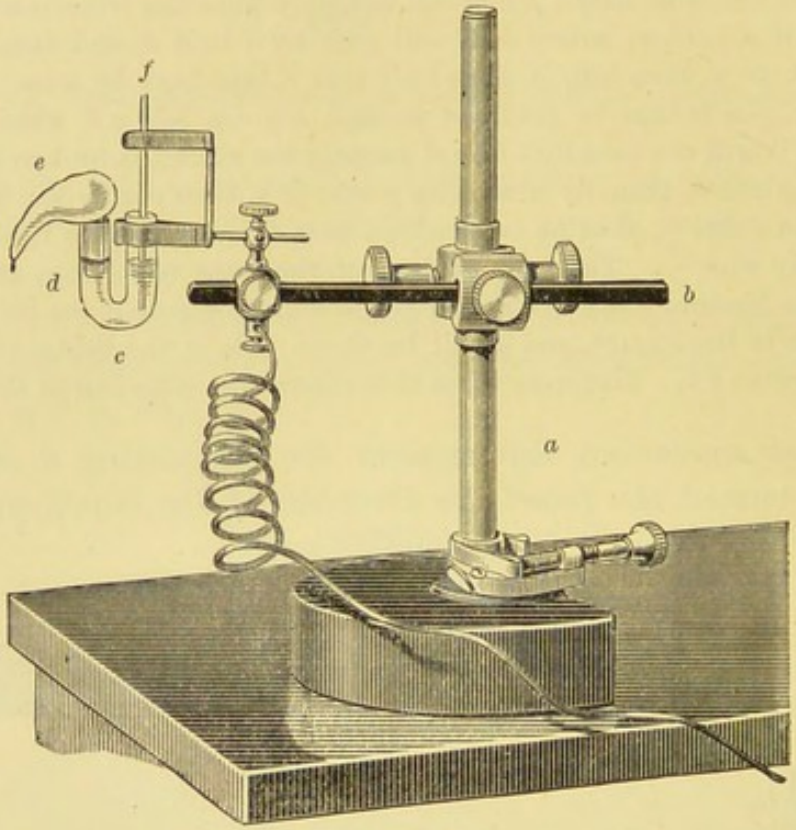


Fig. 230.—Non-polarizable Electrodes of Burdon-Sanderson.

·75 per cent. solution of common salt, and containing a saturated solution of pure sulphate of zinc. Into the other end of the glass tube, there is dipped a small rod of amalgamated zinc. Such an arrangement, carefully prepared, is absolutely non-polarizable, so that if a nerve or muscle were laid across two such electrodes, and a current from a battery or inductorium were passed through the nerve or muscle, the latter would be stimulated by the current from the electrical apparatus, and by nothing else.

Several electrical appliances are shown in Fig. 231, and reference is made to the description of the Figure.

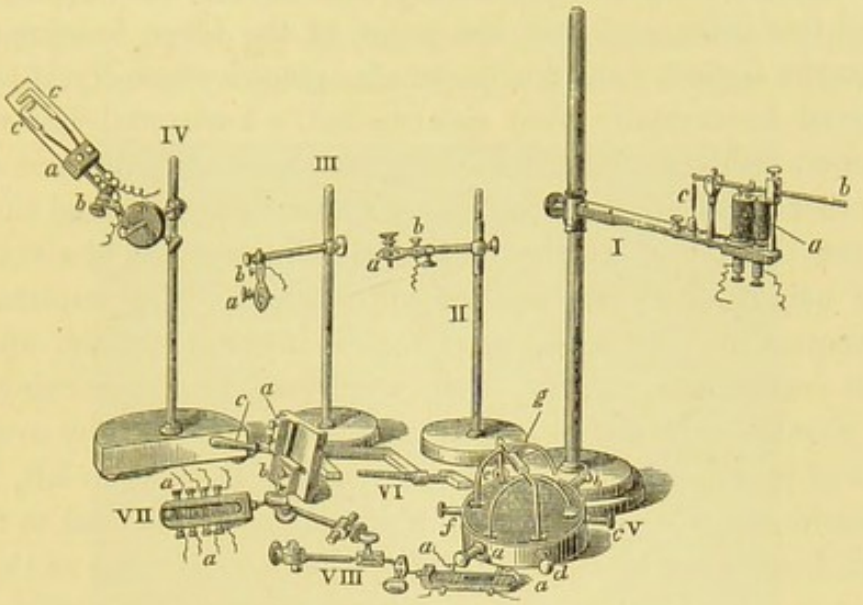


FIG. 231.—Apparatus for electrical experiments on muscle and nerve. I. Electro-magnetic signalling apparatus for recording seconds on a revolving cylinder; *a*, bobbins covered with wire; *b*, brass rod carrying pencil or pen; *c*, steel spring. II. and III. Brass forceps for holding leg of frog; *a*, forceps; *b*, binding screw for wire. IV. Platinum electrodes for stimulating nerve; *a*, piece of vulcanite carrying glass plate *c c*, on which are the copper wires terminating in rectangular platinum points; *b*, universal joint. V. Pohl's commutator for reversing the direction of electric currents; *d* and *e* are connected with the battery or induction coil; *b c* and *a f* are binding screws for attaching wires in different circuits; *g*, bridge of copper wire, divided and insulated in the centre by glass tube, for the purpose of sending the current entering by *d e*, either in the direction of *b c* or *a f*. VI. Du Bois-Reymond's key, consisting of a piece of vulcanite on which there are two rectangular pieces of brass *a b*, each having two binding screws. The two pieces of brass are connected by an arm of brass, the handle of which is seen at *c*. VII. and VIII. Two forms of apparatus for stimulating nerve, consisting of vulcanite troughs, into which are fixed platinum wires, which may be attached to the wires coming from the battery by binding screws at *a a a a*.

CHAP. IV.—THE GRAPHIC METHOD.

Many movements are too rapid to be followed in all their phases by the unaided eye. For example, the prongs of a tuning-fork are in active periodic motion during the time that a tone is emitted by the fork, but the prongs may appear to the eye to be stationary. Again, the movements of a bird's wing or of the limbs of a quickly-trotting horse are too rapid to be followed by the eye. The motion of the living heart exposed in the living animal seems so irregular and tumultuous as to make one with no experience of scientific methods doubt the possibility of tracing its various movements. Even when the isolated muscle of a frog's limb is caused to contract by irritating the nerve supplying it, the eye cannot tell whether the contraction occurs in a shorter time than the relaxation, and still less whether the con-

traction is at a uniform rate in time, or whether it contracts faster at the beginning and more slowly towards the end, or the reverse. Suppose the muscle were hung vertically, and that it was connected at its lower end to a lever, and that the point of the lever, bearing a pencil, were brought against a sheet of paper also placed vertically, if the paper were moved horizontally from right to left, a horizontal line would be drawn upon it by the pencil so long as the muscle did not contract. On the other hand, suppose the paper to be stationary and the muscle to contract, the pencil at the end of the lever would draw a vertical line, the height of which, making allowance for the amplification of the movement by the lever, would be a measure of the amount of muscular contraction, or, in other words, of the shortening of the muscle. Finally, suppose that, while the muscle shortened by contraction, the sheet of paper moved at a uniform speed from right to left, then the pencil would describe a line passing obliquely upwards, and to the right (Fig. 232, from below the line B, in direction *a*, *c*), so long as the muscle contracted, and a line obliquely downward, and also to the right, during the relaxation of the muscle. Thus, by a series of contractions, the zig-zag line would be drawn. If the motion of the contracting muscle were more uniform, then a line like the curve A would be described. This illustrates the essential principle of the graphic method, by which movements are recorded on a surface, which may be either stationary or moving with a uniform velocity, a method which has been of great value in all sciences dealing with movement, and not least to physiological science.

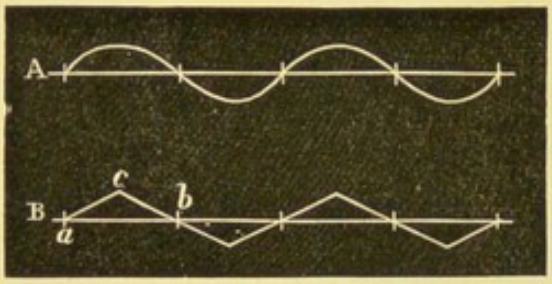


FIG. 232.—Diagram illustrating the graphic method. No muscle would actually record such curves.

An excellent illustration of the graphic method is seen in the tracing of harmonic motions. Suppose two pendular motions acting on the same point, and that the times of the motions were as 1 to 1, or as 1 to 2, or as 2 to 3, or as 3 to 4, and that the surface over which the point travelled was stationary, then the beautiful figures seen in Fig. 233 will be described. If, however, the recording surface be moving from right to left, then the forms of the curves described by the combination of the same motions will be those shown in Fig. 234.

This illustration shows that, however complicated may be the movements of a point, these movements may be recorded, and that the character of the tracing on the recording surface will vary according as the recording surface is stationary or moving.

The method of the graphic registration of movement is not entirely

modern. In 1734, the Marquis d'Ons-en-Bray described an anemometer which recorded its movements on a sheet of paper, rolled round a cylinder, moved by clockwork. Magellan, in 1779, made designs for an instrument he called a "Meteorograph" for recording automatically many meteorological phenomena. It was then a dream to attempt to

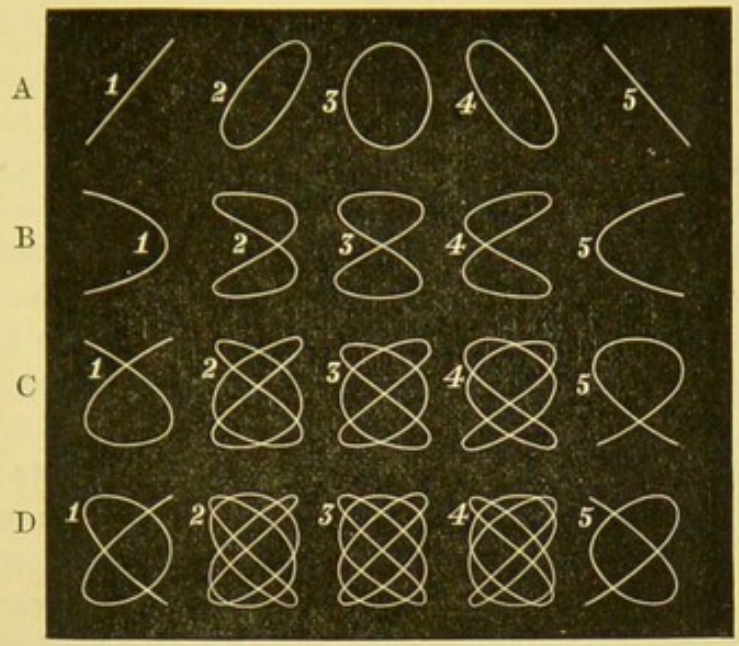


FIG. 233.—Lissajous' figures of harmonic motions. A, 1 : 1, unison ; B, 1 : 2, octave ; C, 2 : 3, fifth ; and D, 3 : 4, fourth. The figures 1, 2, 3, 4, and 5 in each row represent the phases of the harmonic motions.

record such phenomena, but it has become a reality in these latter days. A thermometer was described by Rutherford in 1794, by which the curves of fluctuating temperatures were recorded on blackened paper. About 1800, Thomas Young showed how time can be measured on the surface of a cylinder moving at a uniform speed. The celebrated James

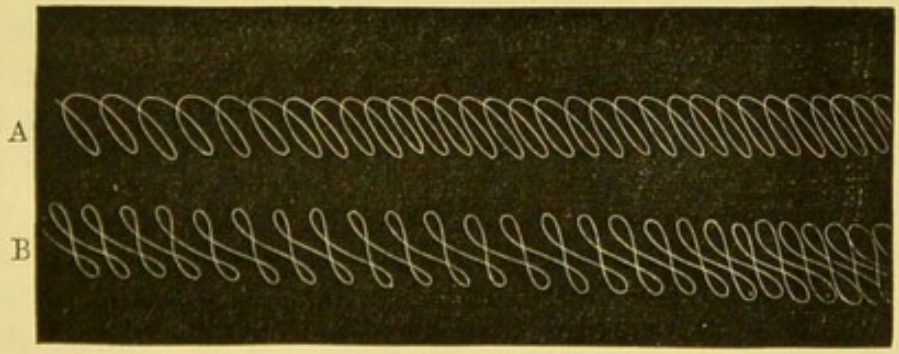


FIG. 234.—Figures of harmonic motions, with recording surface moving from right to left. A, Unison ; B, 1 : 2, octave.

Watt, at a date I have been unable to fix, devised a method of tracing the movements of the indicator of his engine on a cylinder rotated by the engine itself. Thus, he obtained a curve representing variations of

steam pressure at different times.[1] A method of recording the oscillations of mercury in a manometer was invented by Ludwig, and resulted in the kymograph, or recorder of blood pressure, and during the past twenty or thirty years numerous ingenious instruments have been invented by physiologists for recording movements. No physiologist has done more in this direction than Marey, to whom we are largely indebted for the development of the graphic method to its present condition of precision and convenience.

1. Measurement of Time or Chronography.

As many of the phenomena of nervous and muscular actions are of such short duration as to make it impossible to observe their phases with the unaided senses, it is necessary to construct apparatus for the measurement of minute intervals of time. Accordingly, in recent years, much ingenuity has been expended in the construction of chronographic or time-measuring instruments, of which the following are examples—

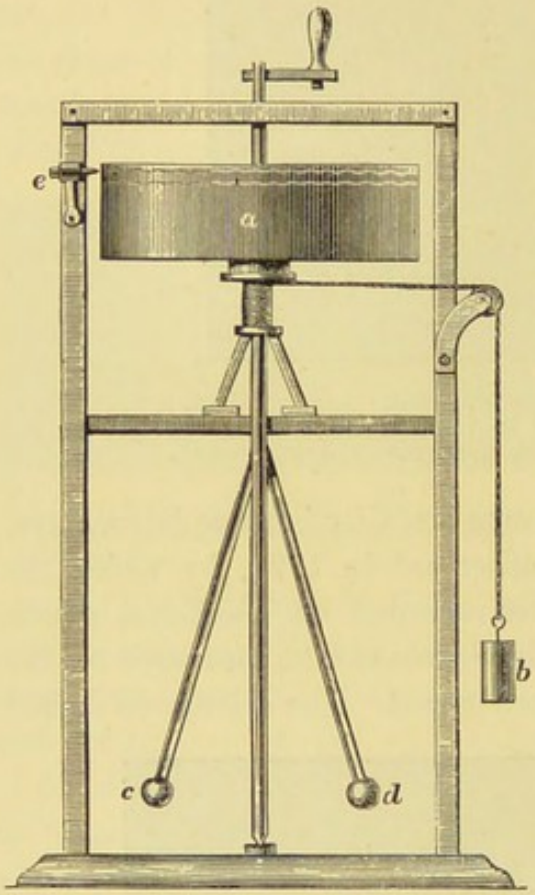


FIG. 235.—Original Chronometer devised by homas Young for measuring time. *a*, Cylinder rotating on vertical axis; *b*, weight acting as motive power; *c*, *d*, small balls for regulating by centrifugal action the velocity of the cylinder; *e*, marker recording a line on the cylinder. This illustration is given to show the form of the first apparatus used for chronographic tracings.

(*a*) *Recording Cylinder.* — This is a cylinder revolving at a uniform rate by means of clockwork. Suppose the surface of the cylinder to be divided by sixty lines, parallel with its axis, at equal distances from each other, and that the cylinder makes one revolution in a second, the distance between two of the lines will represent the $\frac{1}{60}$ part of a second, and any phenomenon recorded on the moving cylinder between the two lines must have happened during that interval of time. It is evident that by means of such an arrangement, seen in Fig. 235, first suggested by

[1] Marey, *La Méthode Graphique dans les Sciences Expérimentales*, p. 113. For a view of Watt's Indicator used with the steam engine, as constructed by Watt, and improved by M'Naught and Richards, see Clerk Maxwell's *Theory of Heat*, third edition, p. 154.

Thomas Young,[1] intervals of time, even to the $\frac{1}{1000}$ of a second, may be measured with accuracy. The difficulty in such graphic measurements of time is to cause the cylinder to revolve at a uniform rate. This is accomplished by means of regulators, of which the simplest is that of Foucault, now attached to all revolving cylinders used for physiological purposes. To obtain, on a revolving cylinder (the time occupied by a revolution of which is not known) continuous registration of minute intervals of time, it is necessary to make use of a chronograph.

(*b*) *Chronographs.*—Thomas Young was also the first to devise the method of inscribing upon a rotating cylinder all the vibrations of a metallic rod bearing a very light style or marker. When these vibrations are isochronous, each of the undulations traced upon the cylinder corresponds to a regular interval of time. Duhamel was the first to apply to one of the limbs of a tuning-fork a small marker, which traces with great regularity the vibrations of the tuning-fork, and in this way, if the surface receiving the tracing is moving with sufficient rapidity, intervals of the $\frac{1}{500}$ of a second, or less, may be readily measured. An example of tracings thus obtained is seen in Fig. 236.

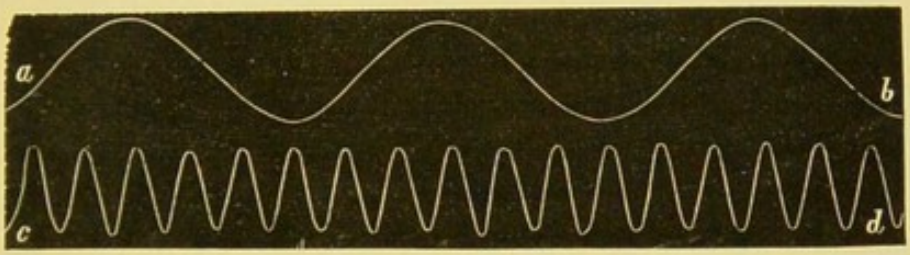


FIG. 236.—Tracings of the vibrations of a tuning-fork, 10 vibrations per second. *a*, *b*, cylinder moving rapidly; *c*, *d*, cylinder moving slowly.

It is difficult to apply the vibrating limb of a tuning-fork to a revolving cylinder or other moving surface, more especially if other recording apparatus is adjusted to the cylinder at the same time. To record more easily vibrations indicating time, an arrangement consisting of a marker, vibrating in unison with a tuning-fork, which is kept in action by the interruptions of an electric current, is much employed. The apparatus, as applied to a revolving cylinder, is seen in Fig. 237. The arrangement of apparatus consists of three parts—a battery, an interrupting tuning-fork, and the chronograph. The latter, seen in Figs. 238 and 239, consists of a very fine stylet fixed at the extremity of a steel spring, and armed with a small mass of steel, somewhat wedge-shaped, which fits in between two small keepers, *b b*, of the

[1] Thomas Young's *Lectures on Natural Philosophy;* Lecture xvii., on Time-keepers. Plate xv. Fig. 198.

electro-magnets, *a a*. The tuning-fork interrupts the current from the battery. This it does automatically. When the iron of the electro-magnet between the limbs of the tuning-fork becomes magnetic, the limbs are approximated, and a small piece of platinum wire affixed to one of them is removed from contact with a platinum surface (Fig. 237, *i*), so as to

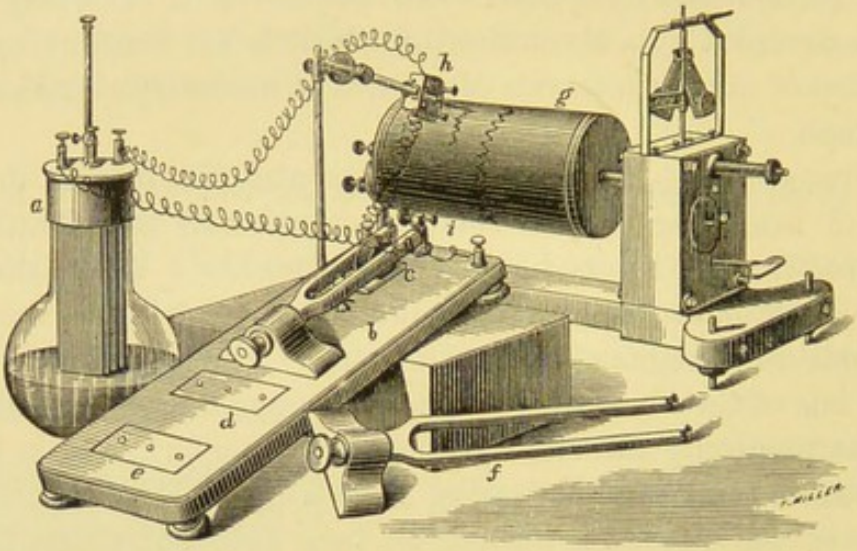


FIG. 237.—Chronograph applied to revolving cylinder. *a*, Grenet's element; *b*, wooden stand bearing tuning-fork, vibrating 200 times per second; *c*, electro-magnet between limbs of the fork; *d*, *e*, positions for tuning-forks of 100 and 50 vibrations per second; *f*, tuning-fork lying loose, which may be applied to *d*; *g*, revolving cylinder; *h*, chronograph vibrating synchronously with the tuning-fork. Current is interrupted at *i*. Foucault's regulator is seen over the clockwork of the cylinder, to the right of *g*.

break the circuit. On the circuit being thus broken, the electro-magnet ceases to act, the limbs of the tuning-fork recede from it, so as to bring the platinum wire again into contact with the platinum surface, and thus again to complete the circuit. The chronograph thus vibrates in

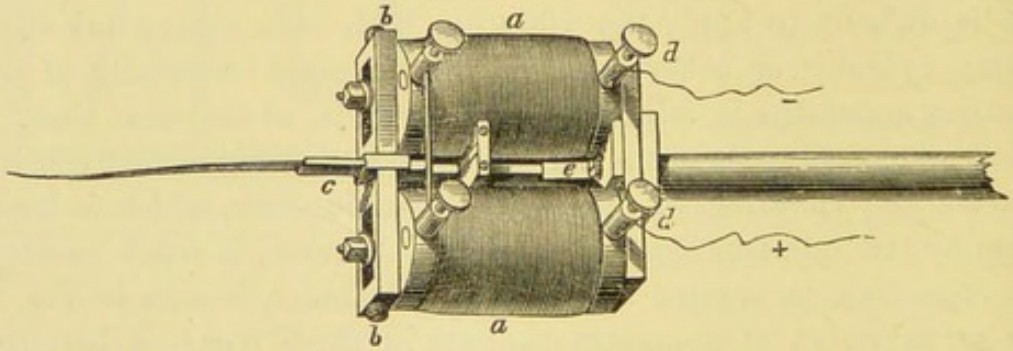


FIG. 238.—Side view of chronograph. *a a*, coils of wire; *b b*, keepers of electro-magnets; *c*, vibrating style fixed to steel plate *e*; *d*, binding screws for attachment of wires; +, from tuning-fork; −, passing to battery.

unison with the tuning-fork. The great advantages of this apparatus are its accuracy and facility of ready adjustment, and it can frequently be applied where it would be extremely difficult to bring the tuning-fork into direct contact with the moving surface.

A very convenient form of interrupting tuning-fork, made by the Cambridge Scientific Instrument Company, is shown in Fig. 240, and it is here introduced to indicate the position of the electro-magnet between the limbs of the fork. Along with the interrupting tuning-fork, the same makers supply a time-marker (Fig. 241) for recording on a surface of smoked paper, and a time-marker (Fig. 242), having at the end a kind of syphon pen, for recording in ink on a long continuous band of white paper. The latter arrangement is especially useful where it is necessary to obtain a long continuous tracing. The mechanism of both instruments is seen on inspecting the figures.

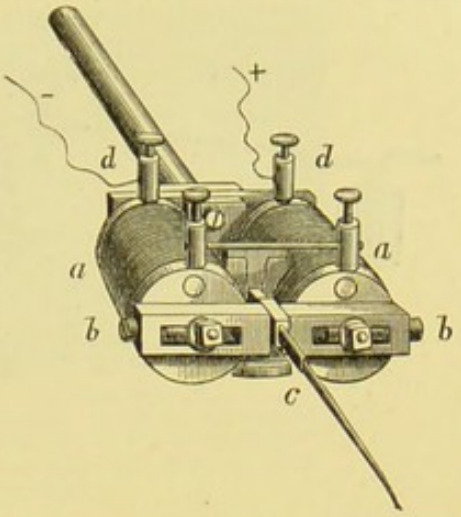


FIG. 239.—Front view of chronograph, to show position of vibrating marker. Same description as given of last figure.

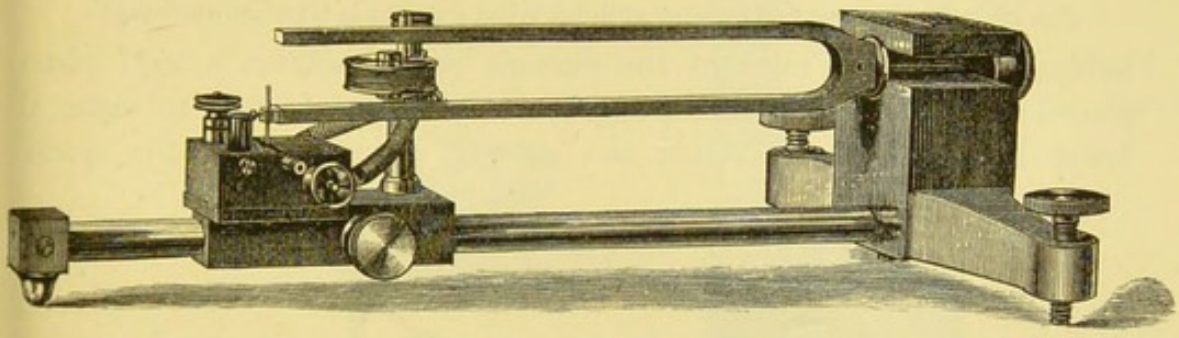


FIG. 240.—Interrupting or Chronographic Tuning-fork of Cambridge Scientific Instrument Company.

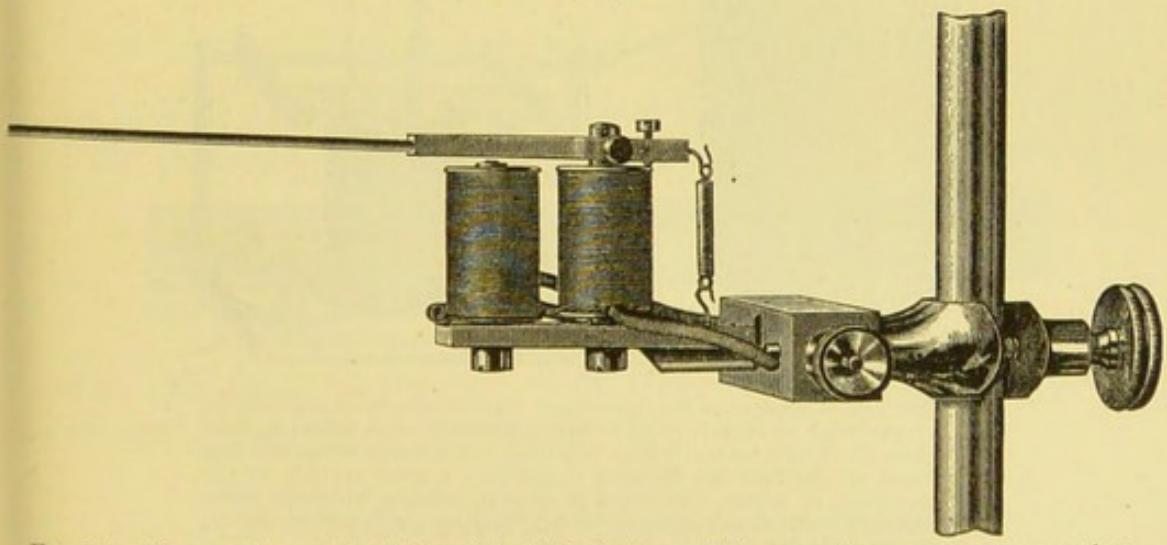


FIG. 241.—Time-marker of Cambridge Scientific Instrument Company for recording on a surface of smoked paper.

(*c*) *Electrical Signals.*—It is often of great importance to determine the moment of the commencement or termination of a phenomenon.

This is readily done by means of electro-magnetic arrangements, such as are seen in Fig. 243. The apparatus consists of two electro-magnetic

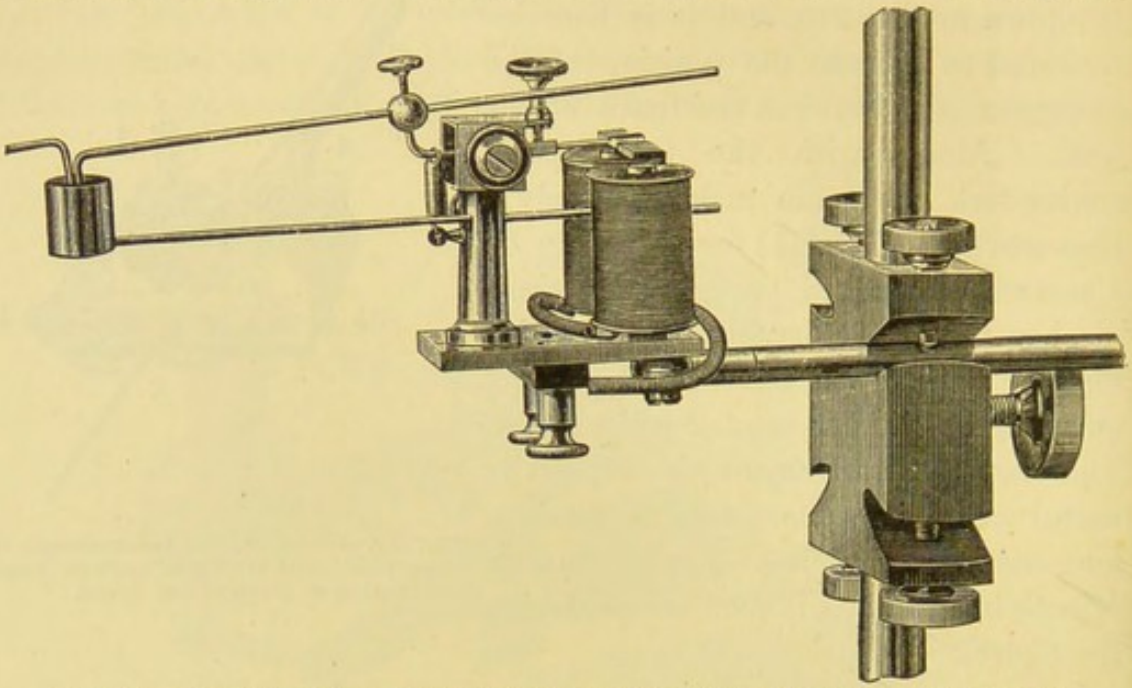


FIG. 242.—Time-marker for recording with ink when a tracing is taken on white paper.

bobbins, which, the moment the current passes, attract a steel plate placed above them, and draw down the writing stylet, so as to make a lower horizontal line. When the current is interrupted, the spiral

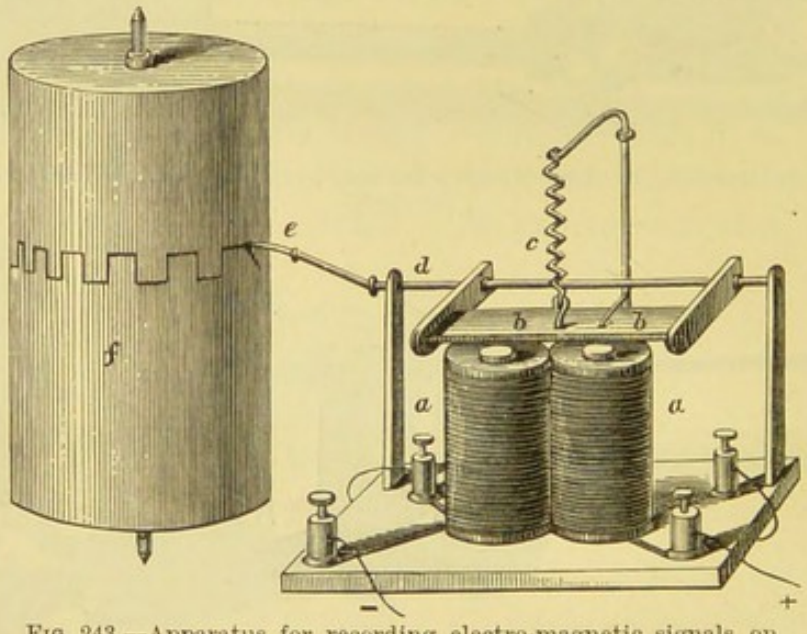


FIG. 243.—Apparatus for recording electro-magnetic signals on a revolving cylinder. *a a*, bobbins covered with wire; *b*, steel plate fixed in a frame, which is pulled downwards when the iron cones of the bobbins become magnetic; *c*, steel spring, by the elasticity of which the plate is drawn quickly upwards when released from the bobbins; *d*, rod, bearing a marker, recording on the cylinder *f* at *e*.

spring elevates the lever, which traces an upper horizontal line until the current is again closed. The current may be opened or closed by means of the seconds pendulum of a clock, by a tuning-fork (Fig. 240),

or by a metronome (Fig. 226). By such an arrangement for closing or opening the circuit, the instant of the commencement or termination

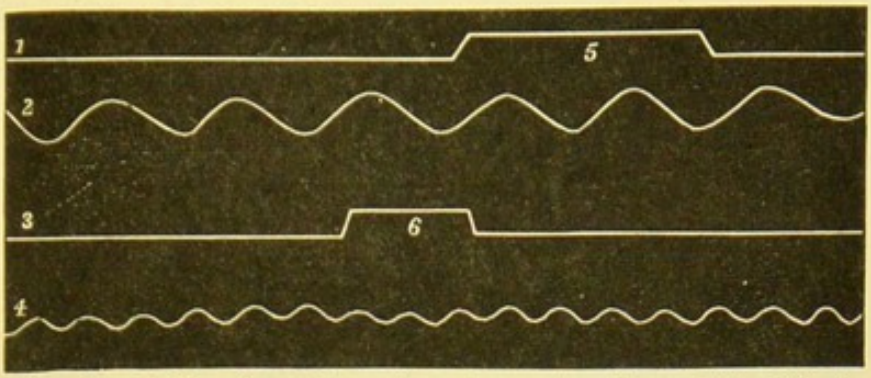


FIG. 244.—1, Line drawn by marker of apparatus shown in Fig. 243; 2, vibrations of tuning-fork, 10 vibrations per second; 5, signal, the rise in tracing 1 indicates the interruption, and the fall shows the formation of the current, whilst the cylinder was rotating quickly; 3, 4, 6, show similar tracings, but with cylinder going more slowly.

of any phenomenon will be recorded. An example of a tracing obtained by such an apparatus is given in Fig. 244.

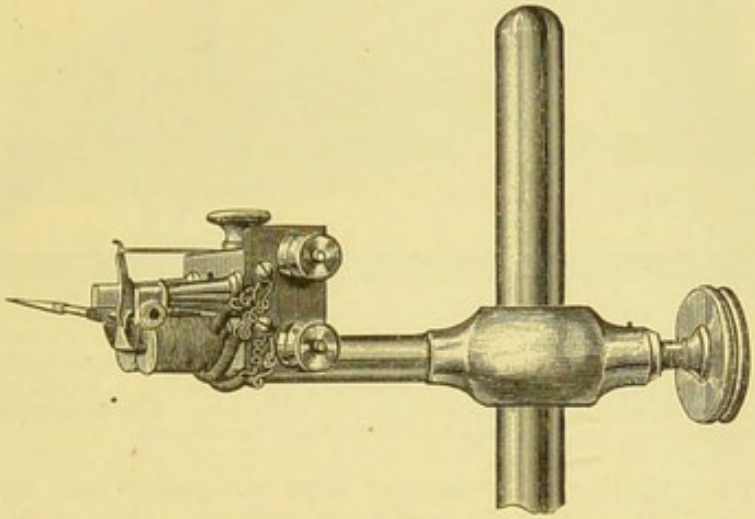


FIG. 245.—Signal of Deprez, as made by the Cambridge Scientific Instrument Company.

A delicate form of apparatus for recording signals, similar in principle to the chronograph shown in Figs. 238 and 239, but much smaller, is

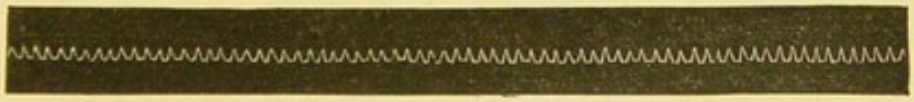


FIG. 246.—Tracing taken with the signal of Deprez, acted on by a current interrupted by a tuning-fork, vibrating 500 times per second.

called the *signal* of *Deprez*. It is represented in Fig. 245, and an example of a tracing is given in Fig. 246.

2. The Direct Recording of Movement.

Movements may be recorded if we attach a lever to the moving structure, as near the fulcrum as possible, and bring the other end of the lever into contact with the recording surface, or the lever may

simply be laid on the moving part, so that it may rise and fall with each movement. In the case of muscular movements, special registering instruments have been invented termed *Myographs*. The first myograph, invented by Von Helmholtz, consists of a brass framework, Fig. 247,

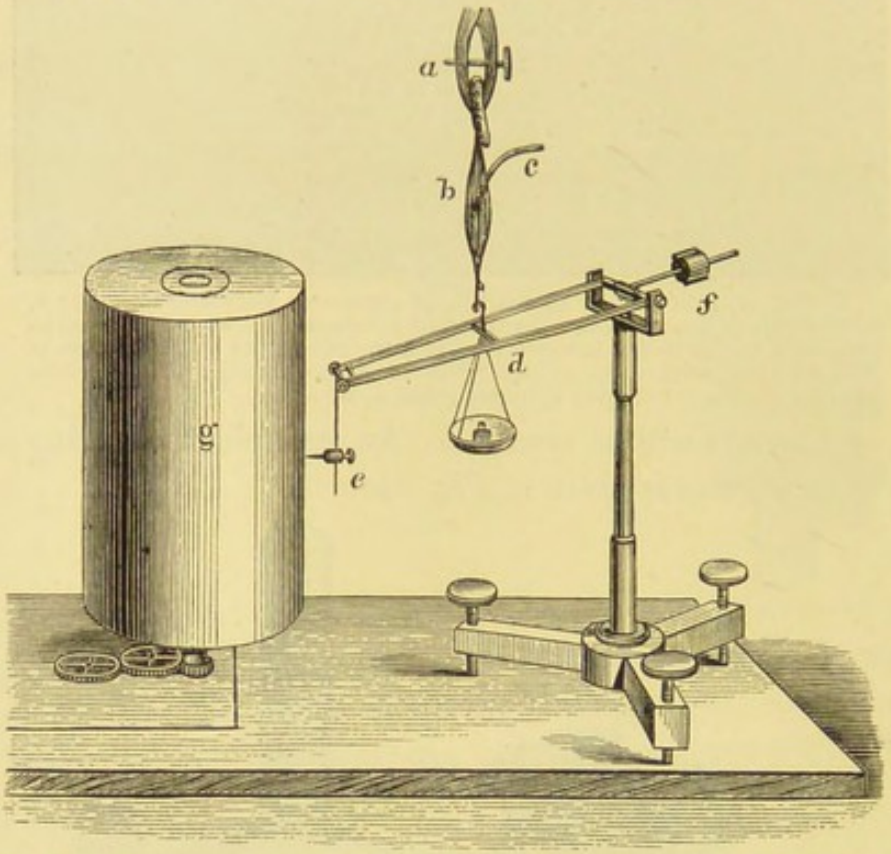


FIG. 247.—Myograph of Von Helmholtz, shown in an incomplete form. *a*, forceps for holding femur of frog; *b*, gastrocnemius muscle; *c*, sciatic nerve; *d*, pan for weights; *e*, marker recording on cylinder *g*; *f*, counterpoise, by moving which the weight of the framework may be reduced.

movable round a horizontal axis, and kept in equilibrium by a counterweight. The tendon of the muscle is fixed by a hook to the middle of the frame, and a balance for the purpose of carrying weights is attached underneath. From the other extremity of the brass framework there hangs a marker, which traces upon any surface the movements of ascent or of descent of the muscle. The registering surface may consist either of a stationary plate of smoked glass, as in the arrangement of Fick (see Fig. 15, p. 28), or of a vertical rotating cylinder (Fig. 256). When the tracing is obtained by Fick's method, it consists of a series of vertical lines, as seen in Fig. 16, p. 29.

A convenient form of myograph is the *spring myograph* of Du Bois-Reymond, shown in Fig. 249. This consists of a rectangular glass plate, *b*, moving horizontally along two slender steel wires, *d*. It may be impelled horizontally by the recoil of a steel spring, *c*, when a check is set free at the other end of the apparatus. Thus applied, the muscular contraction will produce a curve. (See Fig. 248.) By removing the rod

carrying the steel spring, the smoked glass plate may be slowly moved in front of the stylet by means of a long screw attached to the plate, the handle of which is seen a little below *c*. The spring myograph is shown on a larger scale in Fig. 248. See description of Figure.

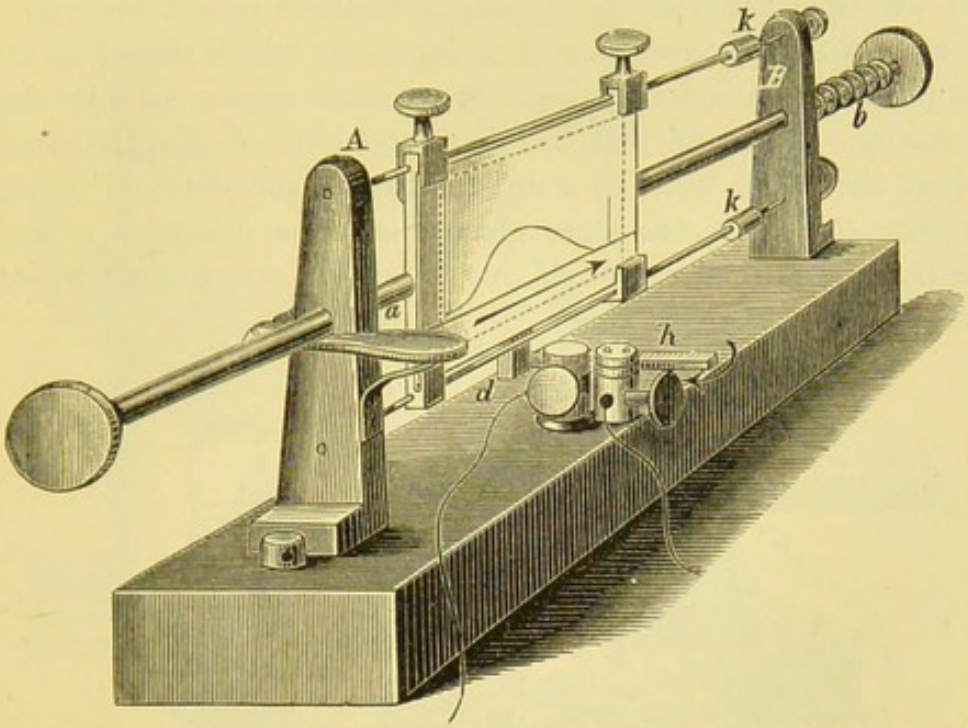


FIG. 248.—Spring Myograph of Du Bois-Reymond. *A B*, metal pillars to which the wires, *k k*, are attached, and along *k k* the frame carrying the glass plate slides in the direction of the arrow when the spring *b* is released; *d*, short bar of metal projecting from lower surface of frame which opens the key *h* when the frame is carried across by recoil of spring *b*. Thus the primary circuit of an inductorium may be mechanically interrupted.

In Von Helmholtz and Fick's arrangement, whether applied to a cylinder or to a moving glass plate, the muscle must be placed in a

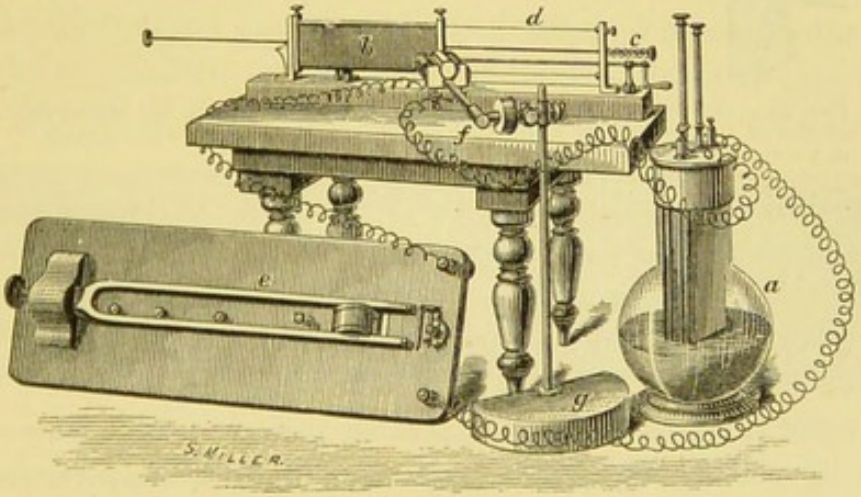


FIG. 249.—Spring Myograph, showing Chronograph applied to it for the purpose of ascertaining the velocity with which the blackened glass plate *b* is drawn across by the recoil of the spring *c*.

vertical position and have a certain weight to bear. Nor can the apparatus be conveniently applied to the muscle while *in situ* in the body

of the animal recently killed. There are thus a want of sensitiveness and a want of convenience, both of which are to a great extent obviated by the myograph of Marey. The arrangement of this apparatus for an experiment is seen in Fig. 250.

The principal piece of the apparatus consists of a horizontal brass plate supporting the axis of a registering lever, which moves in a horizontal plane. Consequently the lever registers upon a cylinder moving horizontally. A thread attached to the tendon of the gastrocnemius muscle of the frog is fixed by a small button to the lever. Fixed to the brass plate, and upon the same plane, there is a flat piece of cork on which the pithed or decapitated frog is laid (in which consequently *all sensation has been abolished*).

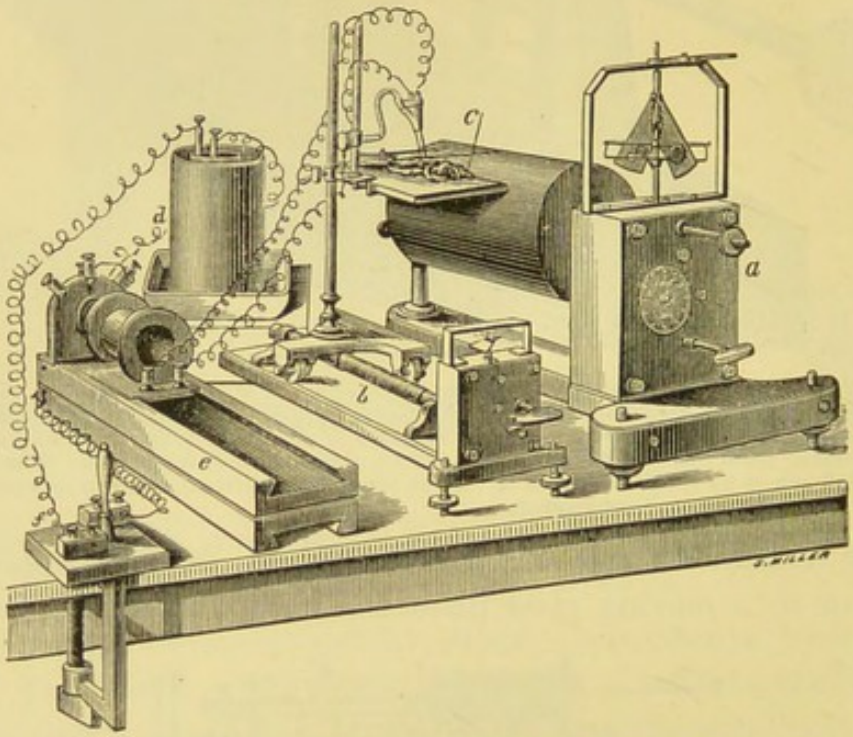


FIG. 250.—Arrangement of apparatus for experiment with the Myograph of Marey. *a*, recording cylinder; *b*, railroad carrying the myograph *c*; *d*, galvanic element; *e*, induction coil; *f*, key.

A view of the apparatus is shown in Fig. 251. Attached to the side of the cork plate, there is a brass support bearing the electrodes for stimulating the nerve or muscle. When the muscle is stimulated electrically, it contracts and moves the horizontal lever, which traces a curve upon the moving cylinder. If it be desirable to have a series of tracings from a muscle for a considerable period of time, these may be obtained by placing the entire apparatus upon a support, moved by clockwork upon a little railroad (see Fig. 251) parallel with the registering cylinder. Marey has also devised a double myograph, which differs from that just described only by having a second lever, so that the two gastrocnemei of a frog may be attached each to a lever; the two levers

are superposed, and the tracings are close together but still distinct. By such an arrangement it is possible to study graphically the forms of the curves obtained by the contractions of two muscles under different conditions, say the one poisoned and the other normal.

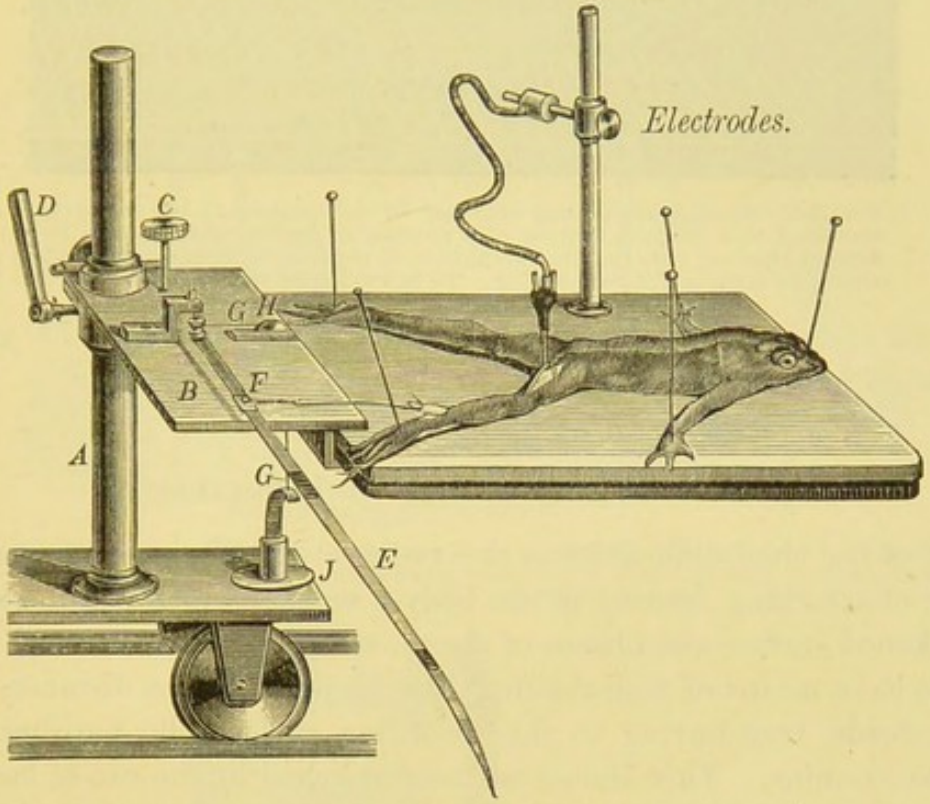


FIG. 251.—Marey's myograph. *A*, pillar; *B*, horizontal metal plate; *C*, screw for adjusting the height of the marker, so that it may, *E*, descend on the surface of a horizontally moving cylinder, by moving the lever, *D*; *H*, pulley for thread; *F*, thread from gastrocnemius to lever; *G*, *J*, spring for bringing lever back to original position during relaxation of muscle.

The great advantage of Marey's method is that it registers the curve of muscular contraction so as to give its different phases of movement. This will be appreciated upon comparing the two tracings seen in Figs. 252 and 253, which show the changes in muscular contraction under

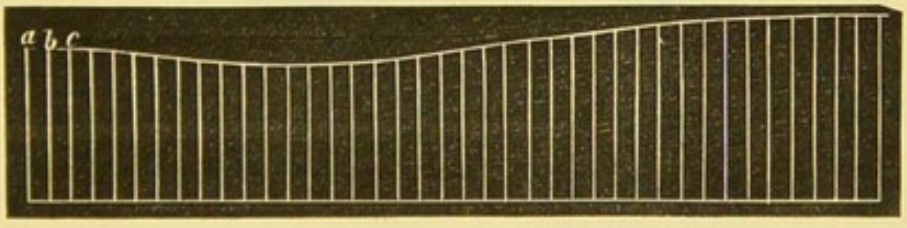


FIG. 252.—Myographic tracing obtained by the method of Fick: changes in the amplitude of contractions of a muscle under the influence of a gradually increasing temperature. *a*, *b*, *c*, individual contractions. To be read from left to right.

the influence of a gradually increasing temperature. It may be observed in both that the rise of temperature increases the amplitude of the contraction, but the vertical lines do not indicate whether or not any change has taken place in the duration of the contraction. It will be

seen, however, by studying Fig. 253 that heat not only changes the amplitude, but causes the contraction and relaxation to occur in shorter

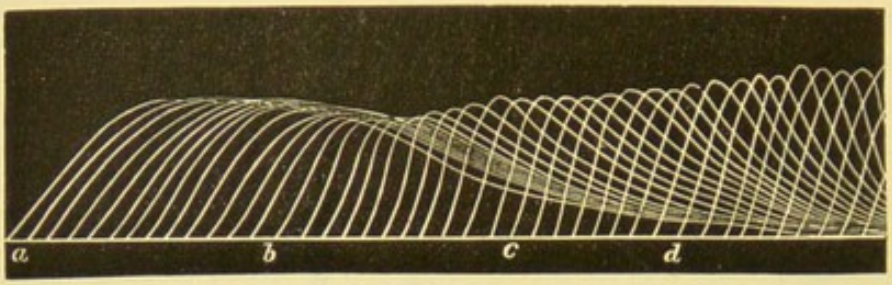


FIG. 253.—Myographic tracing obtained by the method of Marey, corresponding to a gradual heating of a muscle, as in Fig. 252. It will be noticed that not only does the amplitude of the contractions change, but also their form and their duration. To be read from left to right.

intervals of time. This is a good example of the value of the graphic method of registration.

3. The Transmission of Movement.

One of the chief difficulties in the registration of animal movements is that of attaching directly to the body a marker which will inscribe on a blackened surface the phases of the movement. It is therefore necessary to have means of transmitting the movement to a distance, or, in other words, transferring to the recording surface the movement we wish to examine. This Marey has accomplished by the use of *tambours*, or drums, united by tubes containing air. One of these tambours is seen in Fig. 254. It consists of a shallow metallic case, or drum, the upper surface of which is formed by a thin indiarubber membrane sup-

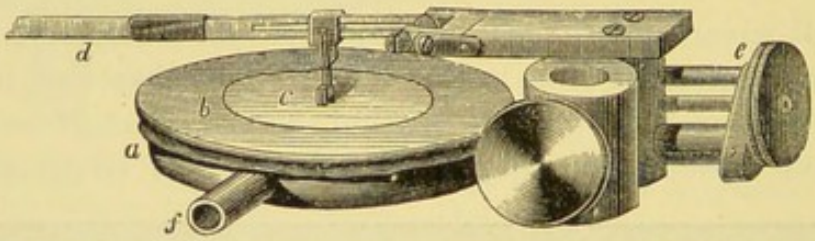


FIG. 254.—Tambour of Marey. *a*, metallic case; *b*, thin indiarubber membrane; *c*, thin disk of aluminium supporting the lever, *d*, only a small portion of which is represented; *e*, screw for adjusting the support of the lever vertically over *c*; *f*, metallic tube communicating with the cavity of the tambour for attachment to an indiarubber tube.

porting the writing lever. Two tambours, each having a short metallic tube communicating with its interior, may be connected together by carrying an indiarubber tube from the one metallic tube to the other, as represented in Fig. 255.

When the membrane of the first tambour is depressed, part of the air which it contains is expelled; this air passes through the indiarubber tube into the second tambour, the membrane of which, carrying the lever, is elevated. On the contrary, when the first tambour is not acted

upon, the membrane of the second returns to its former position. The first may be termed the *receiving*, and the second the *registering*, tambour. It will be observed that, as above arranged, the movements of

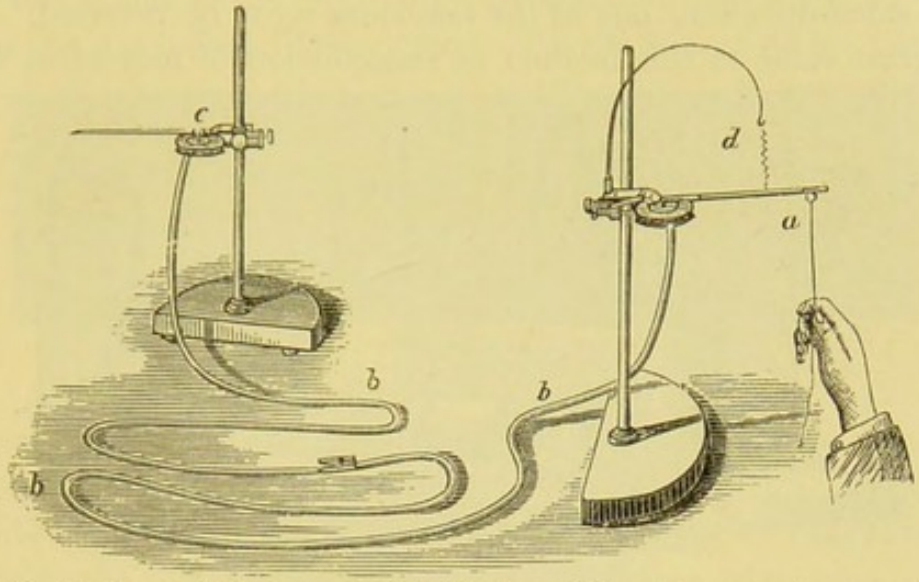


FIG. 255.—Tambours of Marey arranged for the transmission of movement. *a*, receiving tambour; *b*, indiarubber tube; *c*, registering tambour; *d*, spiral of wire, by the elasticity of which, when the tension is removed from *a*, the lever ascends.

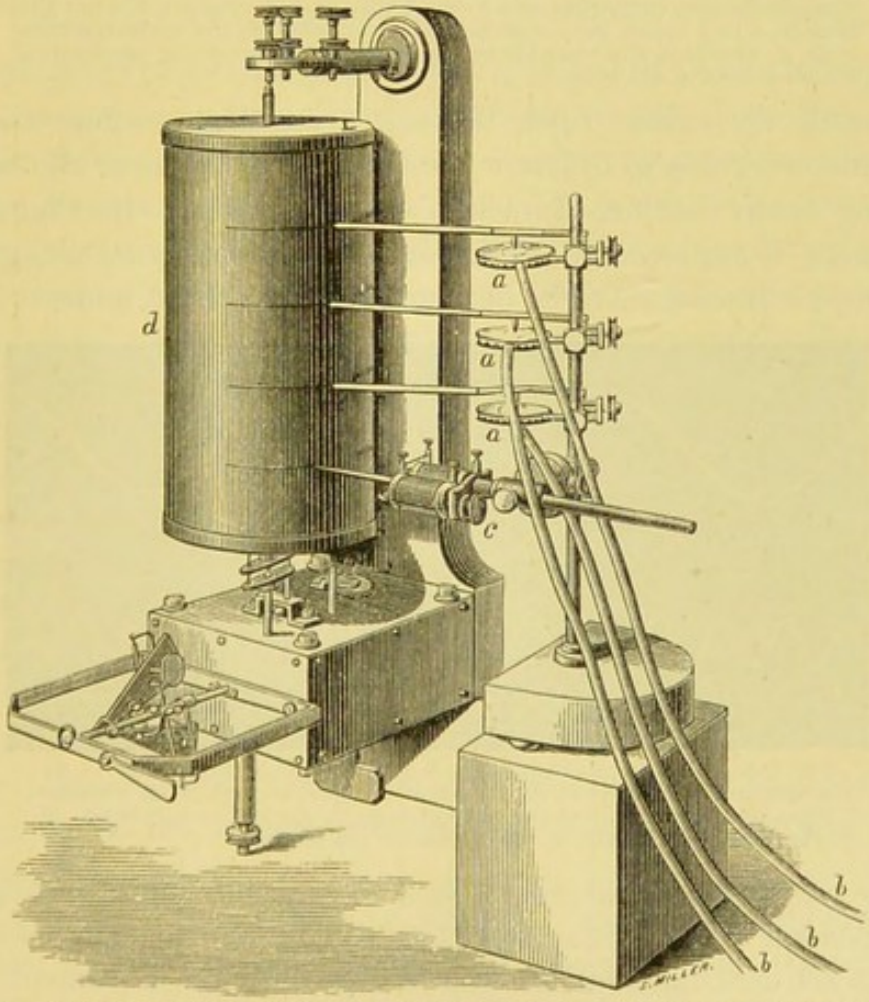


FIG. 256.—Arrangement of registering tambours, *a*, *a*, *a*, for recording three movements on cylinder *d*. The tubes communicating between the recording and registering tambours are seen at *b*, *b*, *b*. The chronograph is represented at *c*.

the registering tambour are in opposite directions: when the membrane of the one is drawn downwards, that of the other is forced upward. When it is desirable to obtain the movements of the levers in the same direction, one of the tambours must be reversed. What lends great value to this method of transmission of movement is that

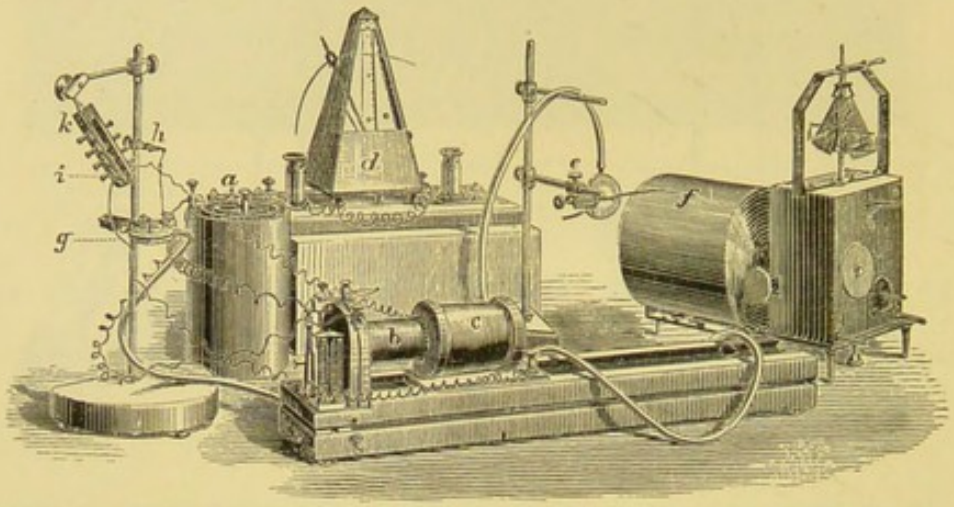


FIG. 257.—Arrangement of apparatus for transmission of muscular movement by tambours. *a*, voltaic element; *b*, primary coil; *c*, secondary coil of inductorium; *d*, metronome for interrupting primary circuit, when induction current is sent to electrodes *k*; *h*, forceps for femur; the muscle, which is not here represented, is attached to the receiving tambour, *g*, by which movement is transmitted to the recording tambour, *e*, which writes on the cylinder, *f*.

it is possible to register upon the same recording surface a series of movements occurring in different localities, so as to have the means of comparing their rhythm, duration, and character. In Fig. 256, an arrangement is represented by which three movements, along with a chronographic tracing, may be obtained on the same cylinder.

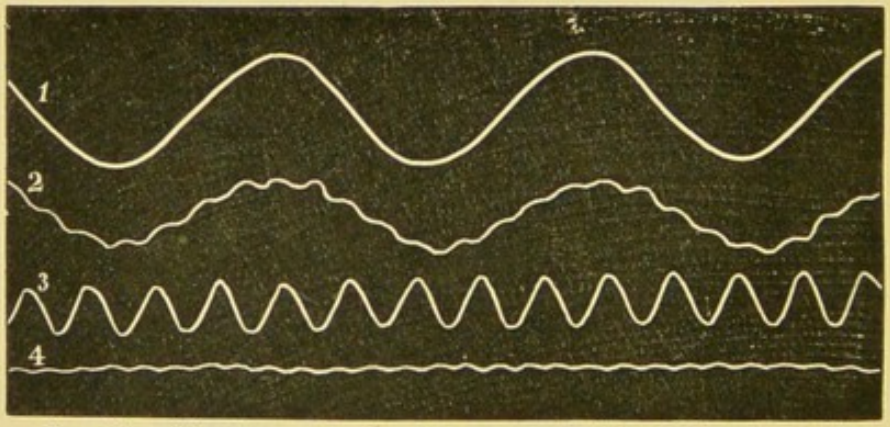


FIG. 258.—Vibrations of tuning-fork transmitted by tambours. 1, 10 vibrations per second; 2, 10 vibrations per second, with smaller vibrations at rate of 80 per second seen on the larger curves; 3, 50 vibrations per second; 4, 100 vibrations per second.

Marey has also applied his method of transmission of movement to the phenomena of muscular contraction. By causing the contracting muscle to act on a tambour, as seen in Fig. 257, the movement may be carried to a distance to a recording tambour in connection with a cylin-

der. By this ingenious method it is possible to subject the muscle to different temperatures, or to the action of various gases, without interfering in any way with the recording apparatus.

Numerous adaptations of Marey's method will be pointed out in discussing the physiology of the organs. It suffices to state here that the

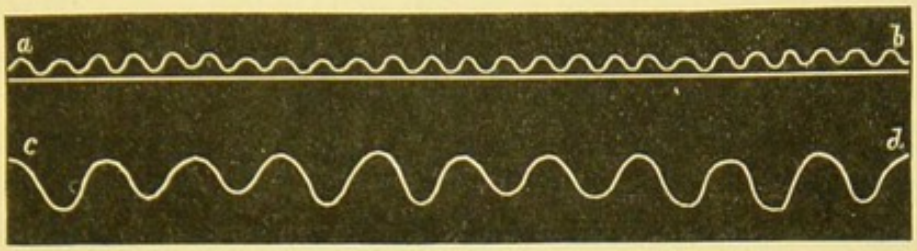


FIG. 259.—Tracing taken with tambours, showing in line *a* beats of the anterior fontanelle of an infant of 6 weeks during sleep; and in line *c* the thoracic movements in breathing.

tambours, when in good order, are remarkably sensitive. Thus they may record even the vibrations of a tuning-fork as shown in Fig. 258, and they can record the delicate pulsatile movements seen in the anterior fontanelle of a child's head (Fig. 259).

CHAP. V.—THE PHYSICAL PROPERTIES OF MUSCLE.

The physical properties of muscle which are of physiological interest are consistence or degree of firmness, cohesion, and elasticity.

1. *Consistence.*—When a muscle is in the contracted condition, it is hard and resistant, and when it is relaxed it is soft. The matter within the sarcolemma is soft and jelly like, but in the state of rigidity which follows death it undergoes a change and becomes firm and almost solid. The semi-fluid condition of the matter of living muscle is indicated by various phenomena. Thus, it may be squeezed out of the sarcolemma by the use of a compressor whilst under the microscope. Wave-like movements, indicating a semi-fluid condition, occur in living muscle. A parasitic worm (*Myorectes Weismanni*) has been observed to push its way through the jelly-like matter of a living muscular fibre, not by boring a tunnel as it would have done in a solid substance, but moving freely as if it were in a viscous mass. Streaming movements or currents have also been detected, flowing from the positive to the negative pole, when a voltaic current was passed from end to end of a living fibre. This is termed *Porret's experiment*, and shows clearly that the physical constitution of the muscle substance is semi-fluid like that of other varieties of protoplasm.

2. *Cohesion.*—The cohesion of muscular tissue is much more feeble than that of the connective tissues, and especially than that of tendon. According to E. Weber, a square centimetre of frog's muscle will support

the weight of a kilogramme without rupture. The disappearance of muscular irritability, which occurs as a muscle gradually dies, is accompanied by a diminution of cohesion. The gastrocnemius of a dead frog, for example, which has lost its irritability, may be torn across by a weight of about 260 grammes, while a similar muscle from a frog just killed may sustain without rupture a weight of 1½ kilogramme. This experiment not infrequently fails from the muscle being torn across at the junction of the muscular fibre with the tendon.

3. *Elasticity.*—The elasticity of *living* muscle in a state of *rest* is small, but nearly perfect; that is, the muscle will elongate easily under the influence of very small weights, and will return on their removal exactly to its former length. We may readily study this physical question by using the myograph of Von Helmholtz. The amount of stretching caused by different weights, placed in the pan (see Fig. 15, p. 28), may be readily recorded on the smoked glass plate. It will then be seen that the elongations of the muscle are not proportional to the weights which stretch it. A rough experiment may show that the amount of stretching is proportional to the amount of heavier and heavier weights; but careful observation proves that the amount of extension or stretching diminishes as the weights increase, so that the curve of muscular extensibility approaches that of a *hyperbola*, and is not a straight line as is the case with most inorganic bodies. To obtain the curve, mark in a horizontal line, as abscissæ, successive units of weight, and from these draw lines or ordinates of lengths corresponding to the amount of extension. A curve touching the ends of these ordinates will be found to be the curve of a hyperbola. In other words, the *amount* of the elasticity of a muscle increases as it is stretched. The elasticity of *dead* muscle is very much greater than of living muscle, but it is imperfect. That is to say, a dead muscle requires a greater weight to stretch it to a given amount, and when the weight has been removed it remains elongated to a considerable extent.

The *limit* of elasticity of living muscle is readily passed. Marey has shown that the gastrocnemius of a frog, weighted with 100 grammes, will not return to its primitive length.

According to Weber, the elasticity of an *active* muscle, that is of a muscle in a contracted condition, is diminished. He arrived at this result by tetanizing the *hyoglossus* muscle of a frog securely fixed at one end and having weights attached to the other. He compared the amount of elongation during the state of contraction with elongations caused by the same weights upon the same muscle in repose. He found, by this method, a greater elongation in the contracted muscle produced by a weight of one or two grammes than when the same weights were

attached to the muscle in repose. He also made the following curious observation, often termed *Weber's paradox*, that if a muscle in repose is heavily weighted, and then stimulated, it may become longer instead of shorter; that is to say, the shortening due to contraction is not sufficient to compensate for the elongation due to diminution of the elasticity. Volkmann pointed out, after an elaborate research, in which he did not employ tetanic excitations, as Weber had done, but isolated opening induction shocks, that Weber had exaggerated the diminution of elasticity in active muscle, and that the results are largely affected by the condition of fatigue. Wundt has also arrived at results contrary to those of Weber. He adopted the method of arresting by an overweight the contraction of a muscle, and he found that the diminution of muscular elasticity does not depend upon the state of activity, but simply upon shortening of the muscle. He says, "If the diminution of elasticity depends upon the state of activity, it would follow in my mode of experimenting, that at the moment of excitation there ought to be an elongation of the muscle, its elasticity having been diminished—a result which never happens." (Wundt.) He arrives at the conclusion that the elasticity of active muscle is practically the same as that of muscle in repose. One can conceive a muscle so loaded that at the moment of contraction no change in its length could take place, because the energy tending to shorten the muscle, that is its contractile power, would be exactly equal to the energy tending to lengthen it, against its elastic force. These results have been confirmed by the research of Donders and Van Mansveldt, conducted upon the flexor muscles of the fore-arm of man. After a muscle has been once stretched by a considerable weight, and the weight has been removed, it may not return to its original length for days or weeks. This is called an *elastic after effect.* This is especially noticeable after the use of very heavy weights. To recapitulate: (1) the elongation of muscle is within certain limits proportional to the weight; (2) the modulus of elasticity is very nearly the same for different degrees of contraction; and (3) fatigue and defective nutrition diminish elasticity. (Roy.)

According to Marey, the elasticity of muscle favours the economical expenditure of muscular work, in virtue of a law which he thus formulates: when a force of short duration is employed to move a mass, a more useful effect is obtained when it acts upon this mass through the intervention of an elastic body.[1] The feeble elasticity of muscle is such as to oppose very little resistance to antagonist muscles, and when the contraction of the antagonist ceases, it restores the muscle to its natural length without loss of force.

[1] Marey, *Physiologie Expérimentale*, 1875.

In the living being, muscles are more or less stretched between their two attachments. Thus they are in a state of tension or *tonicity*, sometimes termed *muscular tonus*, it may be by the contraction of antagonist muscles, or even by the weight of parts of the skeleton or of soft parts. When a muscle is divided transversely, or its tendon is cut, it therefore immediately contracts, and the two parts separate to some extent from one another. Thus a muscle, even at rest, is in a favourable condition for producing by its contraction a given movement without loss of energy. A muscle is always *taut*, never in a state of looseness, and it is therefore ready to efficiently exert mechanical force the moment it begins to contract. The sphincters are the only muscles which, during repose, do not appear to be stretched. Their tonicity operates only when they are dilated.

Various controversies have arisen upon the question as to whether or not muscular tonus is under the influence of the nervous system. Brondgeest divided the spinal cord of a frog below the *medulla oblongata*, and then divided the nerves of the leg on one side; on fixing the frog firmly against a board, he found that the muscles of the leg on the side of the operation were loose and flaccid, and that this leg was longer than the other leg. He therefore concluded that the spinal cord furnished the muscles, especially the flexors, with permanent innervation. Heidenhain, on the other hand, found that when a muscle is stretched by a weight, it does not elongate after section of the nerve supplying it. The probability is that both innervation and the circulation of the blood have a certain influence upon muscular tonicity, by affecting the nutrition of the tissue.

Chap. VI.—MUSCULAR IRRITABILITY.

By this phrase is meant the property which living muscle possesses of responding to a stimulus, the visible indication of a response being a change of form or a contraction. The term *excitability* is sometimes employed to denote the same fact. Irritability is a property inherent in the muscular fibre, a doctrine first clearly enunciated by Haller. The best experimental proof of this fact was first given by Claude Bernard. He showed that if we poison an animal with curare, stimulation of the motor nerves produces no effect, while the direct excitation of the muscle substance is at once followed by a contraction. It might be objected to this experiment that the persistence of irritability may depend on the integrity of the motor end-plates of the muscle, which may not have been affected by the poison; but, as was first shown by Kühne, muscular fibres exist in which he could not find motor end-plates, but these were still capable of contracting

on the application of a stimulus. Bernard's experiment may be readily shown if we ligature the sciatic artery in the leg of a frog and then inject a minute dose of a solution of woorari or curare[1] under the skin of the back by means of a hypodermic syringe, such as is shown in Fig. 260. The ligature round the artery prevents the poison from entering the tissues of the leg supplied by it. Curare is known to have the effect of paralyzing the motor end-plates in muscles. (See p. 358.) After sufficient time has been allowed to elapse for the poison to act, an indication of which may be seen in the animal being completely paralyzed, two ordinary nerve-muscle preparations are made, similar to that shown in Fig. 261, the one from the poisoned and the other from the unpoisoned limb. Each limb is connected with a useful apparatus for demonstration, shown in Fig. 262, termed *a muscle telegraph*. The two telegraphs are placed close beside each other, so as to make it possible to stretch the two sciatic nerves across Du Bois-Reymond's platinum electrodes, Fig. 229, p. 379, and arrangements are made by the use of Pohl's commutator (Fig. 228, p. 378), so that an induction shock may be transmitted either to the two muscles or to the two nerves at pleasure. It will then be found that when both nerves are stimulated only the muscle of the limb that received no poison will contract, but, on the other hand, when the shock is sent through both muscles, both contract, that is to say, the muscle in which all the nerve-endings have been paralyzed by curare has still the property of irritability. It may also be shown that the muscles of the limb that received no poison will respond to a feebler induction shock than that necessary to excite the muscles of the poisoned limb. This is due,

FIG. 260.—Hypodermic syringe. Observe the piston rod is graduated.

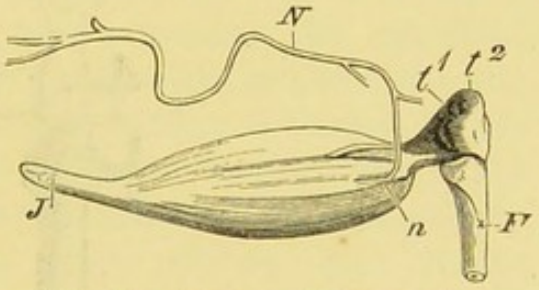


FIG. 261.—Nerve-muscle Preparation. *F*, femur; *N*, nerve; *J*, aperture for hook.

[1] Curare, curara, ourali, woorali, woorari, urari, tigunas, or Indian arrow-poison is the inspissated juice of *strychnos Crevauxi* (?). It contains an alkaloid named curarin. A dose of 2 to 5 m.gms. is sufficient for the experiment above described. An extract or solution is made by dissolving it in water with the aid of a few drops of alcohol and of glycerine. The strength of the solution should be 1 per cent.

as was first shown by Rosenthal, to the nerve-endings in the sound limb being more excitable than the muscle substance, so that a feeble shock sent through the muscles of both limbs may excite only the sound limb

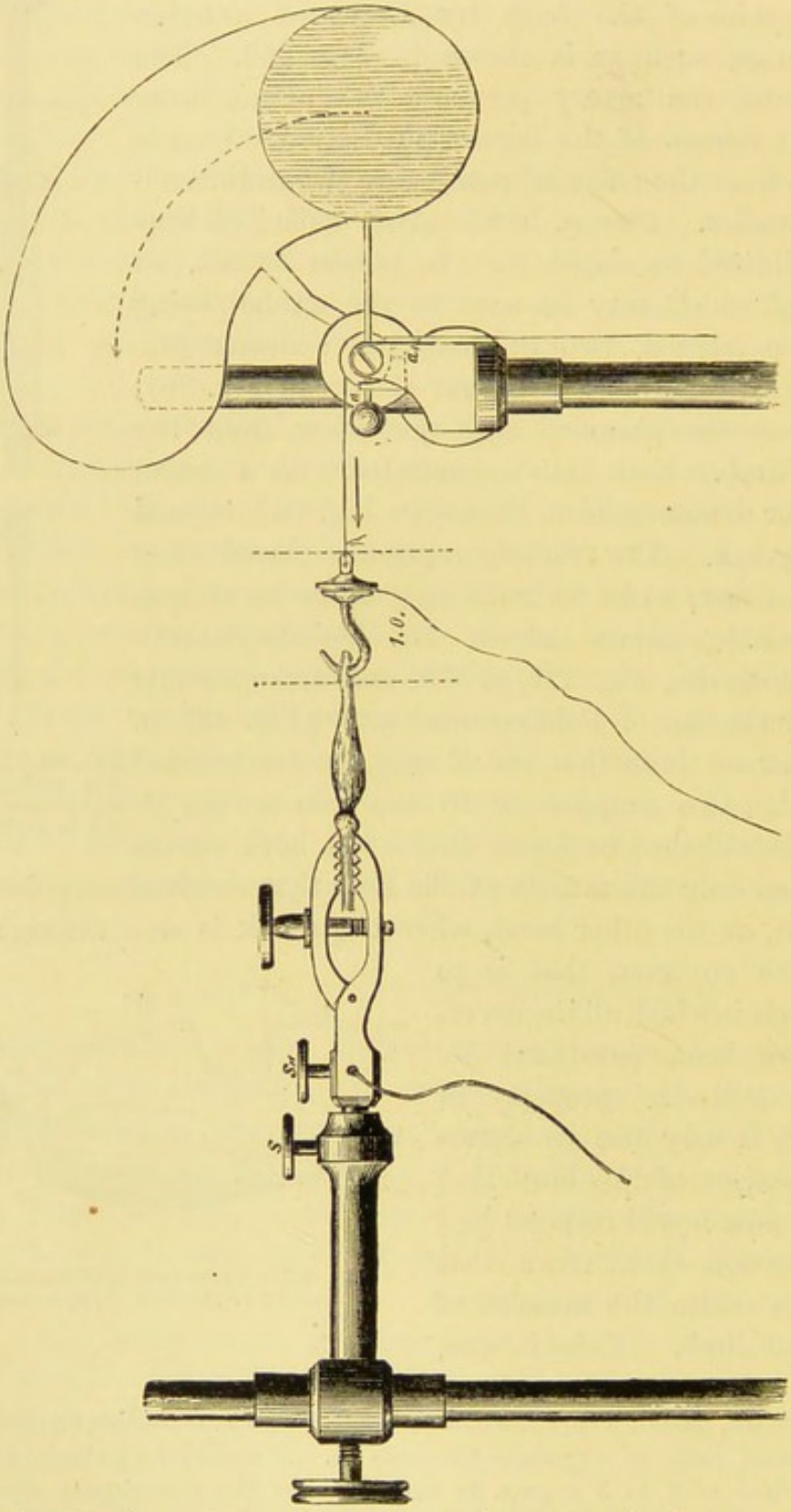


FIG. 262.—Muscle Telegraph, showing the gastrocnemius muscle in position with the wires from the battery or inductorium arranged so that currents will pass through the muscle. The thread passing over the pulley on the right has at its lower end a small pan containing hot to act as a weight.

by irritating its nerve-endings, but when the shock is made stronger, then it is sufficient to excite the protoplasm of both muscles, and then both contract.

The view of the inherent irritability of muscle is also supported by many other facts. Thus sulphocyanide of potassium abolishes irritability without affecting the activity of the nerve, and carbolic acid and ammonia, directly applied, excite the muscle substance without irritating the nerve; electric currents of very short duration may excite the nerve without exciting the muscle; and irritability may last several weeks after section of the motor nerves, while the excitability of the nerves is lost in about four days. Dr. John Reid showed that after having exhausted the nerve supplying a muscle by repeated shocks so that the muscle no longer responded to the nerve, it was still possible to cause the muscle to contract by its direct stimulation. To this experiment there is the objection that the end-plates may not have been completely exhausted by the shocks applied to the nerve. Finally, irritability may be observed in structures having no nerves and consisting only of protoplasm, such as the contractile tissues of plants, the bodies of many of the protozoa and cœlenterata, the hearts of many invertebrates, the fœtal heart, the colourless blood corpuscles, spermatozoids, and cilia. When we remember that the essential constituent of muscle is protoplasm, one of the prominent phenomena of which is its capability of responding to a stimulus, it requires little evidence to convince us of the truth of Haller's doctrine.

As already explained at p. 29, in discussing the relations of energy to living structures, stimuli are to be regarded as liberators of energy. The protoplasm of muscle is not to be regarded as endowed with a special property in the sense of a metaphysical entity, but the use of the word irritability merely indicates that muscle is a substance in which molecular disturbances may be started by various kinds of stimuli. Irritability is simply the term expressing this condition. With this explanation of what is meant by irritability and by a stimulus, we now proceed to state that muscular irritability is excited by the stimulus propagated along a nerve, or by nervous action. This is the normal stimulus; but muscle may also be stimulated mechanically by tension or stretching, by percussion, or by pricking, etc.; by the action of electricity and heat; or chemically by such agents as ice-cold water, weak solutions of metallic salts, dilute acids, alkaline chlorides, ammonia, etc. The effects of these agents will be afterwards discussed.

Muscular irritability may be increased or conserved for a considerable time by the following conditions:—(1) by an afflux of blood: if the lumbar nerves of a frog are divided on one side, the capillaries become dilated from paralysis of the vaso-motor nerves, and the irritability of the muscles is then greater than on the uninjured side; section through

one half of the *medulla oblongata* and of the *corpora bigemina* is followed by hyperæmia, or increase of blood, and increase of irritability in one half of the body; (2) by rest, that is to say, after a muscle has become exhausted so that its irritability has been much reduced, it will recover after a period of inactivity; (3) by the presence of oxygen; muscles retaining their irritability longer in oxygen than in ordinary air, and still longer than in air deprived of its oxygen; the injection of oxygenated blood into a limb entirely separated from the body maintains irritability for a considerable time; (4) by the effect of gentle stimulation, so that while a muscle may not respond to the first or second of a series of feeble stimulations, it may contract with the third or fourth, and then there may be a number of contractions greater than the first one, showing that the irritability has become increased; (5) by the action of certain substances, such as theine and veratrin; and (6) by the passage through the muscle in a longitudinal direction of a very feeble continuous current of electricity.

Irritability is diminished or destroyed by (1) arrest of the circulation; (2) fatigue; (3) a temperature considerably above or below the mean temperature of the muscle, which varies in different species of animals; (4) the presence in excessive quantity in the muscle of such substances as carbonic acid, lactic acid, phosphate of lime, and certain alkaloids. It is said that muscular irritability is almost instantaneously destroyed by large doses of sulphocyanide of potassium, all the salts of potash, the bile, emetine, upas antiar, etc.

After division of the nerves supplying it, and when the circulation is cut off, the irritability of a muscle, as a rule, diminishes during the first four or five days. Then follows a period of several weeks (from 6 to 8) in the human being, during which there is an increased irritability to mechanical stimulation and to the opening and closing shocks of a constant voltaic current, while induction shocks from an inductorium have little or no effect. This stage merges into one in which the irritability slowly diminishes until it may entirely disappear.

Irritability disappears more quickly after death from warm-blooded than from cold-blooded muscles. The muscles of many amphibia, if kept in a cold place, will remain excitable for eight or ten days. According to Brown-Séquard, irritability lasted in the muscles of the following animals for the following periods—

Rabbit,	8½ hours.
Sheep,	10½ ,,
Dog,	11¾ ,,
Cat,	12½ ,,
Frog,	24 to 40 ,,

About 1½ to 3 hours is the average time during which most of the muscles of warm-blooded animals remain irritable. In man it has been observed to last for even so long a period as 26 hours after death, but in most cases it disappears in the course of five or six hours. The muscle of the heart remains longer irritable than any other muscular structure, continuing, even in the human being, for two hours. It vanishes quickly from the muscles of persons who have died from exhaustive diseases, but it may last a long time in well nourished muscles.

CHAP. VII.—GENERAL PHENOMENA OF MUSCULAR CONTRACTION.

When a muscle, say the gastrocnemius, removed from the leg of a recently killed frog, is caused to contract, it assumes the form of a globular mass which is about one third of the total primitive length of the muscle. In the living body, however, the shortening is never so great, as the two ends are stretched by the elastic force of the antagonist muscles and the resistance at the points of insertion. The *amount of contraction* of each muscle depends upon its length, and, for a given muscle, the shortening increases with the intensity of the stimulus, and diminishes with the fatigue of the muscle. With the same strength of stimulus, and supposing the muscle not to be fatigued, an elevation of the temperature up to 33° C. increases the amount of contraction. Above this point, the amount of contraction diminishes.

When a muscle contracts, the *absolute volume* diminishes very slightly, so that the specific gravity of the muscle is 1062, instead of 1061, in the inactive state. This may be demonstrated by placing the muscle in a small bottle filled with water, through the walls of which two fine platinum wires are fused for the purpose of conveying an electric stimulus to the muscle. The stopper of the bottle is drawn out into a long capillary tube, in which the fluid in the bottle ascends. It will then be found that, on causing the muscle to contract, the fluid in the capillary tube, when examined with a microscope, descends very slightly—that is to say, there is a diminution in volume, by about the 1000th part.

Another mode of observing muscular contraction is to examine under the *microscope*, with a magnifying power of 300 diameters, a living muscular fibre removed from the leg of an insect. On stimulating the fibre at one end, an undulation will be observed to travel along the whole length of the fibre, and the transverse striæ approach each other. This phenomenon will be best observed when the muscle is slightly stretched. On adapting the polarizing apparatus to the microscope, it

will be observed, as has been already stated, that the anisotropic portion, or sarcous element, is the seat of the contraction (Fig. 166, p. 311). From a careful observation of the appearances seen with polarized light, Engelmann is of opinion that, during contraction, the singly refractive portions shrink, while the doubly refractive become larger. I have been unable to observe this phenomenon, but the movement seemed to me to consist in a change of form of the anisotropic portion. During contraction, the latter became broader in proportion to its shortening. As to the notion that at any period the isotropic more fluid part penetrates the anisotropic portion, I have seen nothing to lend it any support.

A.—Phases of a Single Contraction or Twitch.

The phenomena already mentioned may be noticed by the unaided eye or by the use of the microscope, but as they do not include all the phases of muscular contraction, many of which are of very short duration, recourse must now be had to various methods of accurate experiment. These are conducted with the aid of myographs, by which a graphic representation of the curve of muscular contraction may be obtained. The arrangement of the apparatus in this experiment is shown in Fig. 250, p. 392. (See also Fig. 251, p. 393.)

The experiment is conducted as follows—The gastrocnemius muscle, the contraction of which is to be studied, is fixed by its tendon to the lever of the myograph (*c*), and the nerve is brought into contact with the electrodes; the recording cylinder (*a*) is then allowed to rotate, and when it has attained sufficient velocity, the key (*f*) in the primary circuit of the coil (*e*) is opened; a single induction shock is thus transmitted to the nerve, which causes the muscle to contract and to describe, by moving the lever of the myograph, the curve *a c d e*, in Fig. 263.

On analyzing this curve, we find it to be composed of three unequal periods—

(*a*) Suppose the muscle has received the shock at *b*, there is a short period represented by the line from *b* to *c*, in which no contraction occurs. This time, usually about the $\frac{1}{100}$ of a second, is called the *period of latent stimulation*. Its commencement and termination are easily registered if we interpose in the primary circuit a signalling apparatus, which would draw on the cylinder the line *f g h i k;* while the time is recorded by a chronographic tracing *l m*. It is during this period, short as it is, that molecular changes occur in the muscle preparatory to contraction, and, as will afterwards be seen, an important electrical phenomenon, the *negative variation* of the muscle current also takes place. The latent period in human muscles, and probably also in the muscles of mammalia in general, is shorter, varying from ·004

to ·01 of a second. The application of heat and an increase in the strength of the stimulus increase, and an increasing weight, cooling, and fatigue diminish, the length of the period. The period is longer in red than in pale muscles. When metabolism in the muscle is interfered with, either by changes in the muscle substance itself, or by changes in its relation to the stimulus of the nerve, the latent period is increased. Thus it is lengthened in paralysis agitans, progressive locomotor ataxia, muscular atrophy, and in cases of degeneration of the spinal cord after apoplexy. It is said to be shortened in senile chorea and tabes dorsalis. (For results obtained by Yeo and Cash, see Appendix III.)

(*b*) A second period, corresponding to the *contraction* of the muscle, indicated by the ascent of the curve *c d*, the duration of which varies from ·03 to ·4 of a second. A fresh active muscle contracts more rapidly than a fatigued one.

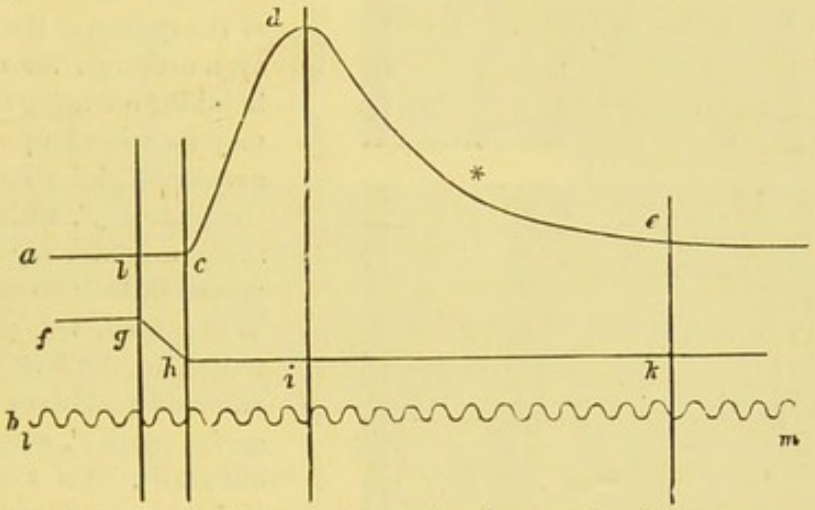


Fig. 263.—Curve of a muscular contraction, produced by a single induction shock. *a b c d e*, line drawn by the lever of the myograph; *f g h i k*, line drawn by electric signalling apparatus; *l m*, chronograph registering 100 vibrations per second.

(*c*) A third period of *relaxation*, represented by the descent of the curve *d e*, in which the muscle returns to its primitive length; this period is usually shorter than the second, but in the tracing shown in Fig. 263 it is longer, because the strength of the stimulus employed was rather strong. The descending curve should have reached the level of the line *a c*, about the position of the asterisk (*). In most cases, one or two short vibrations may be seen after the end of the relaxation of the muscle. These have been termed *residual contractions*, or *after contractions*. They are due to the elasticity of the muscle, and are consequently most pronounced when the muscle has made a strong contraction and has lifted only a small weight. They are often seen in tracings taken with the rapidly moving pendulum myograph (Fig. 264). On examining the curve, we see that the height of the line *d i*, indicates the amount of contraction.

The curve of a single muscular contraction may also be recorded on the pendulum myograph, shown in Fig. 264. It consists of a stout board *A A* fixed to the wall of the laboratory. This carries a strong rectangular metal frame *C C C*, having poised on it, on friction rollers, at *a* and at the other end, an axle *E E E* from which is suspended by stout double rods *F F F F*, a pair of heavy glass plates *g g* and *g' g'*, which are smoked in the flame of a paraffin lamp before a tracing is taken. These constitute the bob of the pendulum, and to maintain the centre of gravity of the system at the same place, even although for convenience in taking a tracing the plate *g g* may be moved up or down by a rack and pinion movement *b c*, the plate *g' g'*, which is of the same weight as *g g*, may be moved in the reverse direction. When used, the pendulum is pulled to the left towards *H*, and it is held in position by the spring catch *e'*, which is turned outwards. On the pendulum making a swing, it is caught at the extreme limit of its swing by the catch *e'*, which is also turned outwards. The under surface of the frame carrying the glass-plates has two little projections *f f'*, each moving on a delicate hinge, and when the pendulum swings across, these strike the arm *b* of a break-mechanism placed immediately below *f f'* in the position occupied by these in Fig. 264. This break-mechanism is shown in Fig. 265. In newer forms of the myograph than that shown in Fig. 264, the break-mechanism slides on a horizontal bar, so that it can be brought into operation at any

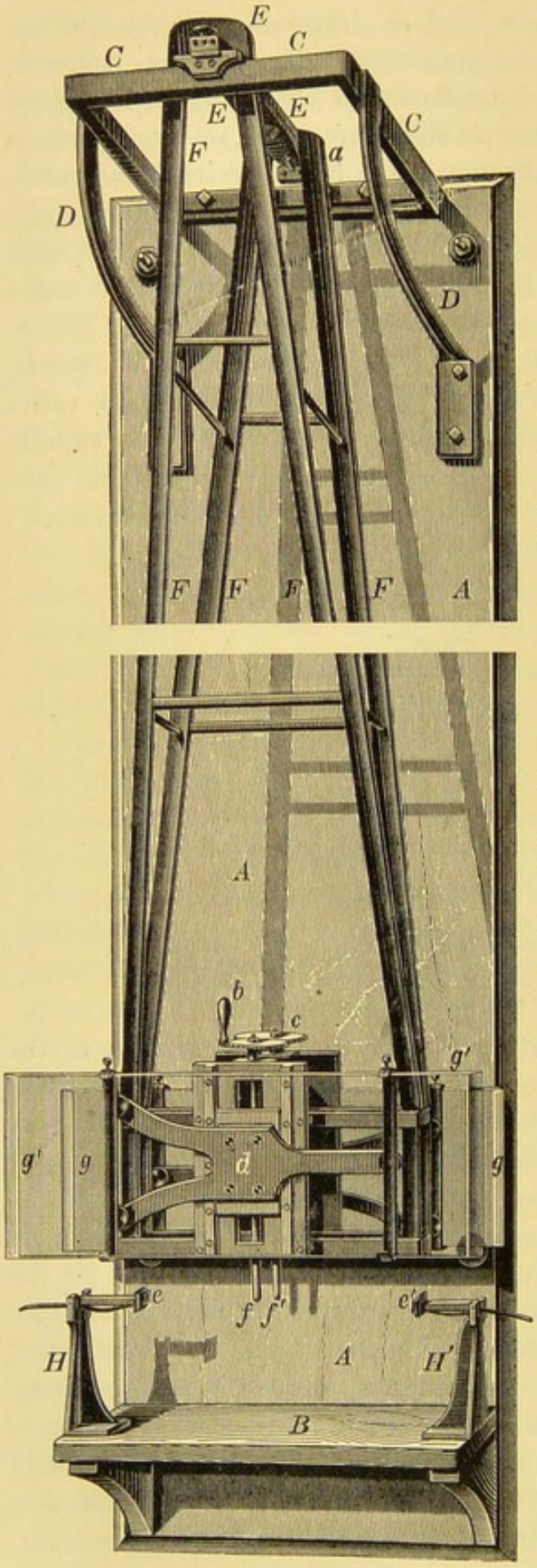


Fig. 264.—Pendulum Myograph. For description, see text.

point in the swing of the pendulum. The break-mechanism is simply a little arm *b*, Fig. 265, which is knocked aside by *ff'* in Fig. 264. The primary circuit of an inductorium passes from the binding screw *d*, Fig. 265, through pillar *g* to *a*, thence through *b* (when in contact with *a*), and then back to the battery, and *f* is insulated from the rest of the instrument. When *b* and *a* are in contact, the primary circuit is formed, but when *b* is knocked away from *a* by *f* in Fig. 264, it is opened. The opening shock of the inductorium is sent to the nerve-muscle preparation connected with a myograph placed in front of the front glass-plate, the muscle contracts, and a curve is described on the glass-plate, such as is shown in Fig. 266. As this instrument has been of great service in measuring

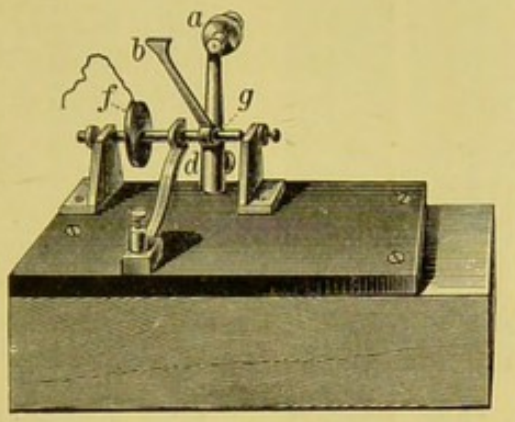


FIG. 265.—Break-Apparatus of the Pendulum Myograph.

the rate of the transmission of the nerve current, it will be further described when we consider the functions of nerve in Vol. II. The pendulum makes a complete swing from one catch *e*, Fig. 264, to the other catch *e'* in one second, and the time of any part of the swing may be recorded by means of a chronographic tracing, as shown in Fig. 266.

The pendulum myograph is usually provided with two "break" mechanisms, similar to that shown in Fig. 265, but so arranged that the one is opened by the swing of the pendulum a little sooner than the other. Thus, by having two circuits, a nerve may be stimulated by one induction shock, and then by another happening the $\frac{1}{100}$th of a second later, and the muscle-curve may then be similar to that shown in Fig. 276, p. 416. The instrument has also been used by Burdon-Sanderson as a *rheotome*, that is an instrument for opening and closing two independent circuits, at very short periods

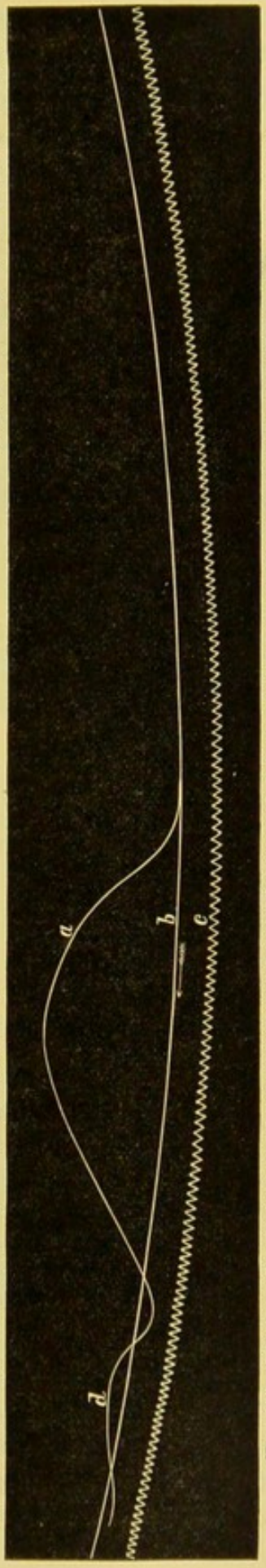


FIG. 266.—Tracing of a single muscular contraction taken with the pendulum myograph. *a*, muscle curve; *b*, basement line; *c*, chronographic tracing, 200 vibrations per second. The ← indicates the direction of movement of the glass-plate. Reduced to half natural size.

of time.[1] Thus the time relations of various electrical phenomena may be investigated.

For purposes of comparison, it is often important to obtain a series of curves in juxtaposition. We may get this by using the travelling

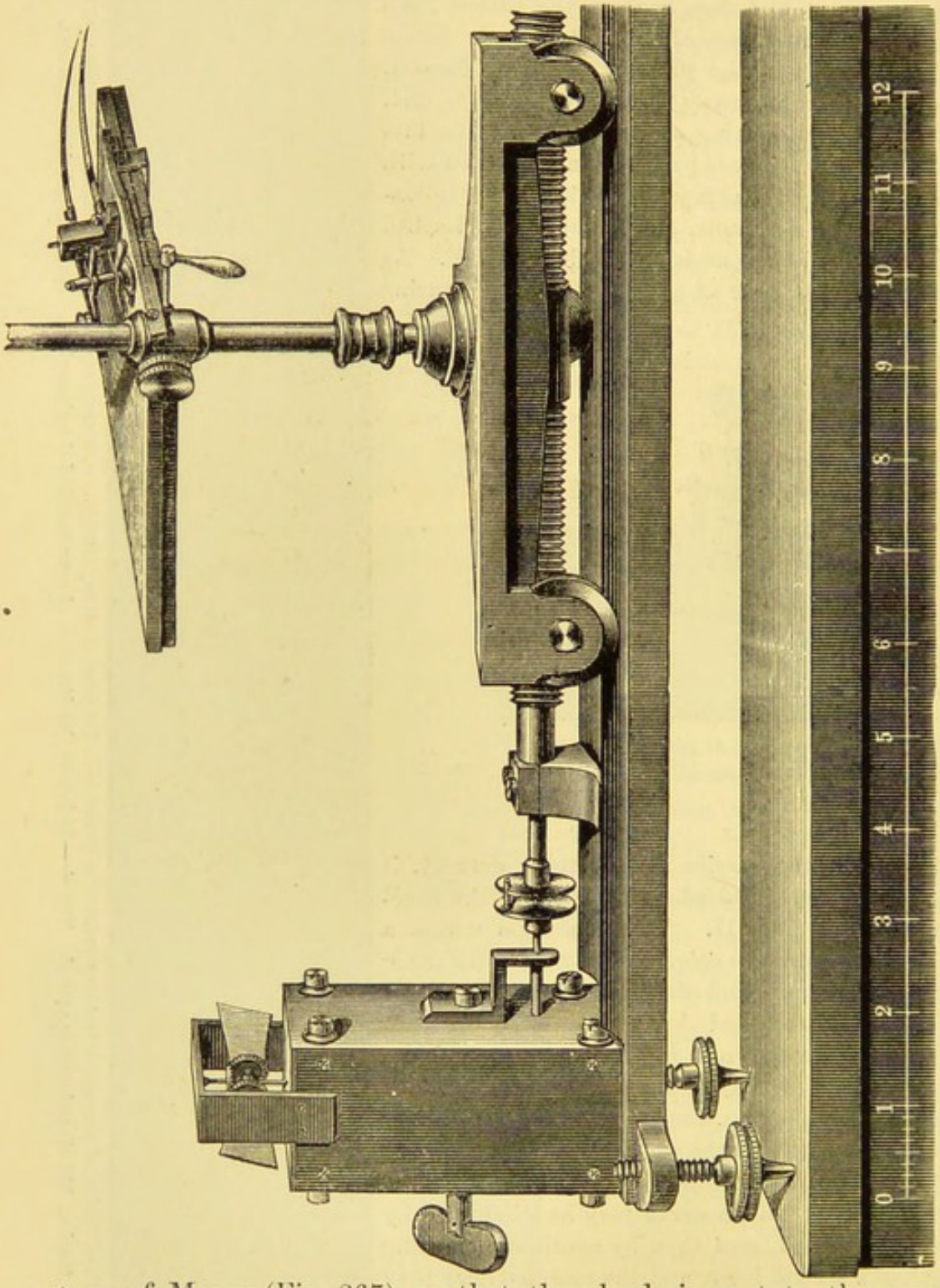


FIG. 267.—Travelling stage or railroad apparatus of Marey, by which the myograph is carried by the revolving screw, driven by clockwork, horizontally alongside of the recording cylinder. The stage travels from right to left, so that during the time of a revolution of the recording cylinder, the stage will have been carried so far to the left that successive tracings made by the marker of the myograph are distinct and superposed.

stage of Marey (Fig. 267), so that the shock is sent to the muscle a little later in each revolution of the cylinder.

[1] Burdon-Sanderson. On the electromotive properties of the leaf of Dionæa. *Phil. Trans.* Part I. 1882.

It can easily be arranged that the recording cylinder carries a wire attached to its axle, through which the current passes when the wire dips with each revolution into a cup of mercury interposed in the circuit Thus each revolution of the cylinder makes and breaks the

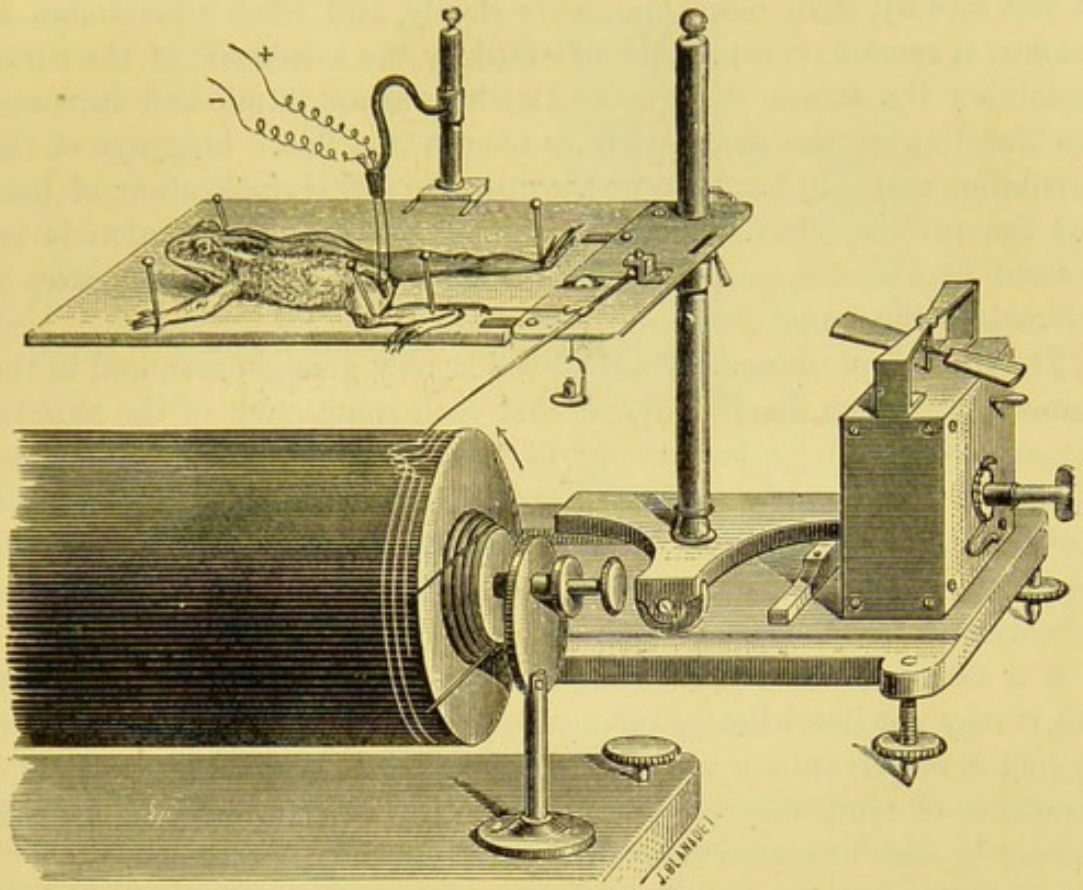


Fig. 268.—Arrangement for recording consecutive contractions of a frog's gastrocnemius muscle, each contraction occurring a little later in time than the one immediately preceding it.

current passing through the primary coil of an inductorium, either at the same moment, or a little later, with each successive contact. The arrangement for the latter purpose is shown in Fig. 268.

By this method, tracings may be obtained, such as those shown in

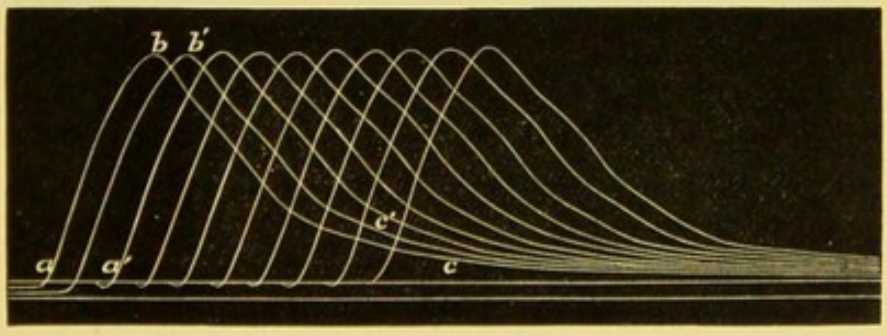


Fig. 269.—Tracings of muscular contractions produced by successive single induction shocks, to be read from left to right, thus—*a*, *b*, *c*.

Fig. 269, and Fig. 283, p. 429, an inspection of which at once elicits facts of physiological interest.

The *duration* of muscular contraction varies in different species of animals; it is very short in birds, somewhat longer in mammals and fishes, and still longer in reptiles. The amplitude of the curve, indicating the *amount* of muscular contraction, increases with increase of the stimulus, at first rapidly, then more and more slowly, and when a maximum is reached it remains constant, as indicated by the amplitude of the curve remaining the same. Fatigue diminishes the amplitude and increases the duration of the contraction, as seen in Fig. 283. Stoppage of the circulation and cold has the same, whilst the gentle application of heat has the reverse, effect. The duration of muscular contraction is increased in diabetes, jaundice, cerebral hemiplegia, and in some cases of sclerosis of the spinal cord.

The *rapidity* of muscular contractions is very great in man and in the mammalia. Thus, Landois found that each contraction of the muscles in writing the letter *n* lasted only ·0564 of a second. A striking illustration of the rapidity of muscular contractions is seen in the rapid movements of the fingers of a violinist or pianoforte player, or in the rapid movements of the muscles of the larynx when a singer trills in the execution of vocal music.

If a fatigued muscle has been strongly stimulated, it may contract, but it may not immediately relax when the stimulus has ceased to act, so that it may require a weight to stretch it to its original length. This condition of temporary contraction is called the *contraction remainder*. It may be seen to some extent in muscles not greatly fatigued.

B.—Propagation of a Wave of Contraction.

When a living muscle is stimulated, it not only contracts, but becomes thicker. The curve of the movement of *thickening* has been found to be generally the same as that of contraction. This may be shown by the arrangement seen in Fig. 270.

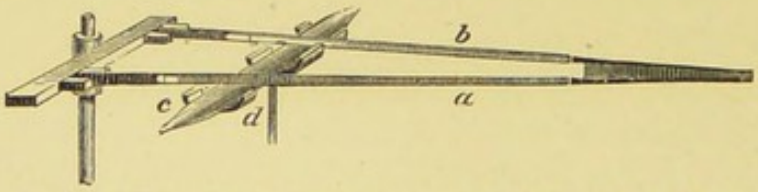


Fig. 270.—Diagram showing apparatus for recording the curve of thickening. *a b*, Two light levers joined at the point on the right and resting on the muscle, *c d*. The muscle is caused to contract by sending an induction shock through it. The tracing is recorded and compared with that obtained by a myograph.

As has been already mentioned, if a stimulus is applied to one end of a living muscular fibre, a wave is seen to propagate itself towards the other end. This has been termed the *wave of contraction*, and Marey has

measured its rapidity by grasping it at two places with instruments called *myographic pincers* (Fig. 271, 1, 2). Each of these is connected with a transmitting tambour. The recording tambours, 1′, 2′, trace the curves on a cylinder. When we stimulate one of the ends of the

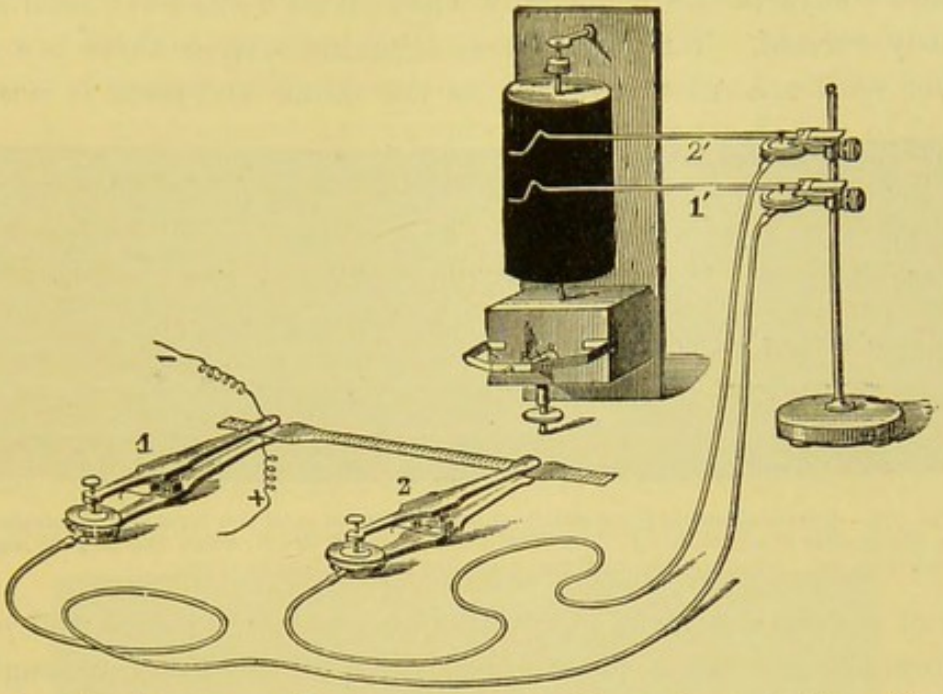


Fig. 271.—Arrangement for tracing the wave of muscular contraction. The muscle is diagrammatic.

muscle, the thickening which accompanies the contraction, as it passes from one end to the other, acts through the pincers upon the two receiving tambours successively, and a tracing is obtained such as is seen in Fig. 272.

It will be seen that the two curves do not coincide, and the distance between their summits gives the rapidity of propagation of the muscular wave, which is from 1 to 3 metres per second. The wave in the

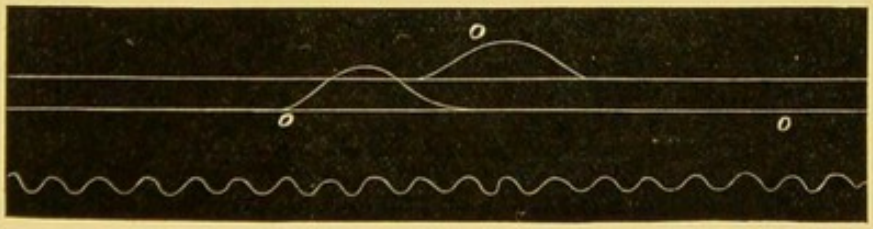


Fig. 272.—Tracing of the propagation of the muscular wave. Chronographic tracing, 100 vibrations per second underneath. (Marey.)

muscle of the heart travels slowly, being only at the rate of 10 to 15 mm. per second, and in the muscles of the lobster it progresses at the speed of 1 metre per second. In living human muscle it is very rapid, 10 to 13 metres per second—so rapid that some have denied its existence. The wave-length has been estimated at only 206 to 380 mm. Cold diminishes the rate, as shown in Fig. 273.

Fatigue, the tendency to coagulation of the myosin so as to produce *rigor mortis*, veratrin, and sulphocyanide of potassium also delay the progression of the wave. The wave of contraction excited in a muscular fibre is limited to it alone, and is not transmitted to neighbouring fibres. A muscle contracts as a whole only when all its fibres have been simultaneously excited. When the nerve stimulus acts, as there is a nerve fibre for each muscular fibre, and as the motor end-plate is near the

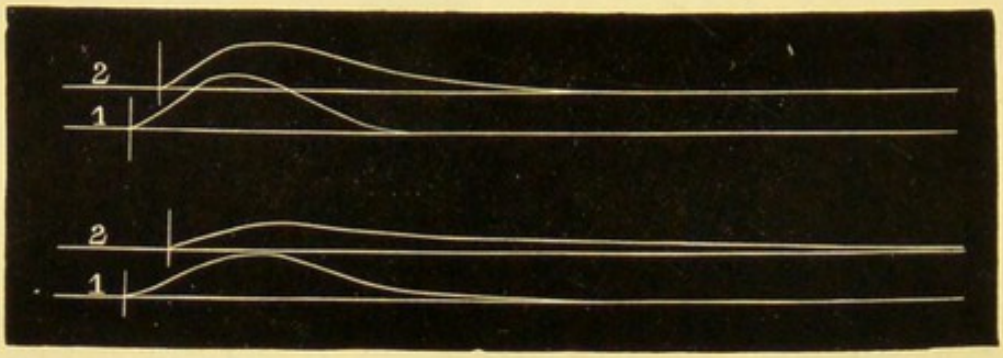


FIG. 273.—Curves showing the rapidity of the transmission of the wave of contraction in the muscle of a rabbit. A, muscle in normal condition; B, when the muscle was cooled by ice. Observe that in B the wave has travelled more slowly.

centre of the fibre, a wave of contraction proceeds to each end of the fibre from the end-plate. Each fibre is from 30 to 40 mm. in length, so that even with this comparatively short length it can scarcely be said to contract instantaneously as a whole. Practically, a muscle contracts as a whole when it receives the nerve stimulus, but in many muscles, and in long muscles especially, the distance of individual muscular fibres from the main nerve trunk will vary so that they will not receive the nerve stimulus precisely at the same instant.

CHAP. VIII.—THE GENESIS OF TETANUS.

If a rapid series of induction shocks be sent to the muscle of a frog from Du Bois-Reymond's inductorium, seen in Fig. 222, the muscle will pass into a state of strong contraction, in which it will remain a considerable time, so long as induction shocks are transmitted to it. This state of strong contraction is termed *tetanus* or *cramp*, and is quite different in character from the short contraction caused by a single shock, which we have termed a *twitch*. In the first place, it may be observed that the shocks sent by the apparatus, when the primary circuit is automatically closed and opened, may be regarded as instantaneous excitations. When excitations, by induction shocks, amount in number to three or four per second, the muscle contracts more strongly, and the contraction continues for a longer time than if the muscle had received a single shock of the same intensity. The height

of a contraction caused by a single shock is usually from a third to a half that caused by a rapid series of shocks, or tetanus. We may readily make this observation by interrupting the current of the induction coil by means of a metronome or by a vibrating spring, so that the number of shocks sent per second may be varied at pleasure. It will then be found that the opening shock is more powerful, as shown by the amount of muscular contraction, than the closing shock, and an explanation of this phenomenon has already been given (p. 374). When the shocks succeed each other regularly, at the rate of about fifteen per second, and in such a way that the duration of the interruption is short compared with the duration of the shock, the muscle remains persistently contracted. The phases of this state of permanent contraction must be carefully studied.

When a muscle is stimulated by an inductorium, it draws a curve on the smoked surface of a revolving cylinder which is different in character from the curve produced by a single shock, described in the last chapter (Figs. 263 and 266). The curve of tetanus is shown in Fig. 274.

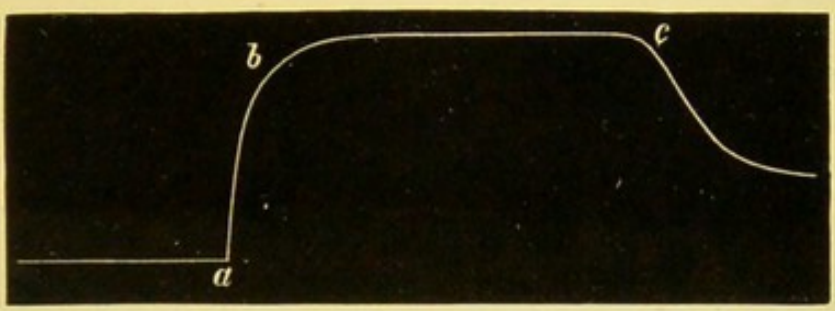


FIG. 274.—Tetanus produced by numerous shocks from an inductorium. The interrupted current acted a little before *a*, and after a period of latent stimulation, the muscle began to contract so that the lever rises rapidly, and at *b* the muscle reaches the maximum of contraction. The contraction lasts until *c*, when the current is shut off and the muscle slowly relaxes.

It is important to note that when a muscle is tetanized by an induction current, it contracts suddenly, as indicated by the rapid rise of the lever, that tetanus is a condition which lasts for a considerable time, and that the muscle relaxes slowly. The curves in Figs. 274 and 275 should be compared.

When a series of induction shocks is more slowly transmitted to the muscle in contact with Marey's myograph, the primary current being interrupted by a metronome, a tracing is obtained, as in Fig. 275. A number of distinct oscillations will be observed in the ascent of the curve. Each oscillation is somewhat shorter than the one immediately preceding it. Three cases in which a muscle is excited by successive shocks present themselves for consideration.

1. When a second excitation acts after the termination of the contraction occasioned by the first, it produces a second muscular

contraction having the characters of the first, and so on, as regards successive excitations, until the muscle becomes fatigued.

2. If the second excitation acts during the period of latent stimulation following the first, the shortening is not greater than for one excitation, and the curve of contraction is the same.

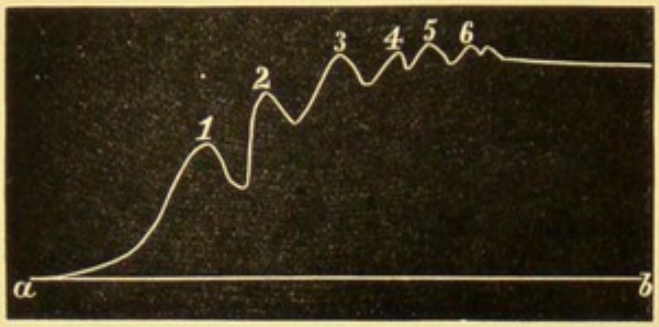


FIG. 275.—Tracing of a muscle passing into a tetanic state, when the primary current of an inductorium is rapidly made and broken by a metronome. The first shock was transmitted to the nerve at *a*, the second an instant after 1, the third an instant after 2, and so on. It will be observed that with each succeeding shock the muscle becomes shorter, though the amount of shortening at each shock is less.

3. If the second excitation acts during the two last portions of the preceding contraction, the shortening corresponding to the second excitation is added to that of the first. The curve thus produced is seen in Fig. 276. If other excitations quickly follow, each capable of causing a partial contraction, there is then a summation of the effects of the individual excitations, and a state of permanent contraction or tetanus is produced, which is the fusion of the partial contractions resulting from the successive shocks. This is well illustrated by varying the number of

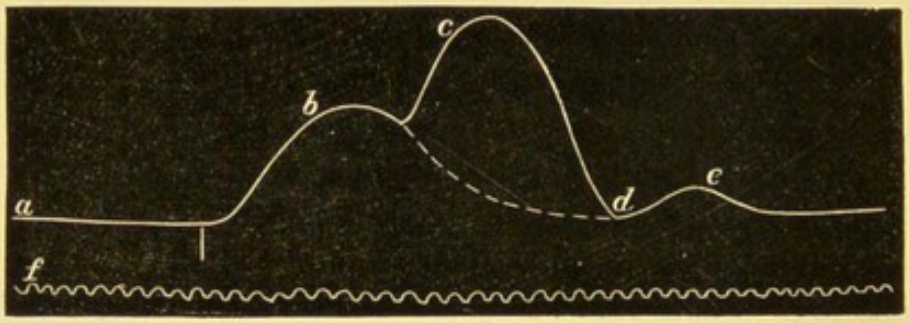


FIG. 276.—Tracing of a double muscle curve. While the muscle was engaged in the first contraction (whose complete course, had nothing intervened, is indicated by the dotted line), a second induction shock was sent to it, at such a time that the second contraction began just as the first was beginning to decline. *a*, *b*, first contraction; *c*, *d*, second contraction; *f*, chronographic tracing.

excitations transmitted to the muscle. Thus, we may obtain a curve like that shown in Fig. 276 if the second excitation has been sent just when the muscle was beginning to relax from the effects of the first excitation, and like those in Figs. 275 and 277, if the excitations followed faster, but not too fast, so that the contractions caused by individual excitations appear like teeth on the tracing, and, finally, like Fig. 278, when the excitations follow in very rapid succession, and individual contractions cannot be seen. Tetanus may be maintained for a considerable time, which varies according to the degree of vitality

of the muscle, but by degrees it passes off, and the muscle slowly returns to its original length, under the influence of fatigue. A study of the genesis of tetanus clearly shows that it is a condition produced by a fusion of individual contractions.

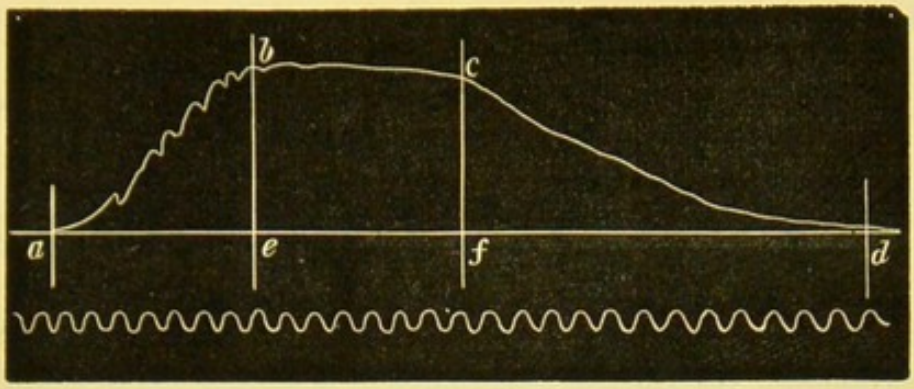


FIG. 277.—Curve showing the genesis of tetanus. *a* to *b*, individual contractions; *b* to *c*, muscle now tetanic, but wavy line still indicating individual contractions—the slope of the line from *b* to *c* showing that the muscle is becoming fatigued; *c*, indicates time when induction shocks were stopped; *c*, *d*, slow relaxation of muscle.

The number of shocks required to cause tetanus varies. From 16 to 30 per second are sufficient for the gastrocnemius of the frog, while 10 per second may cause tetanus of the frog's hyoglossus muscle. Kronecker and Stirling found incomplete tetanus of the red muscles of the rabbit to be caused by 4 shocks per second, while 10 shocks per second caused complete tetanus. On the other hand, the pale required over 20 per second. It is curious that, according to Marey, the muscles of

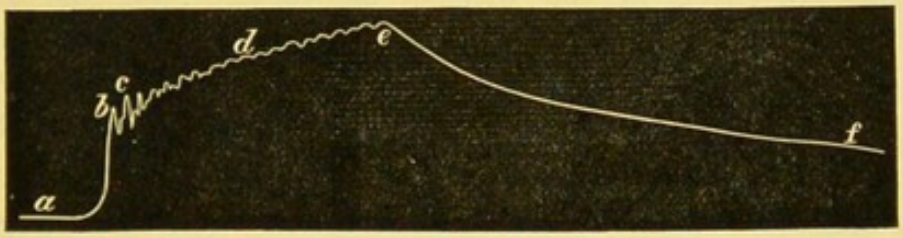


FIG. 278.—Curve showing genesis of tetanus. *a* to *b*, result of first shock; then observe the cumulative effect of the successive shocks, as shown by gradual ascent of curve from *b* to *e*; stoppage at *e* of induction shocks; *e*, *f*, gradual relaxation of muscle.

birds are not tetanized by even 70 per second. If shocks are sent to a muscle at the rate of from 220 to 360 per second, instead of tetanus, there may be only a momentary contraction. This result was obtained by Bernstein, but Kronecker and Stirling, by using an ingenious instrument invented by them, termed a *tone-inductorium*,[1] produced tetanus by shocks so rapid as 24,000 per second.

The study of tetanus becomes of great interest when we remember that many, if not most, of the muscular movements occurring in the body are of this character. A simple muscular movement, such as

[1] See Landois' and Stirling's *Physiology*, vol. i. p. 717.

flexing the arm, throws certain muscles into a condition of normal tetanus. This is proved by Kronecker's experiment of placing two needles, one in a human muscle and the other in its tendon, and connecting both with a telephone. If, then, the muscle be tetanized by induction shocks coming at the rate of about 100 per second, a sound will be heard. The sound is a proof of vibrating motion. The phenomena of shivering of muscles is an example of incomplete tetanus.

Chap. IX.—Modes of Exciting Muscular Contraction.

In addition to the normal stimulus transmitted by a nerve, muscular contraction may be excited by electrical, mechanical, thermal, and chemical stimuli or excitants.

1. *Electrical Stimuli.*—An electrical stimulus acts most effectively on muscle when the current passes in the direction of its fibres. We have already seen that a single *induction* shock of sufficient strength will cause a single contraction or twitch, and that a number of shocks, coming at short intervals in succession, will cause tetanus, spasm, or cramp. As already explained, p. 374, the opening shock produces a more powerful effect than the closing shock, but the two may be made nearly equal by the use of Von Helmholtz's arrangement. When a current from a battery of voltaic elements is passed through a muscle, no effect in the way of contraction can be observed, except at the moments of opening and of closing the current, or when the current is very suddenly and largely increased or diminished in strength. If the muscle forms part of an electrical circuit and the current is opened and closed, the resulting contraction shows a muscle curve differing from that produced on irritating the nerve, by the contracted muscle relaxing slowly, so that the time of relaxation is very much longer than the time of contraction. The passage of the current, however, causes electrolytic changes, and no doubt there will be also the production of heat owing to the resistance opposed by the muscle as a conductor to the passage of the electrical current.

Suppose a muscle to be traversed by a voltaic current, it is stimulated only near the negative pole when the circuit is closed, and near the positive pole when the circuit is opened, and if the motor end-plates have been paralyzed by curara, a wave of contraction may be observed starting from the *negative* pole on closing, while it starts from the positive pole on opening. (Rutherford.)

It is important to observe that a muscle, with paralyzed nerve-endings, responds much more readily to the opening and closing shocks

of a voltaic current than to the shocks of induced or Faradic currents. This is probably owing to the muscle-protoplasm not responding to the very short and sudden stimulations of induced currents, while, on the other hand, the motor end-plates and the nerves themselves readily respond to sudden shocks of this character. It would appear that all kinds of protoplasm, except that of nerve and nerve-endings, are more susceptible to the more sluggish opening and closing shocks of a voltaic current.

2. *Mechanical Stimuli.*—The effect produced by a mechanical stimulus depends upon the intensity of the stimulus and the rapidity with which it acts. Pressure upon a muscle, if slowly applied, may not excite a contraction, but if the pressure be made suddenly, contraction may be the result. When a muscle is stimulated by any mechanical excitant, the result is not only a contraction passing from the excited point through the whole of the muscle, but a permanent contraction at the point touched. This contraction, termed by Schiff, its discoverer, *idio-muscular contraction*, may be observed in the living man when a blow is struck with a blunt body across a muscle, and perpendicularly to the direction of its fibres. This produces a local contraction like a weal, or as if there was a bit of whip cord beneath the skin. There is then a contraction which extends the whole length of the muscle, and when this contraction has disappeared, there remains at the point of excitation a transverse swelling, which lasts for a certain time. A muscle may pass into a state of tetanus, if the nerve supplying it is irritated by successive mechanical shocks.

3. *Thermal Stimuli.*—The sudden passage of a muscle from one temperature to another considerably higher or lower than the first may excite muscular contractions, and as the temperature is raised from 28° C. to 45° C., the individual contractions become stronger. About 60° C., tetanus may be produced. Above this limit, the myosin or other albuminous constituent of the muscle coagulates, and the muscle becomes stiff. The effect of heat on a muscular structure is strikingly illustrated by the action of heat on the heart of a frog, an experiment which may be readily performed by means of the arrangement shown in Fig. 279. In this case, however, the heat acts not merely on the muscular fibre, but also on the intrinsic nervous mechanism or ganglia of the organ. (See Heart.)

4. *Chemical Stimuli.*—A great many chemical agents cause contractions by their influence on the chemical composition of the muscle or of the nerve supplying it. Amongst the agents which excite contraction are the fixed alkalies, the mineral acids, acetic, oxalic, tartaric, and lactic acids, alcohol, ether, creosote; the neutral

alkaline salts, such as chloride of sodium, and sulphates and carbonates of the alkalies; metallic salts, such as those of iron, zinc, copper, silver, and lead, and concentrated solutions of urea, sugar, and glycerine. These excite contraction when used as weak solutions, and in the case of several, the solution must be considerably stronger before it has any effect on the nerve of the muscle. A weak solution of bile also stimulates muscular fibre. Neutral alkaline salts act to the same extent on muscle and nerve. The vapour of hydrochloric acid causes single con-

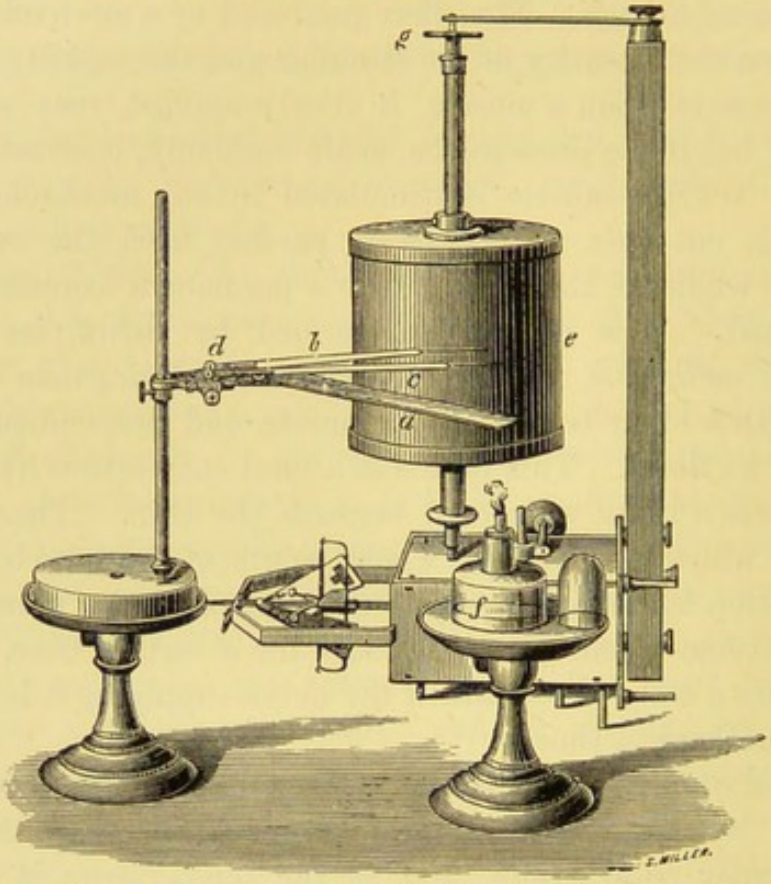


FIG. 279.—Arrangement for studying the action of heat on a frog's heart. A copper plate, carrying at one end two delicate levers, *b*, moving on a joint at *d*; the heart is placed underneath the levers near *d*, one on the auricular and the other on the ventricular portion, and the movements of the levers are recorded on the cylinder *e*. When heat is applied by the spirit lamp, *f*, to the end of the copper plate, the heart beats faster and faster until it passes into a state of tetanus, from which it may recover if the copper plate is cooled with a piece of ice.

tractions, while chlorine gas produces tetanus. It is said that the vapour of bisulphide of carbon stimulates the nerves and not the muscular fibres. Very dilute solutions of caustic soda or caustic potash first stimulate and then kill both nerve and muscle.

Certain solutions cause rhythmic movements in muscle. The most efficient fluid for this purpose is called *Biedermann's fluid*, prepared by dissolving 5 grammes of chloride of sodium, 2 grammes of alkaline phosphate of soda and ·5 gramme of carbonate of soda in 1 litre of water If the sartorius muscle of a frog, to which curare has been

given so as to paralyze the motor end-plates, is immersed in this solution so that about one half of the long thin muscle dips into it, the muscle begins to contract rhythmically in a manner analogous to the pulsations of the frog's heart. This is an interesting experiment, as suggesting that rhythm may, to some extent at least, depend on the chemical composition of the fluids circulating through the rhythmic organ. All of these substances possibly excite either the nerve or the muscle by absorbing water from them, as the contractions resemble those following rapid drying of a muscle or nerve by exposure to the air. Distilled water injected into the blood-vessels of a muscle excites the contraction of individual fibrillæ, producing the local quiverings called fibrillar contractions; but it rarely excites contraction when applied directly to the nerve or muscle.

Chap. X.—THE PRODUCTION OF HEAT BY MUSCLE.

The apparatus required in investigating the thermal phenomena of muscle consists of a galvanometer of small resistance, and a thermo-electric pile, or thermo-electric needles. A thermo-electric *needle* is composed of an iron wire, to each end of which is soldered a copper or German silver wire. Several such needles are passed through the muscle so that one set of junctions of iron with copper, or iron with German silver, is embedded in the muscle, and the other set is exposed to the air. The adjacent ends of the needles, composed of copper or silver, are in connection with two wires which go to the galvanometer. This arrangement is not very delicate, and for more refined investigations a thermo-electric pile is necessary. A *pile* consists of short bars of bismuth and antimony, having each end of a bar of bismuth soldered to the end of a bar of antimony, the bars lying parallel. Thus there are two sets of alternate junctions, one of which may be applied to the body giving off heat, while the other is kept at as near a uniform temperature as possible. In these circumstances, if the one set of junctions becomes hotter than the other, a current of electricity is generated which deflects the needle of the galvanometer. By such arrangements, it will be found that there is a deviation of the galvanometer needle when the muscle contracts, owing to the development of a thermo-electric current, from the metallic junction in, or applied to, the muscle being raised to a higher temperature than the other junction kept at a constant temperature in a vessel of boiling water, or in melting ice. Heidenhain has shown, by the use of extremely delicate arrangements of this nature (Fig. 280), that the instantaneous excitation

of the nerve supplying a muscle is attended by a rise of temperature in the muscle.

The development of heat in a muscle depends—(1) upon its tension; (2) on the work done; and (3) on the state of fatigue of the muscle.

1. *Tension and Heat.*—The more a muscle is stretched, the greater will be the amount of heat developed when it contracts. According to Béclard and Heidenhain, a muscle develops its maximum amount of heat, supposing the intensity of the stimulus to remain the same, when it is so stretched as to be unable to contract at all. This occurs

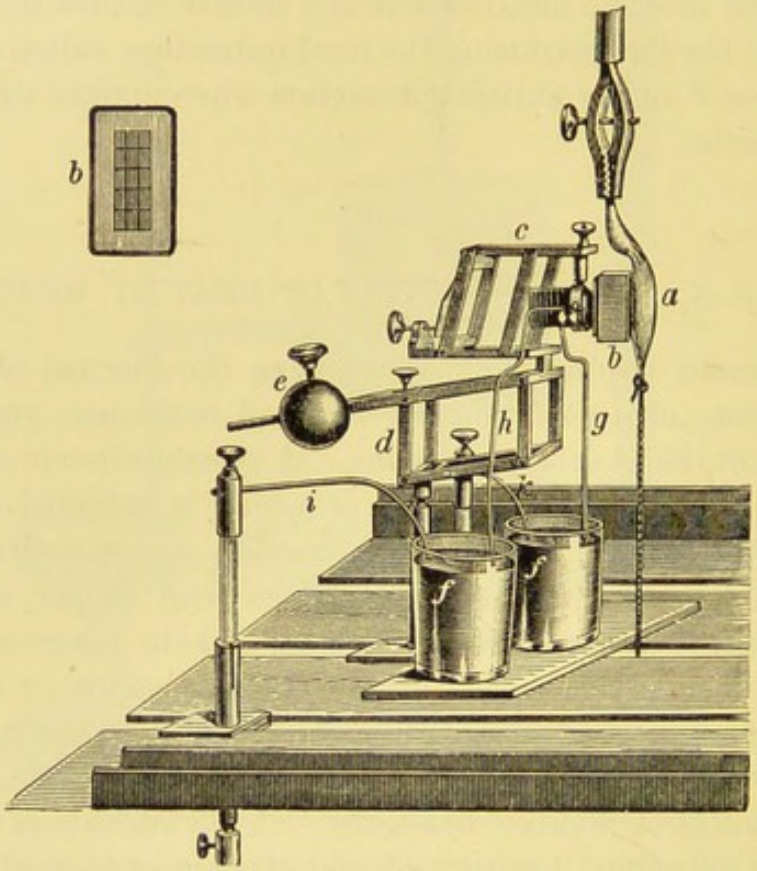


FIG. 280.—Heidenhain's arrangement for studying the heat-phenomena of muscle. *a*, gastrocnemius muscle of a frog having a small thermo-electric pile, *b*, in contact with it. The figure *b* in the upper left-hand corner shows the surface of the thermo-electric pile, consisting of 15 junctions. *c*, is a frame bearing the pile; *d*, support of the frame *c*, with a counterpoise *e* attached to it; *f*, *f*, two glasses containing melting ice to give a uniform temperature to the other thermal junctions, *g*, *h*.

in tetanus of a limb when the antagonistic muscles oppose each other, a fact which accounts for the high temperature observed in cases of tetanus.

2. *Work Done and Heat.*—As to the relation between the heat produced in, and the work done by, a muscle, when the muscle remains contracted supporting a weight sufficient again to stretch it, no effective work is done, and energy appears only as heat. Energy appeared as motion during the contraction, but this portion disappeared when the

weight restored the muscle to its original length. Again, the energy of a contracted muscle appears as heat, and that of a contracting muscle either as heat or mechanical work, or both. If we add the energy appearing in the form of heat in a contracting muscle to the energy liberated as mechanical work, the sum is the total amount of energy expended during the contraction. When a muscle lifts a series of gradually increasing weights up to a given height, the amount of heat increases with the work done up to a maximum point peculiar to the condition of the muscle at the time. If the weight be still increased, it will not be lifted so high, but the work done will be greater and the amount of heat generated in these circumstances will be less. It follows that the maximum production of heat by a given muscle is reached sooner than its maximum amount of work. The greater the heat, the smaller will be the mechanical work, and *vice versa*. The heat, therefore, produced during work is, in the same time, inversely proportional to the amount of work. Several large contractions liberate more heat than a greater number of small contractions, even although the amount of work done be the same in both cases, or in other words, large contractions cause greater changes in the muscle substance than a greater number of small contractions. Lastly, suppose a muscle does work by a number of small contractions in a given time, in each case lifting a weight, more heat will be produced than if the muscle kept up the same weight for the same time by being in a state of tetanus.

3. *Fatigue and Heat.*—With regard to fatigue, it may be stated that as a muscle becomes exhausted by successive excitations, or by the approach of death, both the amount of work done and the production of heat simultaneously diminish. The two quantities, however, do not diminish equally: heat diminishes more rapidly than work, so that it is possible to have a muscle capable of performing a small quantity of mechanical work, but at the same time producing no appreciable amount of heat.

Heat to a small amount is also produced by the relaxation of a muscle, indicating that this is not merely a passive process, but that it also involves a small amount of metabolism.

The venous blood, coming from a muscle in a state of activity, is *cæteris paribus* warmer by ·6° C. than that flowing from a muscle at rest; and it has been ascertained that the gastrocnemius of a frog, deprived of blood, will show an increase of about ·001° to ·005° C. for each contraction, until it becomes much fatigued. Tetanus of the same muscle for two or three minutes will produce a rise of temperature of ·014° to ·018° C. Billroth and Fick determined the heat produced by tetanus of the muscles of mammalia whilst the blood was circulating as equal to a rise of 5° C. Leyden also found a rise of temperature during

tetanus in animals, and Wünderlich observed the same phenomena in patients suffering from tetanus; and, further, that the maximum temperature was not attained, in these cases, until after death.

The heat produced by a contracting muscle is a physical expression of the metabolic changes occurring in it. The chemical energy set free by these metabolic processes appears as heat and mechanical work, and the general law regulating the relation of these is that, within certain limits, the greater the resistance offered to the contraction the greater will be the work done. In these circumstances, as much as one fourth of the total energy may appear as work, the remaining three fourths appearing as heat—a remarkable result when compared with that of the best constructed engines, which yield as work only one ninth part of the total energy supplied to them by the oxidation of the fuel, while the remaining eight ninths are manifested as heat.

Chap. XI.—THE WORK DONE BY MUSCLE.

The amount of work, W, done by a muscle when it contracts is measured by the product of the load, l, and the height, h, through which it is lifted. Thus, $W = l \times h$. Hence if $l = o$, that is if the muscle has lifted no weight, no work has been done; and again if the muscle be so over loaded that $h = o$, there will be no work, as the muscle has not contracted. The work done by a healthy muscle in lifting a weight will vary according to the strength of the stimulus and the weight to be lifted. A few experiments made with Von Helmholtz's myograph (Fig. 15, p. 28) in which the muscle is stimulated by single induction shocks of equal intensity, will show this clearly.

a. *The Contraction as a Function of the Stimulus.*—Suppose the gastrocnemius muscle of a frog is loaded with say a weight of 10 grammes, and the apparatus is arranged for a single induction shock. Removing the secondary coil of the induction machine to a considerable distance from the primary, let a single shock be sent to the muscle. If no contraction follows, let the secondary coil be moved nearer the primary until the first visible contraction is obtained and recorded. This point has been termed the point of *liminal intensity* of the stimulus. Then advancing the secondary coil onwards definite distances nearer the primary, let each contraction be recorded as an ordinate on the abscissa line at distances proportional to the distances the secondary coil has been moved.

Such a method shows that the amount of contraction increases with the increase of stimulus, at first rapidly, then more slowly, until a maximum is reached; the maximum remains for some time, and afterwards

the amount of contraction may become less owing to the fatigue of the muscle from repeated stimulations. As will be seen in the course of this discussion, the *amount of contraction* alone is no measure of the amount of muscle work. This depends, as already pointed out, on the product of the amount of the contraction and the load lifted.

β. The Contraction as a Function of the Resistance.—Suppose in this experiment the strength of the stimulus is the same, while the load which the muscle has to lift is gradually increased. Let a contraction be recorded when there is no load to the muscle at all. Then load successively with 10, 20, 30, etc. grammes, and record the several contractions at proportionate distances along the abscissa line. It can thus be shown that as the weight is increased from zero upwards, the contraction increases; as the weight continues to be increased, contraction also increases, but more slowly, and beyond a certain point increase in the weight is followed by a diminution in the amount of contraction.

γ. The Contraction as a Function of the Time between Successive Stimulations.—In a third experiment, it is interesting to observe the effect of varying the time between successive stimulations, keeping the load or resistance and also the strength of the stimulus the same. This may be done by sending shocks to the muscle at intervals of a second, half a second, a third of a second, and so on. The result shows that if a certain amount of time is allowed to elapse between successive shocks, the same amount of work may be over and over again repeated, but when the shocks come too quickly, the work quickly diminishes, or, in other words, the muscle becomes fatigued.

δ. Measurement of the Work Done.—The amount of work done by a muscle at a given time will depend on the strength of the stimulus, the amount of the resistance to be overcome, and the condition of the muscle as regards fatigue, and no doubt there will be for a given muscle, after a period of rest, a maximum amount of possible work corresponding to a certain strength of stimulus and a certain load to be lifted, or resistance to be overcome. As already pointed out, the work done is ascertained by multiplying the actual amount of shortening of the muscle by the weight lifted. Suppose, for example, a muscle lifts 10 grammes 20 millimetres in height, the work done is 200 gramme-millimetres. This may also be shown diagrammatically by drawing an abscissa line, marking off upon it the distances proportionate to different weights, and drawing as ordinates the actual work done in the case of each weight. A line drawn through the summits of the ordinates will give the curve of the work done with the same stimulus and increasing loads. Such a line would of course slope upwards. The heights, however, of the marks made on the smoked glass-plate of the myograph would diminish

as the amount of work increased. Thus, suppose the diagram in Fig. 281 represents the result of an experiment of a muscle lifting gradually increasing weights. The figures at the side indicate millimetres, and the figures along the base line indicate grammes. It is clear that the

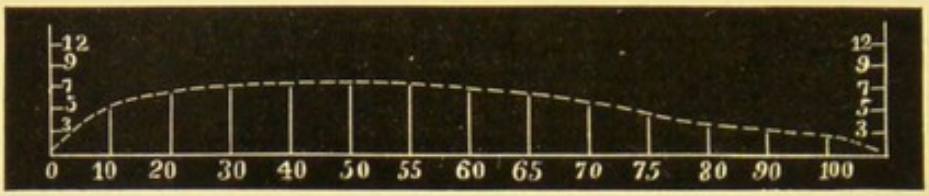


Fig. 281.—Diagram to show the mode of measuring muscle work.

work done as indicated by the first line on the left is $10 \times 5 = 50$ gramme-millimetres; the next, $20 \times 5 = 100$ gramme-millimetres, etc.; while the last on the right, $100 \times 3 = 300$ gramme-millimetres.

Weber has given the following figures with reference to the work done by the gastrocnemius muscle of a frog:—

Weight Lifted in Grammes.	Height in Millimetres.	Work done in Gramme-Millimetres.
5	27·6	138
15	25·1	376
25	11·45	286
30	7·3	219

This table shows that the work increases with the weight up to a certain maximum, after which a diminution occurs, more or less rapidly according as the muscle is fatigued.

To maintain a muscle in a tetanic state, successive stimuli are required. The first few stimuli cause the muscle to contract, and then work is done, but no work is done during the tetanic state. The chemical changes set up by the successive stimulations required to keep up tetanus find expression as heat and as continued contraction. A tetanic contraction will do more work than will be done by a single contraction caused by a stimulus of the same strength as the individual stimuli producing the tetanus, but the tetanus will of course quickly produce fatigue in consequence of the rapid metabolism taking place in the muscle.

Static Force of a Muscle.—In charging a contracting muscle with gradually increasing weights, there arrives a time when the elongation due to tension of the muscle exactly compensates the contraction of the muscle. When this is so, the muscle is in a state of equilibrium, and the weight expresses what Weber termed its static force, or *absolute force*. The force is stated usually as the number of grammes per square centi-

metre.[1] This force for frog's muscle has been found to be 2,800 to 3,000 grammes, and for human muscle, to be from 7,000 to 10,000 grammes, that is to say, a strip of frog's muscle having a transverse section of a square centimetre would, when stimulated, *undergo no change in length* with a weight of say 3,000 grammes attached to one end.

The force of individual muscles in the human body may be measured by means of instruments, termed *dynamometers*, one of which, adapted for testing the muscles of the arms and hands, is seen in Fig. 282. On compressing this instrument with both hands, a strong man may force round the index to 70, representing kilogrammes of pressure. The muscles of women are feebler than those of men. With another kind of dynamometer, a man, by pulling, may move the index to 150 kilogrammes.

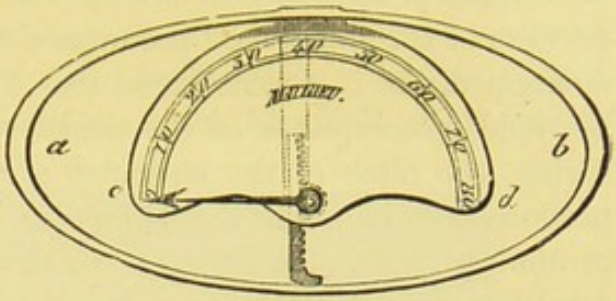


FIG. 282.—Dynamometer. *a*, *b*, strong steel spring ; *c*, scale.

CHAP. XII.—THE MUSCLE SOUND.

When a stethoscope is firmly applied over a powerfully contracting muscle, such as the biceps in a muscular man, a deep tone is heard, which is produced by about twenty (19·5 ?) vibrations per second. This sound was first detected by Wollaston. It may also be heard during profound stillness, as in the middle of the night, if we stop the ears and powerfully contract the muscles of mastication. This tone is the same in pitch as the resonance tone in the ear. The muscular sound, according to Von Helmholtz, is produced by the variations of tension which occur during the formation of a continuous muscular contraction. It is probable that the sound heard is not that due to twenty vibrations per second, which number of impulses on the ear would not produce the sensation of a tone, but to forty vibrations per second, or the first overtone or harmonic of the fundamental tone.

The existence of the muscular tone renders it probable that, to secure the contraction of a voluntary muscle in man during life, about twenty impulses per second are transmitted from the nerve-centres to the

[1] To find the mean transverse section of a muscle—(1) Ascertain the volume by dividing the absolute weight of the muscle by its specific gravity, 1·058 (C.G.S.), and then (2) divide the volume by its length. This gives the mean transverse section. (Landois.)

muscle along the motor nerves. Of course it is understood that the contraction is not merely a twitch, as when one quickly flexes and extends the arm, but such a contraction of the muscle as is necessary to support a weight for a short time. This condition of contraction may be regarded as a kind of *normal tetanus*, and no doubt the great majority of voluntary contractions are of this nature. Irritation of a motor nerve or of the spinal cord produces a muscular sound of the pitch above mentioned, but it is remarkable that if tetanus is produced in a muscle by rapid interruptions of the primary circuit produced by a vibrating spring, the pitch of the muscular sound so produced is exactly that produced by the number of vibrations of the spring, and by increasing or diminishing the number of vibrations of the spring, the pitch of the resulting muscular sound can be raised or lowered in the scale. Thus, a tone may be heard corresponding to a pitch of from 700 to 1000 vibrations per second, when the number of vibrations of the interrupting spring reaches those amounts. If the nerve is irritated, the sound is not so loud.

Chap. XIII.—THE PHENOMENA OF MUSCULAR FATIGUE.

A muscle becomes fatigued after continuous work. Fatigue means a diminished power of work. Up to a certain point, the substances produced in a contracting muscle are eliminated as quickly as they are formed, and at the same time new materials necessary for the repair of the muscle are supplied. There is thus, as it were, a balance between the two processes. When, however, the muscle is excited to very frequent contraction, the products of waste accumulate in the muscle, and probably also sufficient time is not allowed for the supply of reparative material. The waste products are chiefly acid phosphate of potash, and carbonic acid, possibly small amounts of free phosphoric acid, and such nitrogenous matters as are found in extract of meat, creatin, creatinin, etc., p. 361. The injection through the vessels of a muscle of a ·6 per cent. solution of common salt, a weak solution of sodium carbonate, or a weak solution of permanganate of potash, removes these substances, and the muscle may again become fit to do work. As oxygen is necessary for the metabolic processes occurring in a muscle, it is evident that if the supply of this gas be much diminished, the muscle will rapidly become fatigued. Further, as the muscle becomes fatigued, it consumes less oxygen and produces a smaller quantity of waste products.

Fatigue diminishes the cohesion of muscle, and makes the muscle more easily stretched by weight; it has apparently little or no effect

upon its elasticity, it lowers irritability, and it lengthens the period of latent stimulation. If a muscle is stimulated by shocks sent to it at equal intervals of time, it will contract with each shock, and, for a short time, the *amount* of each contraction may slightly increase. The *duration* of each contraction steadily increases. By-and-bye, however, as the muscle becomes fatigued, the amount of contraction diminishes until the muscle does not contract at all, but the duration increases throughout the whole experiment. The phenomena in the first part of the experiment are illustrated by the tracing in Fig. 283. This tracing

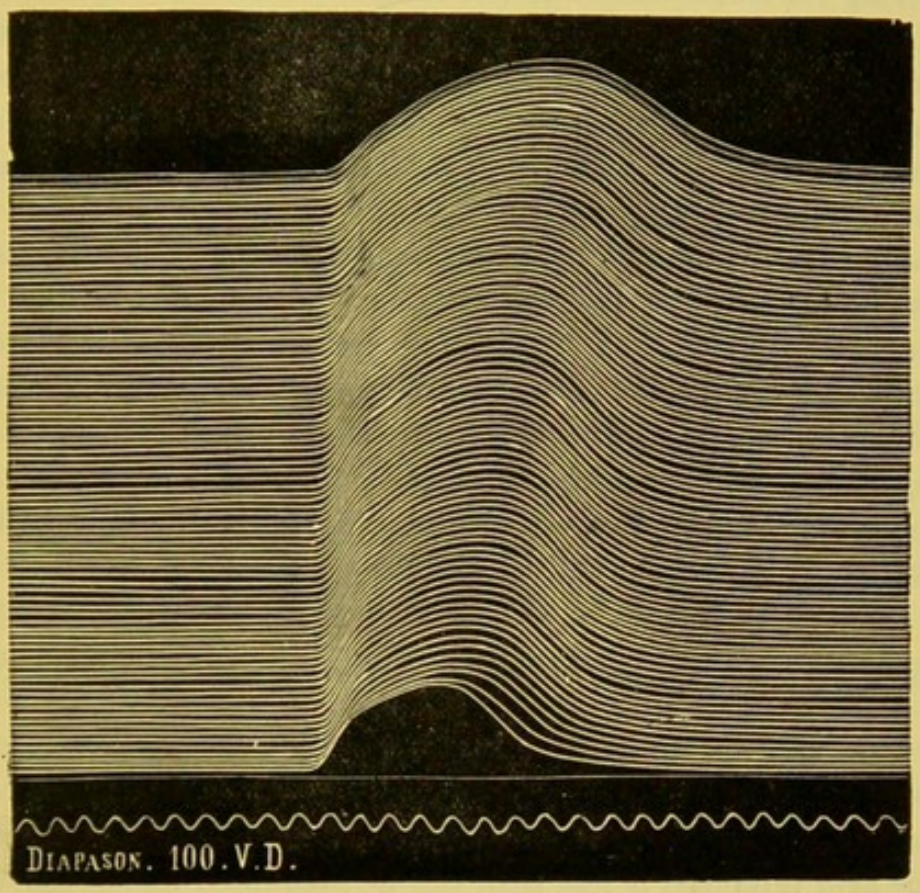


FIG. 283.—Consecutive tracings of the contractions of the gastrocnemius muscle of a frog, showing the effects of fatigue. The beginning of the contraction is the commencement of the curve on the left. The first contraction produced the lowermost curve, and the uppermost curve was the last made in the experiment. Chronograph, 100 vibrations per second.

was taken by means of the arrangements described on p. 410, with the aid of the apparatus shown in Figs. 267 and 268. It shows (1) the gradual lengthening of the period of latent stimulation, as indicated by each contraction beginning a little later than the one before it; (2) the gradual increase in the amount of contraction, as the curves at the top of the diagram are higher than those at the foot; and (3) the gradual lengthening of the contraction, by the muscle losing time during relaxation. The experiment was not carried far enough to show the diminution

in the amplitude of the contraction caused by fatigue. During fatigue, a muscle returns to its original length more slowly after a contraction. As has been already stated, the work done diminishes rapidly during fatigue. The absolute muscular force is diminished by fatigue.

According to Kronecker, a muscle is fatigued more quickly when it does work than when it contracts without accomplishing work, or when it is merely tetanized. The fatigue which follows work depends upon the amount of the resistance to be overcome by the work, and the time during which the resistance acts against the muscle. Loading a muscle only during its contraction will not fatigue it so soon as if it is loaded both during its contraction and relaxation, indicating that metabolic changes occur to some extent during relaxation as well as during contraction. A muscle becomes most rapidly fatigued when stimulated to powerful contractions which are not able however to overcome the resistance, say of a heavy weight.

Probably the end-plates become fatigued first, and it would appear that the muscle substance becomes sooner fatigued than the nerve. (Waller.) The circulation of various substances in the blood, such as strychnin, veratrin, ptomains, also affects the metabolism of muscle, as shown by changes in the behaviour of the muscle during fatigue. The capacity for work diminishes with elevation of temperature, and if the temperature does not pass a certain limit, about 40° C. for the muscle of a frog, the muscle may recover its original power on cooling.

Chap. XIV.—THE NUTRITIVE CHANGES OR METABOLISM IN MUSCLE.

The metabolic changes occurring in muscular tissue are very active and very complicated. A muscle in a state of rest, and supplied with blood, absorbs oxygen and eliminates carbonic acid, and the amount of carbonic acid produced is less than can be accounted for by the amount of oxygen used. A resting muscle, even without the blood circulating through it, shows similar gaseous exchanges, as it retains its irritability longer in air, or in oxygen, than in an atmosphere containing no oxygen. It is clear, then, that even during rest metabolism takes place in living muscle; in other words, it respires like a living being.

During contraction, these exchanges are much increased, and the elimination of carbonic acid goes on more rapidly than the absorption of oxygen. A muscle actively contracting uses four or five times as much oxygen as it uses during rest, and it also excretes a much larger amount of carbonic acid. Thus, in the body, the venous blood flowing from an active muscle is richer in carbonic acid and poorer in oxygen than the venous blood flowing from the same muscle at rest.

Further, a tetanized muscle produces more carbonic acid than can be accounted for by the oxygen supplied to it—that is to say, the metabolic processes tend to the production of more carbonic acid. While, therefore, it is correct to say that, normally, muscles require oxygen, we must not assume that the metabolism occurring in active muscles absolutely requires a supply of oxygen from without, at all events for some time, as it is well known that muscles removed from the body may continue to contract in an atmosphere containing no oxygen. In these circumstances, the protoplasm of the muscle must receive oxygen set free by decomposition of the tissue itself. (See pages 182, 183.)

About ·4 per cent. of glycogen has been found in the muscles of the frog, and it is said that the amount is diminished in muscles that have passed through a period of active contraction. Muscles may show contractile power without the presence of this substance. Active muscles also contain a smaller amount of creatin and creatinin and of fatty acids. The amount of urea is not increased. The amount of water in muscle is increased by contraction. It has also been ascertained that during contraction the substances that may be extracted by water from muscle are diminished, while those extracted by alcohol are increased. At the same time, the muscle becomes acid from the development of paralactic acid, and probably of free phosphoric acid, and the amount of acid formed increases with greater muscular activity. The circulation of the blood is more active in a contracting muscle than in one at rest.

According to Bernard, at the moment of contraction, the blood is retained in the capillaries, and does not enter the veins; it passes only into the veins in the interval of muscular contraction, and the blood flowing in the veins from a contracting muscle is dark and distinctly venous, while, if the muscle is in a state of repose, it is more red and arterial. As shown by Gaskell, the small blood-vessels in muscles are dilated during contraction.

The exact nature of the chemical processes occurring in muscle is not understood. The protoplasm of the muscle is probably built up by anabolic processes out of such materials as proteids, carbo-hydrates—as glycogen or sugar—and fats, saline matters and water—and then, in the presence of oxygen, and probably associated with oxidations, when the muscle contracts, various substances are formed by katabolic processes, such as carbonic acid, water, etc. Immediately after a contraction, the substance of the muscle is again rapidly built up, and the waste products are removed. It is not necessary to assume that in these anabolic and katabolic processes the whole of the protoplasm is either built up anew or entirely decomposed, and it is more likely that a substance exists which may be regarded as the organic (or chemical?) basis associated

with these changes. Suppose we call this substance x, the substances above enumerated which go to the building up of muscle x^{i}, x^{ii}, x^{iii}, x^{iv}, etc., and the substances formed as waste products, y, y^{i}, y^{ii}, y^{iii}, y^{iv}, etc., then the anabolic process would be represented by $x + x^{i} + x^{ii} + x^{iii} + x^{iv}$, etc. = muscle protoplasm, and the katabolic, by muscle protoplasm $- y - y^{i} - y^{ii} - y^{iii} - y^{iv}$, etc. $= x$.

Chap. XV.—THE PHENOMENA OF CADAVERIC RIGIDITY.

Soon after death, muscles become firm, thicker, denser, more solid; they oppose a great resistance to extension, are more readily torn, and when extended, do not return to their original length. This state is called *rigor mortis*, or *cadaveric rigidity*. Their tonicity has disappeared, so that when cut across, they do not retract. The muscle substance, when seen in thin section, has lost its transparency. In this condition, also, muscular irritability is lost, but within a certain limit of time after its disappearance it may return on the injection of arterial blood into the arteries. The muscle is strongly acid, a reaction due to the presence of lactic, glycero-phosphoric and carbonic acids, and acid phosphate of potash. Cadaveric rigidity is due to coagulation of myosin from the formation of the acids. The change comes on progressively. At first, the muscle may be slightly rigid, but still irritable, but, as the rigidity increases, the irritability diminishes, until a stage is reached when the muscles are no longer irritable. The sudden stoppage of blood flowing to a muscle at first increases its irritability; this is soon diminished, and rigor begins to appear. If the circulation be re-established during the time when the muscle is even slightly irritable, the muscle may recover and the irritability entirely return, but when irritability has entirely disappeared, establishment of the circulation cannot restore it. Thus, Brown-Séquard removed cadaveric rigidity and preserved the irritability of the muscles of a criminal thirteen hours after execution, and two hours after the appearance of partial rigidity, by injecting into the blood-vessels warm and fresh defibrinated human blood containing oxygen. In this case, however, the muscles were still slightly irritable before the injection, and it can hardly be said that irritability was restored after it had entirely disappeared. Rigidity may commence from a quarter of an hour to twenty hours after death. After the death of a human being, rigor usually appears in the following order—muscles of jaws, muscles of trunk, muscles of lower limbs, muscles of upper limbs. As it supervenes, the body becomes gradually colder, until it reaches the temperature of the surrounding atmosphere, and at the same time irritability, as already stated, gradually diminishes.

Irritability has been stated by Onimus to disappear in the following order—left ventricle, stomach, and intestines, in 55 minutes; bladder and right ventricle, in 60 minutes; iris in 105 minutes; muscles of face and tongue in 180 minutes; extensors of extremities, about one hour before flexors; and in muscles of trunk, in five or six hours. The complete disappearance of irritability coincides with the complete establishment of cadaveric rigidity. This lasts for a period of from one to four or six days, and then it begins to disappear, slowly vanishing from the muscles in the order in which it supervened. Then, in turn, cadaveric rigidity is succeeded by putrefactive changes. As to the relation of these phenomena in time, the general rule is that in cases in which *rigor mortis* supervenes at an early period after death (exhaustive diseases, in animals whose muscles have been exhausted by the prolonged exertions of the chase, and in cases of death by tetanus, cholera, strychnia, or lightning), it soon disappears, and putrefaction comes quickly, whereas in cases in which *rigor mortis* is late in appearing (as in the sudden death by accident of a healthy muscular man) it lasts longer, and putrefactive changes come on late.

Rigidity of muscle may also be caused by heat. Thus, at a temperature of 40° C., the muscles of cold-blooded animals, such as the frog, become rigid, while the same phenomenon occurs with the muscles of birds at 53° C., and of mammals at 50° C. This form of rigidity is not the same as that of *rigor mortis*, because it is no doubt due to the coagulation by heat of other proteids than myosin. (See Chemistry of Muscle, p. 360.) A muscle may also be made rigid by the injection into the vessels of ·1 to ·2 per cent. solutions of hydrochloric or lactic acids, and the rigidity may be removed by the subsequent injection of a 15 per cent. solution of ammonium chloride.

A muscle may be frozen so as to be solid throughout, and yet on careful thawing, it may still be found to be slightly irritable. This kind of rigidity is not, therefore, the same as that now being described. Solutions of quinine, digitalin, caffein, veratrin, hydrocyanic acid, ether, chloroform, oil of mustard, fennel, aniseed, when given internally, favour the appearance of *rigor*, and the same is the result of the direct contact with muscle of potassium sulphocyanide, ammonia, alcohol, and metallic salts. (Landois.)

Chap. XVI.—THE GROWTH AND ATROPHY OF MUSCLE.

The embryonic development of muscle has already been briefly alluded to, p. 312. A fibre of striated muscle is developed from a single cell, which increases in size and becomes fusiform in shape. The nucleus repeatedly divides, and the surrounding protoplasm is differentiated into the disc-like arrangement already described. In the muscles of mammalia, the nuclei leave the centre and pass to the periphery of

the fibre, and are found between the bundles of fibrils, of which the fibre is now composed. The sarcolemma is in all probability the membrane of the cell from which the fibre was formed, but some have supposed that is formed by special cells, which become flattened and blend at their edges to form a membrane. The latter view is probably erroneous. A fully formed muscle consists of a certain number of fibres. It is a familiar observation that functional activity, in a normal condition, will cause a muscle to become larger, thicker, and stronger, and, along with these changes, there is an increase in the number of fibres. This enlargement is due both to the development of new fibres and to an increased thickening of older ones, by interstitial growth. The muscular fibres of a child are shorter and about one fifth of the breadth of those of an adult. Increased and regular exercise causes an increased growth and probably also a development of new fibres from the nuclei under the sarcolemma. Professor Rutherford points out, however, that many muscles, such as those of the muscles of respiration of the fœtus, grow without exercise, in consequence of the "germinal energy of the tissue," influenced probably by the activity of certain trophic nerves. A mere determination of nutritive blood to a muscle will not necessarily cause it to grow, but if, along with this, the muscle is caused systematically to contract, its protoplasm will take up nutritive matter to an increased amount, and the muscle will increase in size and strength, until it reaches a limit beyond which no further growth is possible. A striking example of the growth of involuntary muscular tissue is seen in the development of the muscular wall of the pregnant uterus. During gestation this organ increases from about 1 ounce to from $1\frac{1}{2}$ to 3 pounds, and the increase is largely due to the increase in the muscular coat, the cells of which become enlarged from 7 to 11 times their length, and from 2 to 5 times their breadth in the unimpregnated state. Muscular hypertrophy is also seen normally in striated muscle, in the muscles of the arm of a labourer, and in the limbs of gymnasts and dancers, and in the intermediate variety of muscle in hypertrophy of the walls of the left ventricle of the heart associated with contraction of the orifice of the aorta.

The opposite condition of hypertrophy is atrophy, as seen in the wasted muscles of a paralytic. The muscles become thin, soft, and flabby, and when examined show numerous fatty particles in the muscle substance (Fig. 170, p. 312). The striation also becomes less distinct. The small fat drops, mostly in or near the nuclei, are usually in rows parallel to the longitudinal striæ of the fibre. The small drops may by-and-bye coalesce to form larger ones.

If muscle be divided and the ends be brought into close apposition,

they may be joined by the development of new muscle substance arising from proliferation of nuclei and the formation of new muscle cells. (Kraske.) Various pathologists have held that muscle fibres may be regenerated from migrated colourless blood corpuscles. There is undoubtedly a physiological regeneration of muscle in frogs after the sleep of winter and probably in hypernating animals. A wound in muscle may therefore not be healed by a cicatrix of connective tissue, if the margins of the wound are closely united, but if the wound be left partially open, the cavity will be filled up by connective tissue.[1]

Chap. XVII.—THE PROPERTIES OF NON-STRIATED MUSCULAR FIBRE.

When stimulated, either directly, by mechanical, chemical, or thermal stimuli, or by a nervous impulse, involuntary muscle contracts. The period of latent stimulation is very much longer than in striated muscle, and the contraction takes place slowly, and lasts for a lengthened period. The general character of the contraction may be seen

Fig. 284.—Tracing of the contraction of non-striated muscular fibre. The upper line was obtained from the contracting stomach, and the lower from the contracting bladder, of a dog. The horizontal mark indicates the moment of application of the stimulus. In this tracing, 1 centimetre = 6 seconds.

in the movements of the intestine of a recently killed warm-blooded animal. Paul Bert obtained a tracing of the contraction of non-striated muscle, as manifested by the movement of the walls of hollow organs, containing this kind of tissue. This is shown in Fig. 284.

Marey has shown that the contraction of non-striated muscle is not made up of a series of smaller contractions, as in striated muscle, but is one single contraction, which may be of greater or less duration. According to Engelmann, a stimulus applied to a group of non-striated fibres is propagated to neighbouring fibres, and thus the stimulus may radiate in various directions, even in tissues containing no nerves. The interesting observations of Foster and Dew Smith on the contractions of the heart of a snail, and of G. J. Romanes on the transmission of impulses in the bodies of *Medusæ*, support the statement. Organs containing non-striated muscle frequently show rhythmical movements.

[1] As to the hypertrophy and atrophy of muscle, see Wagner's *General Pathology*, pp. 418-422.

It is difficult to estimate experimentally the work done by this kind of fibre, but when we consider the force of the bladder in expelling urine, and still more the force of the uterus in parturition, it must evidently be very great. Nothing definite is known regarding the conditions of fatigue. According to Beaunis, the state of cadaveric rigidity occurs to a slight extent.

In ordinary circumstances, organs composed of non-striated muscle are only feebly sensitive, but occasionally they become so sensitive as to give rise to ill-defined sensations of discomfort, or even of pain.

Regarding the general physiology of muscular tissue, consult PROCHASKA—De Carne Musculari in Oper. Min., 1800; BOWMAN, in Trans. Roy. Soc. London, 1840, and art. Muscle, in Cyclop. of Anat. and Physiol.; TODD and BOWMAN—Anat. and Phys.; and SHARPEY and SCHÄFER, in Quain's Anat., ninth ed., vol. II., p. 118 *et seq.*; MAREY—Du Mouvement dans les Fonctions de la Vie, 1868; WUNDT'S Physiologie Humaine, p. 372 *et seq.*; BEAUNIS' Physiologie; for full details as to the experimental study of the functions of muscle, see account by MICHAEL FOSTER, in Handbook for the Physiological Laboratory, p. 341; also CYON'S Methodik der Physiologischen Experimente und Vivisectionen, Fünftes Capitel. For sketches of apparatus, see the Atlas of the same work, and also GSCHEIDLEN'S Physiologischen Methodik; MAREY'S Animal Mechanism, chaps. IV., V., and VI. As to the production of heat in muscular work, see MATTEUCCI —Sur les Phénomènes Physiques et Chemiques de la Contraction Musculaire (Comptes Rend., 1856, t. XLII.); J. BÉCLARD—De la Contraction Musculaire dans ses raports avec la Témperature Animale (Arch. Génér. de Méd., 1861, 5e série, t. XVII. p. 24); HEIDENHAIN—Mechanische Leistung, Warmentwicklung und Stoffumsatz bei der Muskelthätigkeit, Leipsig, 1864; WUNDT—Physique Médicale, p. 535. The best general account of the action of poisons and drugs on muscle is to be found in T. L. BRUNTON'S Pharmacology, Therapeutics, and Materia Medica, chap. V. See also papers by GERALD F. YEO and THEODORE CASH, on the relation between the active phases of contraction and the latent period of skeletal muscles, *Journal of Physiology*, vol. iv. No. 2; and H. KRONECKER and W. STIRLING on the Genesis of Tetanus, *Journal of Physiology*, vol. i. Nos. 4 and 5.

CHAP. XVIII.—THE ELECTRICAL PHENOMENA OF MUSCLE.

We have already seen that when the stimulus transmitted by a motor nerve reaches a muscle, molecular changes of a chemical character are set up in the latter which result in the production of motion, of heat, of sound, and of various chemical substances, such as carbonic acid, paralactic acid, etc. One would naturally expect that such changes would be associated with electrical disturbances, seeing that chemical changes are so often, if not invariably, linked with electrical phenomena. The stages, however, by which the existence of electrical phenomena in living muscle and in other living structures has been satisfactorily established

forms a chapter in science so interesting and so suggestive that I shall now place a short account of them before the reader, and gradually lead him to our present position with regard to these phenomena.

The discovery of animal electricity dates from 1786. It is said that Madame Galvani, the wife of Luigi Galvani (1737–1798), in preparing some frogs for culinary purposes, observed that the apparently dead animals became convulsed when brought into the neighbourhood of a frictional electrical machine in action. Her husband, who was professor of anatomy and physiology in Bologna, had his attention directed to these phenomena, and found that the convulsions occurred at the instant a spark was emitted from the conductor, provided some metallic substance was in contact with the nerve of the frog. He tried the same experiment with lightning, and, in the autumn of the same year, endeavoured to discover the action of atmospheric electricity on the prepared legs of a frog when the sky was stormless. On the 20th of September, he suspended three frogs to the iron trellis-work surrounding the roof of his house, by means of copper hooks, and saw, when they were blown about by the wind, that convulsions were caused whenever they came in contact with the iron. This observation convinced him that an electrical machine was not required, the same effect being produced on the contact of dissimilar metals. He at length concluded that the convulsions were due to inherent electricity, that is, electricity, to use his own phrase, secreted in and originating from the animal tissues.[1]

Galvani published an account of his experiments in 1791.[2] At this time the existence of a "nervous fluid," a something which, if not life itself, was considered inseparable from it, was keenly debated by the learned, and contended for by the animists. Galvani's discovery, therefore, riveted the attention of the scientific world—electricity took the place of the nervous fluid, and the source of life, and the origin of the bodily and mental functions were now ascribed to the existence of a new principle, which, from the name of its discoverer, was called *Galvanism.*

Among the many distinguished men whose attention was attracted toward's Galvani's discovery, the most remarkable was Alexander Volta. He was a physicist and professor of natural philosophy in the University of Pavia. At first he entered fully into the views of his countryman,

[1] Most of the historical portion of this chapter was written by the author for Hughes Bennett's Text-book of Physiology in 1871. Assistance was also derived from a paper by Dr. M'Gregor-Robertson, in *Proceedings of the Philosophical Society of Glasgow*, vol. 13, No. 2.

[2] These results were communicated to the Institute of Bologna in 1791, in a paper entitled "*Aloysii Galvani de viribus Electricitatis in motu musculari commentarius.*"

and repeated his experiments. But in his hands galvanism, instead of taking a physiological and medical, took a physical, direction. Finding that muscular contractions in a frog could be produced only by the contact of dissimilar metals, he at length dissented from Galvani's theory of animal electricity. Trying by the same means to produce muscular contractions in the human body he failed. But on placing a metal above and below the tongue, he detected a peculiar taste at the moment of contact. This experiment had been previously recorded by Sulzer, and laid aside only as curious, but in the hands of Volta it became the groundwork of chemical, or what has been called *Voltaic*, electricity. In opposition to Galvani, therefore, he put forth the opinion that the muscular contractions in the frog had nothing to do with animal, but were caused by a very feeble, *artificial*, electricity, which was produced by the application of heterogeneous metals to the limbs of the animals.

In reply to this attack, Galvani pointed out that the contractions might be occasioned by one metal; but Volta then showed that the two extremities or surfaces of one piece of metal might be in different states of tension, and therefore capable of exciting electricity. Galvani then used mercury alone, to which this objection could not apply, and dipping the muscles into one part of a trough filled with it, he thus succeeded in causing contractions. But Volta, by a new series of experiments, demonstrated that mercury was always altering under the action of the air, and that, in conjunction with moisture, it could produce electricity independently of an animal. It now became necessary for Galvani to show that contractions took place without any metal at all, and, aided by his nephew Aldini, he succeeded in doing so, and, as he thought, in for ever silencing his formidable opponent. In fact, he discovered that on dissecting out the sciatic nerve of a frog, but leaving one end in connection with the leg, and bringing this nerve in contact with the muscles externally, the latter were thrown into distinct contractions. He also caused the limb to contract by simply bringing the nerve in contact with the muscle of another animal, which was insulated from the limb of the frog.

His theory of animal electricity now was, that it was produced in the nervous system, and especially in the cerebrum. The internal substance of nerves he considered to have great conducting powers, but their oily surfaces prevented dispersion through the body, and allowed its accumulation in the muscles. These last he compared to a Leyden jar, the outer surface being negative, the inner positive. The nerve was analogous to the conductor of the jar. When the nerve connects the inner with the outer surface, there is a discharge of electricity, causing irritation and contraction of the muscles.

Volta at first endeavoured to meet these facts by the supposition of a mechanical stimulation of the nerves, but at length showed that, if not caused by metals, it was necessary that there should be dissimilar fluids and tissues which were capable of exciting electricity. It was admitted, even by Galvani's followers, that it was essential to the success of the experiment that the muscle with which the nerve was in contact should be moistened with blood or some thick fluid, and if by any accident the limb remained motionless, it was only necessary, in order to induce muscular contractions, that the parts to be in contact should be moistened with saliva, brine, mucus, etc., or still better, with soapy water, and best of all, with a solution of acids or alkalies.

Galvani, in several letters to Spallanzani, endeavoured to weaken the force of these arguments, as did Humboldt, who showed that contractions resulted when the circuit consisted only of nerve and muscle, without the interposition of blood, mucus, etc. About this time, 1798, Galvani fell ill, and died on the 4th December of that year. Volta's experiments, on the other hand, were continued with unabated energy, and, towards the end of 1799, he invented the Voltaic pile, whereby his opinions were supported and the production of electricity by the contact of metals and a fluid was completely proved. This great victory, which Galvani, by his death, escaped the mortification of witnessing, notwithstanding the opposition of Humboldt, Aldini, Pfaff, and a few others, overthrew the idea of an animal electricity for the space of twenty-eight years. During this period, indeed, its existence was generally regarded with incredulity, and the term *animal magnetism*, a designation not infrequently adopted by impostors, only tended to bring it still more into contempt. Volta died in 1826, and it is curious that only a year afterwards, Nobili again revived the idea of animal electricity, by demonstrating the existence of an electrical current in the frog. But in the interval of twenty-eight years, Voltaic electricity had made wonderful progress. In the hands of Sir Humphry Davy, it led to brilliant discoveries in chemistry, and its subsequent practical applications constitute some of the wonders of physical science in the present age.

In 1820, Oersted, a Danish philosopher, discovered electro-magnetism. He showed that when a continuous galvanic current passes along a wire, placed above or below, and parallel to, a magnetic needle, the latter is immediately deflected. This led Ampère to invent the astatic needle, and in turn enabled Nobili to construct a galvanometer, which he rendered exquisitely sensitive by various improvements. He prepared a frog after the method of Galvani, and having introduced its two legs into two glasses of salt water, he united the two vessels by filaments

of moist cotton: the frog's muscles at once contracted. Removing the cotton he connected the two glasses through a galvanometer circuit, and he observed a deviation of from 10° to 30°, showing that *an electrical current was passing from the feet to the head of the animal.* By introducing several frogs into the circuit, he increased the strength of the current. The galvanometer of Nobili enabled physiologists to demonstrate that Galvani and Volta were both right and both wrong. Galvani was right in maintaining the existence of an inherent animal electricity, whilst he was wrong in supposing that the contact of two metals with the tissues gave a proof of this. Volta, again, was right in maintaining that galvanism could be produced independently of animals, but wrong in denying that electrical currents existed in them. By the astatic needle, as Du Bois-Reymond happily remarks, metallic electricity was enabled to atone for the wrong she had done to her more tender twin sister in their earlier years.

While Nobili, by his construction of a galvanometer, was of such essential service, he was led into the error of supposing that the deflection of the needle was due to thermo-electricity. It was ten years later, in 1837, that Matteucci, of Pisa, showed that to obtain a deviation of the galvanometer needle, it was not necessary to prepare the frog according to Galvani's method. On connecting any two parts of its body, as, for example, the head and legs, by means of the galvanometer, he at once obtained a deviation. In 1841, he advanced the following law: "The interior of a muscle, placed in connection with any part whatever of the same animal, such as nerve, surface of muscle, skin, etc., produces a current which goes in the animal from the muscular part to that which is not so." In this paper, he first described his muscular *pile*, consisting of about twenty frogs' thighs roughly cut across, and arranged end to end. On connecting the stump of the knee with one wire leading to the galvanometer, and the section of the femur with the other, the needle was at once deflected so as to indicate a current passing upwards in the pile from the knee to the thigh. He considered this to be a current different from the frog current of Nobili, and he held that the frog had two currents (1) the muscular current (Nobili's) common to all animals; and (2) the special current, evolved by the muscular pile, peculiar to the frog.

In 1841, Professor Emil Du Bois-Reymond, of Berlin, repeated Matteucci's experiments, and further investigated the subject with the aid of most delicate galvanometers, and other ingenious apparatus constructed by himself. From this period a new impulse was given to the experimental investigation of animal electricity.

For the purpose of indicating the current Du Bois-Reymond used a

FIG. 285.—Magnet of Wiedemann's Galvanometer, shown in Fig. 286.

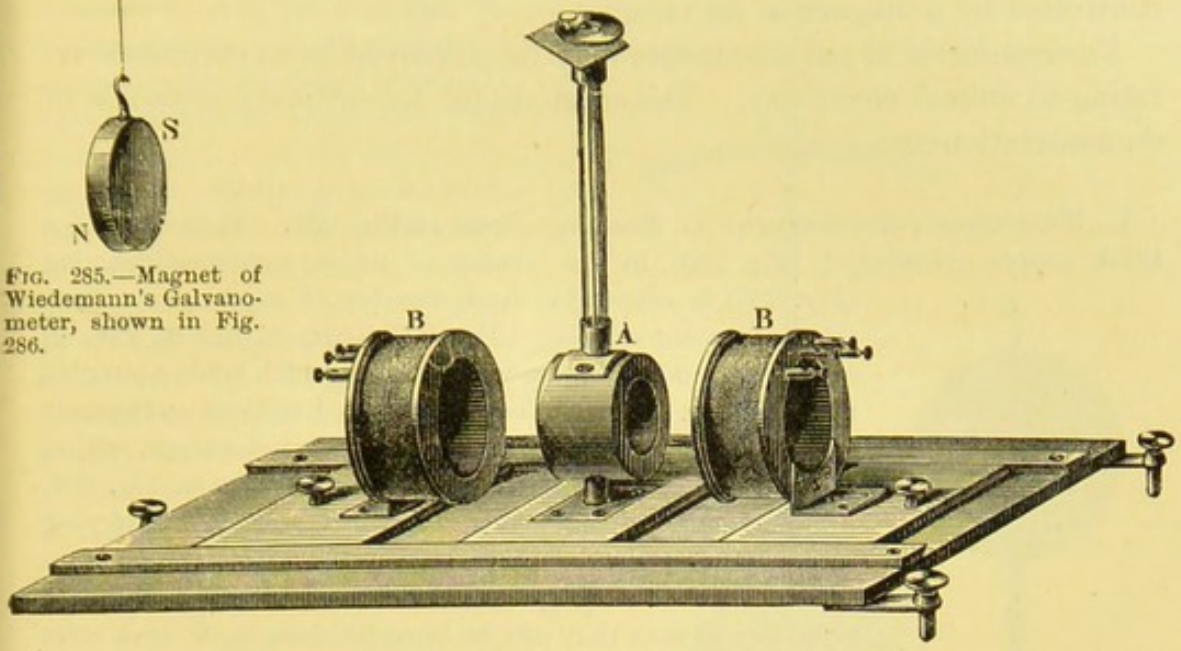


FIG. 286.—Wiedemann's Galvanometer or Boussole.[1]

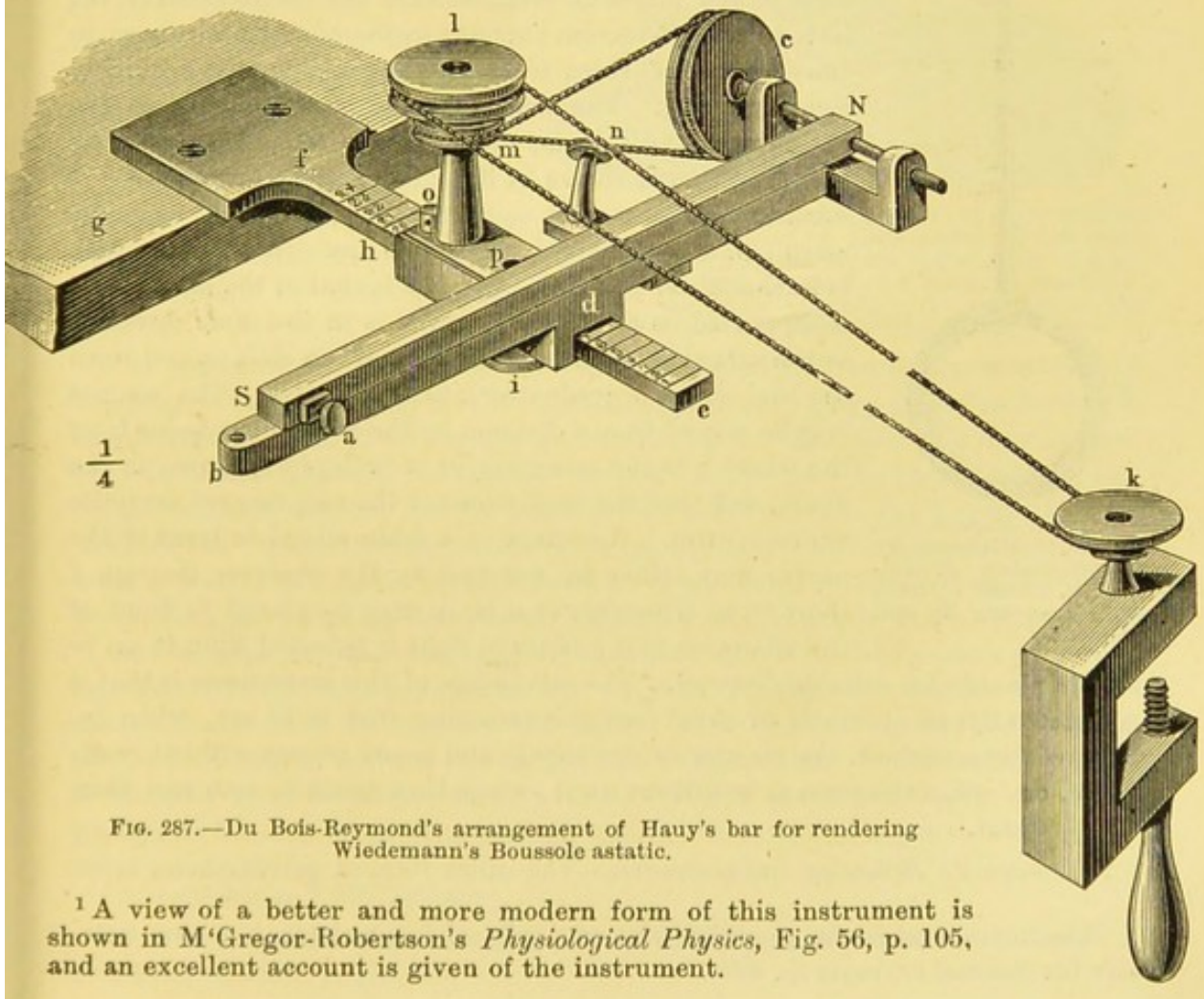


FIG. 287.—Du Bois-Reymond's arrangement of Hauy's bar for rendering Wiedemann's Boussole astatic.

[1] A view of a better and more modern form of this instrument is shown in M'Gregor-Robertson's *Physiological Physics*, Fig. 56, p. 105, and an excellent account is given of the instrument.

reflecting galvanometer of extreme sensibility, whose oscillations were controlled by a magnet in its vicinity.

Various forms of galvanometers may be employed in experiments relating to animal electricity. The most useful for ordinary purposes of demonstration are—

1. *Wiedemann's Galvanometer or Boussole*, shown in Fig. 286. It consists of a thick copper cylinder, A (Fig. 286), in the interior of which a magnetized ring (Fig. 285) is suspended from the top of a glass tube by a single filament of silk. From the ring A, in Fig. 288, an aluminium rod passes up to B, a frame which holds a circular plane mirror. Plugs of copper are used to close up the ends of the chamber in which the circular magnet swings, whilst the chamber containing the mirror (not shown in Fig. 286, but placed a little above A) is closed by a brass cover carrying a thin circular piece of glass, so that the mirror can be seen. On each side of A (Fig. 286) the coils of wire B B slide in a wide slot so that they can be brought close to or even over the copper chamber A. Each coil contains about 30,000 turns of very fine wire, and the resistance is equal to 7,000 ohms for each coil.[1] The copper chamber damps the oscillations of the magnetic ring, because the oscillations of the latter set up induction currents in the opposite direction in the copper, and these react on the ring, so that it quickly comes to rest. The ring magnet (corresponding to the needle in an ordinary galvanometer) is rendered astatic by the arrangement shown in Fig. 287, called Hauy's bar. It consists of an accessory magnet, S N, placed in the magnetic meridian, and therefore parallel to the needle. Its north pole should be pointing north, as is that of the needle. It is supported on the bar e f, which is in the same direction as the axis of the coils, and the magnet can slide up and down the bar, which is graduated into centimetres. The magnet may be moved from a distance by the cord m n, passing from the wheel k to the arrangement of pulleys l c, shown in the figure, and thus the oscillations of the ring magnet are quite under control. An image of a scale placed in front of the mirror may either be watched by the observer through a short focus telescope, or a lamp may be placed in front of the mirror so that a beam of light is reflected from it on to a scale placed at a suitable distance. The advantage of this instrument is that it is practically an aperiodic or *dead beat* galvanometer, that is to say, when influenced by a current, the magnet swings slowly and comes to rest without oscillation, and when the current is withdrawn it swings back again to zero and then comes to rest.

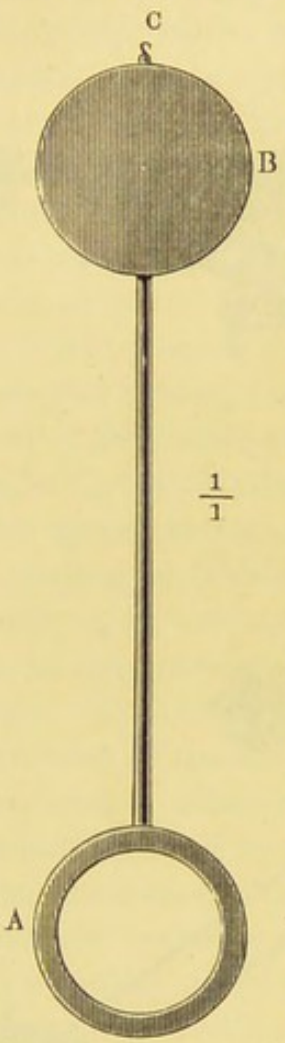


FIG. 288.—Magnet and mirror of Wiedemann's Galvanometer. A, Magnet; B, mirror; C, hook for suspending by silk filament.

2. *Thomson's Reflecting Galvanometer.*—The other form of galvanometer is Sir

[1] The instrument is also provided with two coils of low resistance, 1 ohm, suitable for thermal currents (p. 421).

William Thomson's reflecting galvanometer, seen in Fig. 289, an instrument more sensitive than the one above described and more suitable for refined investigations. This instrument is upon the same principle as the ordinary galvanometer, but it is modified by having the needles constituting the astatic system very short and very light, by having the coils of wire in the bobbins brought as close to the needles as possible, and by having a small silvered mirror attached to the uppermost group of needles. A lamp is placed in front of the little mirror, and a ray of light is reflected by the mirror upon a scale placed at a convenient distance in front of the instrument. The galvanometer made for physiological purposes has a resistance of 8,498 ohms at 18° C. One Daniell's element gives through a circuit of 254,970,000 ohms resistance a deflection of 130 divisions on the scale, and through a resistance of 33,146 meg-ohms[1] a deflection of 1 division. It is therefore an instrument of great resistance and of extreme delicacy.

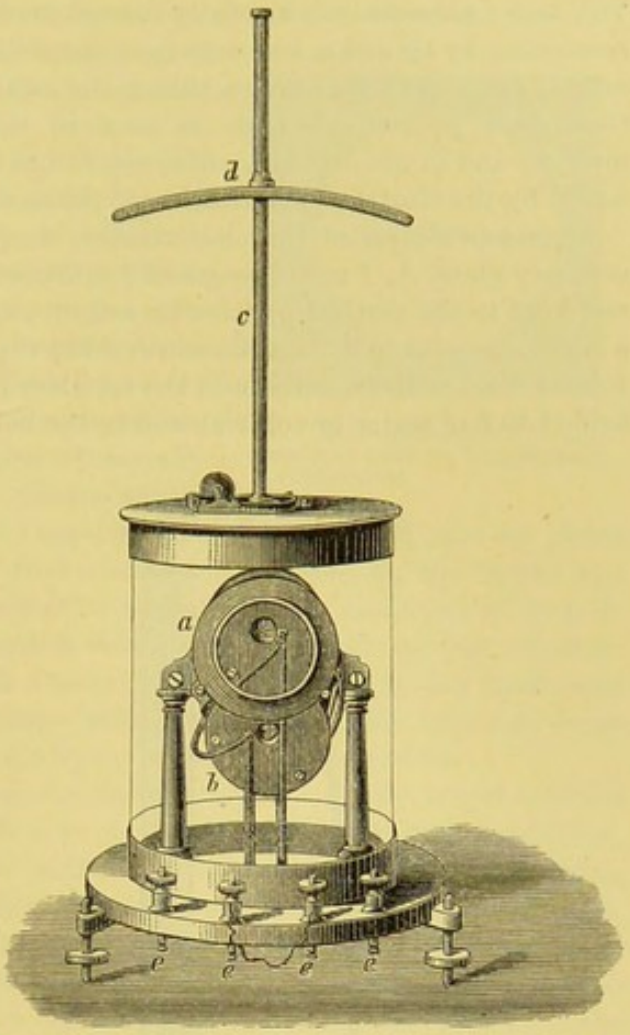


FIG. 289.—Reflecting galvanometer of Sir William Thomson. *a*, Upper, and *b*, lower bobbin of fine wire; a small mirror is attached to the upper group of magnetic needles in the centre of *a*; *c*, brass rod, bearing *d*, a curved magnet for regulating the position of the needles underneath; *e e e e*, binding screws.

3. *Lippmann's Capillary Electrometer.* The action of the substances set free at the electrodes in electrolytic decomposition, and the energy shown as motion in these circumstances, are strikingly manifested by the behaviour of a drop of mercury in dilute sulphuric acid, when the positive pole of a battery is put in connection with the mercury and the negative dips into the acid. The mercury extends towards the negative electrode during the passage of the current, becoming covered with a film of sub-oxide, which dissolves in the acid, leaving a bright surface. By making and breaking the current a series of oscillations is set up. Movements in the mercurial electrode and adjacent acid have been observed by many physicists, and have received various explanations. Erman, in 1809, was the first to observe that when a drop of mercury was placed on a grooved surface between the electrodes it moved towards the negative pole; and he also observed that "a drop of mercury in a horizontal tube, with dilute acid on both sides, moved at the passage of the electric current through the tube toward the negative electrode."[2]

[1] A meg-ohm = 1,000,000 ohms.

[2] For an historical account of this subject, see Lippmann, *Annales de Chemie et de Physique*, 1875, p. 540.

This latter phenomenon was fully investigated by Lippmann,[1] and led, along with researches by Quincke, not only to a theoretical explanation of electro-capillary action, but also to the construction of the capillary electrometer. It is now known that these phenomena, both as seen in the experiment with the globule of mercury and in the capillary tube, are due to a change in the surface tension produced by the electrical polarization of the surface of the mercury.

Lippmann's form of the electrometer, shown in Fig. 290, consists of a tube of ordinary glass, A, 1 metre long and 7 millimetres in diameter, open at both ends, and kept in the vertical position by a stout support. The lower end is drawn to a capillary point, until the diameter of the capillary is ·005 of a millimetre. The tube is filled with mercury, and the capillary point is immersed in dilute sulphuric acid (1 to 6 of water in volume), and in the bottom of the vessel, B, containing the

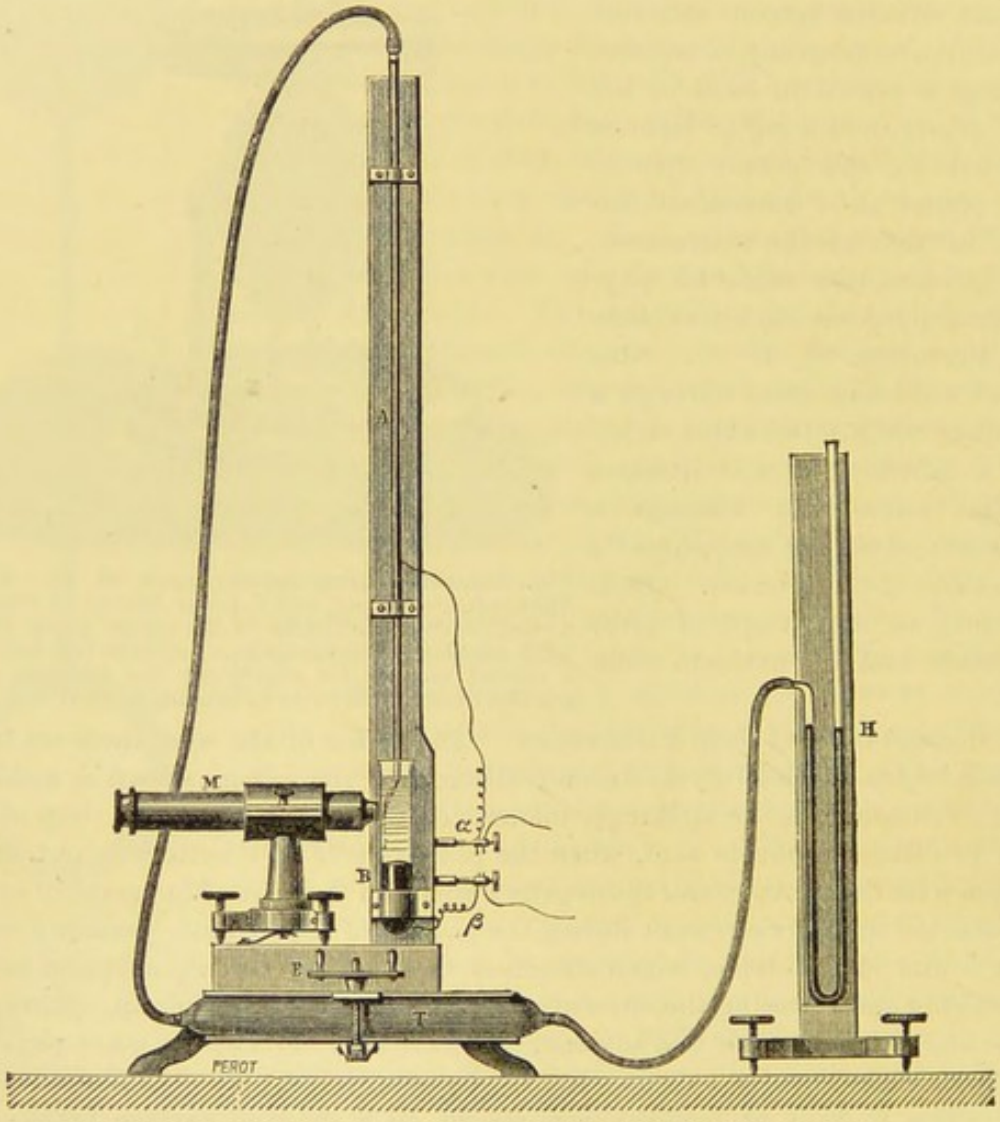


Fig. 290.—Lippmann's Capillary Electrometer.

acid there is a little more mercury. A platinum wire, α, is put into connection with the straight tube having the capillary point, and another platinum wire, β,

[1] Lippmann, *Comptes Rendus*, 1873, p. 1407 ; *Annal. de Chem. et de Phys. op. cit.* p. 494 ; Poggendorf's *Annalen*, cxlix. p. 547 ; also *Phil. Mag.* [4], xlvii. p. 281.

enters the wide tube, B. Finally, arrangements are made by which the capillary point can be seen with a microscope, M, magnifying 250 diameters. The appearance of the tube under the microscope is shown in Fig. 291. Such an instrument is very sensitive ; and Lippmann states that it is possible to determine a difference of potential so small as that of the $\frac{1}{10080}$ of a Daniell. Lippmann made use of the arrangement shown in Fig. 290 for bringing back the mercury to the zero-point when it had been pushed up the capillary tube by the action of a constant electro-motive force in the direction of β to α, and for measuring by means of a manometer, H, the pressure in mm. of mercury required to bring the mercury back. In the apparatus, T is an India-rubber bag or tube capable of being compressed between plates approximated by the screw E, so that pressure is exerted on the upper end of the long vertical tube, and the amount of this pressure is measured in mm. of mercury by the manometer, H. Various simpler forms of the capillary electrometer can be easily made, in which the capillary tube rests in the horizontal position on the stage of the microscope, and in which, also, the tube may be so fine as to bear examination with a magnifying power of 400 diameters.[1]

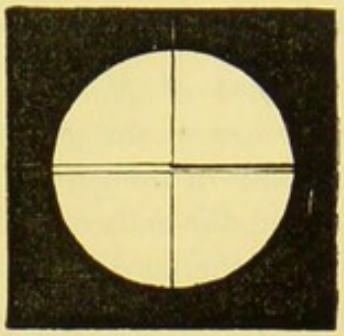


Fig. 291.—Appearance of the capillary tube in Lippmann's electrometer.

The special value of the capillary electrometer lies in the *quickness with which it responds to any sudden variation in the e. m. f.* As observed by Burdon-Sanderson, with reference to changes in potential in the injured heart of the frog while alive, it is striking to notice " that the movements of the mercurial column not only correspond closely in time to the actual changes which they represent, but express with very great accuracy the differences of potential which actually exist in each successive phase of a variation." The oscillations of the column of mercury in the capillary tube may be photographed on a moving sensitive plate, when a picture such as is seen in Fig. 292 will be obtained, which shows at a glance gradual or sudden changes in potential.[2]

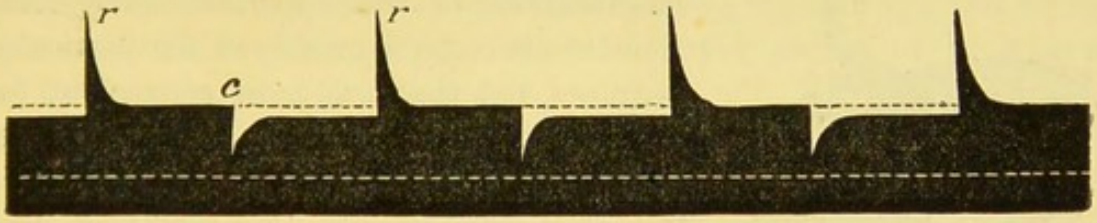


Fig. 292.—Changes in potential caused by the induction currents from the secondary coil of an inductorium at the moments of closing, *c* ; and of opening, *r* ; *r*, the primary current. From a photograph by Marey of the oscillations of the mercury in the capillary tube of Lippmann's electrometer.

4. *The Telephone.* By this instrument sounds may be heard on making and

[1] See author's paper on Lippmann's capillary electrometer, *Proceedings of the Philosophical Society of Glasgow*, vol. xiv. p. 37 ; Burdon-Sanderson, on the electromotive properties of the leaf of Dionæa in the excited and unexcited states, *Phil. Trans.* part i. 1882 ; Lovén, *Nordiskt Medic. Arkiv.* vol. xi. No. 14. As to a form made by Prof. Dewar, see S. P. Thomson's work on Electricity.

[2] Thus Burdon-Sanderson has photographed the oscillations caused by changes of potential in Dionæa and in the frog's heart, *Royal Institution Lecture*, 1882.

breaking the current from a muscle at rest. The current shown by a muscle in action cannot be so detected. The telephone has not been of any special service in the investigations of currents from animal tissues.

Method of Making Experiments with Galvanometer.—The very sensitiveness of the galvanometer is one of its chief sources of error. If the ends of the galvanometer wires are connected with two wires, say of copper, the ends of which are immersed in a conducting fluid, a deflection of the needle at once indicates a powerful current, because the two wires are not in an absolutely similar electrical state; or if the wires are directly connected with the tissue to be examined, another powerful deflection occurs, because electricity is evolved at the point of contact of the wires and the moist tissue.

Further, if metallic conductors, say composed of zinc, coming from the galvanometer, were brought into connection with living muscle, little or no current would be obtained, and even if there were a current, it might be due to contact of the metallic conductors with the living tissue exciting electrolytic decomposition. Hence it is necessary to have a fluid interposed between the metal and the animal tissue, as, for example, the zinc wire or plate forming the terminals of the galvanometer must be immersed in a saturated solution of sulphate of zinc. But as sulphate of zinc solution would have the effect of irritating the living muscle, it is necessary to have an inactive substance between the tissue and the sulphate of zinc solution. All these conditions are fulfilled by the arrangement of Du Bois-Reymond, which is that usually employed, and which may conveniently be termed the *non-polarizable electrodes.* Many modifications of this apparatus have, from time to time, been employed for particular purposes, but the form most convenient for demonstrating the principal electrical phenomena of nerve and muscle is what is here described. It consists (Fig. 293) of two troughs made of zinc, mounted on insulating plates of vulcanite. The inner surfaces of the troughs are carefully amalgamated, and they are filled nearly full of a saturated solution of sulphate of zinc. Into each trough is then placed a small cushion of clean blotting or filter paper, which quickly becomes permeated by the solution. Finally, a small thin film or plate of sculptor's clay, or kaolin, moistened with a half per cent. solution of common salt, or still better, with saliva, is laid on each paper pad. These clay-pads are for guarding the tissue

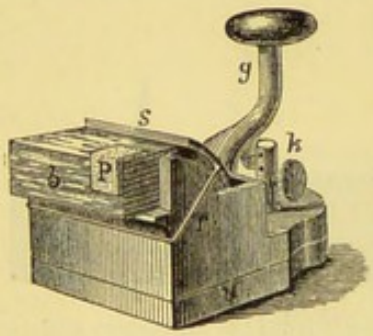


FIG. 293.—Non-polarizable electrode of Du Bois-Reymond. *r*, zinc trough; *v*, vulcanite base; *s*, vulcanite shield to keep pad *b* of blotting paper in position; *p*, moist clay; *g*, handle, at end of which there is a vulcanite knob; *k*, binding screw.

from the irritant action of the sulphate of zinc. Wires are conducted from the trough to the galvanometer, as shown in Fig. 294, and it is

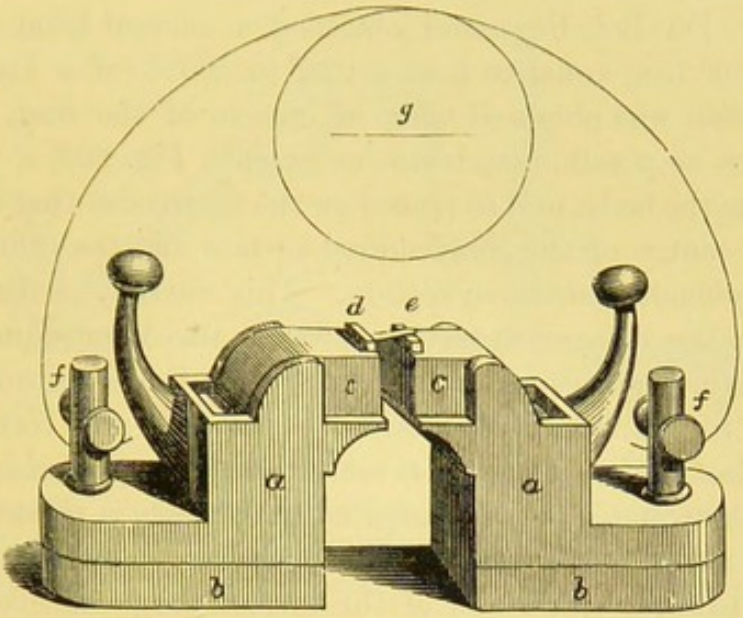


FIG. 294.—Diagram of arrangement of Du Bois-Reymond. *a*, *a*, zinc troughs ; *b*, vulcanite ; *c*, paper pads ; *d*, *e*, moist clay ; *f*, *f*, binding screws for terminals of galvanometer ; *g*, observe the bit of paper conducting *d* and *e*, thus completing the circuit.

convenient to have a key interposed in the circuit, so that any current from the troughs may be permitted to pass to the galvanometer, or be shut off at pleasure. It is essential that no currents are derived from the troughs or from any of their connections, that is, to have the apparatus non-polarizable. This can be done by taking extreme care in all the arrangements, and by connecting the troughs for a few hours before the experiment by a thick copper wire (passing from *f* to *f*), and laying a morsel of moist blotting paper from one paper cushion to the other so as to place the troughs in circuit.

To determine in what direction that current may be, a preliminary observation is necessary. To one wire of the galvanometer a piece of zinc is bound, and to the other a piece of copper. The two being placed in circuit, a current is produced, and the beam of light moves in a particular direction. The copper pole being positive to the zinc, one can find in which direction the beam of light will move if a particular wire is positive. The non-polarizable electrode to which this wire is connected is carefully noted, and one can then always tell in which direction the needle must swing if this be the positive electrode. Into the circuit of the electrode and galvanometer is placed a small resistance box or shunt, graduated for the particular galvanometer used. It is so interposed that one of the brass plugs may be placed in one of four different positions. If in one, the current is short-circuited, and none can pass

to the galvanometer; if in another, a 10th part of the total amount of current is allowed to pass; or in other positions, 100th or 1000th—the remainder being returned to the electrodes. With the aid of such an arrangement, Du Bois-Reymond obtained a current from the inactive muscle of the frog equal to from 0·035 to 0·075 of a Daniell. This maximum effect was obtained when a muscle of the frog, one with as parallel fibres as possible (sartorius or gracilis, Fig. 203, *s*, p. 355), was removed from the body, and so placed on the electrodes that one clay pad touched the centre of the longitudinal surface and the other the centre of a carefully-made transverse section. This current is directed *in* the muscle from the transverse section towards the longitudinal surface—that is to say, it passes *out of* the muscle by the longitudinal surface and returns to the transverse section. Hence the rule formulated is—*The longitudinal surface is positive to the transverse section, which is negative.* A point in the equator of the longitudinal surface is most positive, the axis of the transverse section is most negative. As you proceed from the equator towards either end of the surface the positivity diminishes, and as you proceed from the axis of the section towards the circumference the negativity diminishes. To put this in another way—*On the longitudinal surface, a point at a distance from the equator is negative to a point nearer to it; on the transverse section, a point at a distance from the axis is positive to a point nearer to it.* If two points are taken on the longitudinal surface of a regularly-formed muscle, one on either side of the equator, and both equidistant from it, these two points are iso-electrical, that is of equal potential, and show no current, and similarly with two points on the cross section equidistant from the axis. Fresh cross sections may be made, and fresh longitudinal surfaces may be formed, still the muscle cylinder exhibits the same phenomena, and that whether the surfaces have been formed by cutting, heating, the action of acids, or simply by drying the exposed surface. In the last case, the layer of dead tissue on the surface acts as an indifferent conductor, and leads the current produced in the living portions beneath it.

In the case where the cross section has been made obliquely, Du Bois-Reymond showed that the greatest positive tension is towards the obtuse angle, the most negative point towards the acute angle—*i.e.* a point in the neighbourhood of the obtuse angle is positive to a point in the neighbourhood of the acute angle, even though both are equidistant from the equator. These have been called "inclination currents." The relative electrical condition of different points is best investigated by a modification of the non-polarized electrodes made by Du Bois-Reymond himself. A flattened tube of glass, *r* (Fig. 295), contains a slip of amalgamated zinc. The end of the tube is closed by a moistened

piece of clay, the part projecting from the tube being pressed to a point *t*, and the tube is then filled with zinc solution. The zinc is fastened to a brass support, to which a wire is led (Fig. 295).

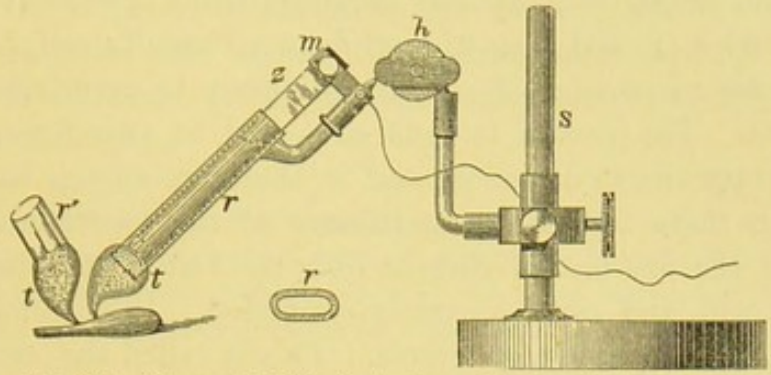


Fig. 295.—Non-polarizable electrodes. For description, see text.

See also the non-polarizable electrodes of the form used by Burdon-Sanderson in Fig. 230, p. 380. Another form which may be used for stimulating protoplasmic bodies under the microscope by non-polarizing currents, or for leading currents from such microscopical structures to a capillary electrometer, is shown in Fig. 296.

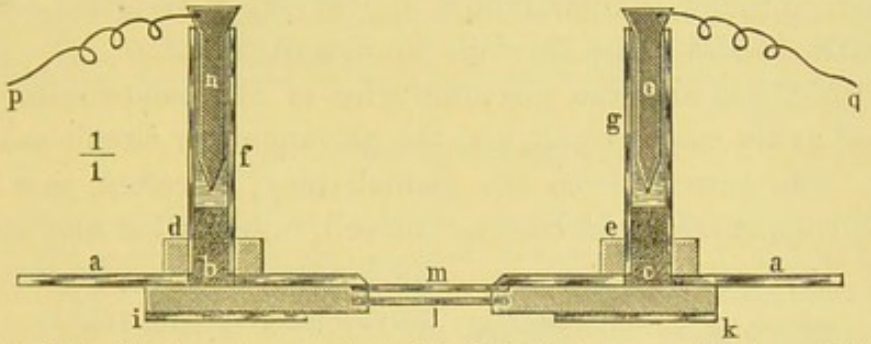


Fig. 296.—Engelmann's non-polarizable electrodes for use on the stage of the microscope. *a a*, grooves in glass slide, with holes in *d e*, into which glass tubes, *b c*, tightly fit; *f g*, tubes containing salt solution, or saturated solution of sulphate of zinc; *b c*, pads of paper: *n o*, zinc rods; *m*, cover glass; *l*, glass on which object is placed. The space between *m* and *l* communicates with *b* and *c*.

The fine clay points can then be placed on any desired part of the muscle. As the muscle preparation slowly dies, the amount of current diminishes, till, on the advent of rigor, it disappears. In regard to temperature, freezing and a high temperature also abolish it, but with moderate degrees of heat it increases.

Differences of temperature produce specially strong currents,[1] a warmer part being positive to a colder, the difference between the two connected points being alone influential, and the condition of the parts lying between being unimportant.

A method of accurately determining the amount of current obtained from muscle or other tissue was devised by Poggendorff. An improved

[1] Hermann, *Handbuch der Physiologie*, 1879, Bd. I. Th. I. *s.* 196.

form of the arrangement, by Du Bois-Reymond, is shown in the accompanying diagram, Fig. 297.

The idea of the arrangement is to send through the galvanometer, B, the maximum strength of current obtained from the tissue M, placed on the electrodes, T, and then to send from a Daniell's cell, K, *but in an opposite direction*, a portion of current sufficient to counterbalance the tissue current. The needle is thus subjected to two forces, equal in amount but opposite in direction, and it therefore swings back to zero, its remaining there indicating the balance of the two forces. By the arrangement the amount of current from the Daniell employed can be regulated at will, and can be exactly estimated. It therefore becomes a measure of the force it counteracts. This is called the compensation method.

From the Daniell, K, wires proceed to the binding screw *a* and *b* at each end of the long compensator, which consists of a stretch of platinum wire of uniform thickness. A slider, bearing a platinum point, *c*, to make contact, is movable up and down the wire. From one binding screw of the compensator a wire proceeds to one terminal of the galvanometer, through a commutator, C, the other terminal being connected with the slider also through the commutator.[1]

The Daniell cell and the platinum wire of the compensator may be considered as the main circuit, and the galvanometer circuit as a branch from it. The current from the Daniell may, therefore, pass straight along the compensator and back to the cell, or a portion may start from the binding screw, *a*, go through the galvanometer, and regain the compensator by the connection with the slider at *c*. The strength of this portion will depend on the resistance in the branch circuit. By varying the position of the slider, the relative amounts of resistance in the two circuits are altered, and so a stronger or weaker current can be sent through the galvanometer from the Daniell, according as the slider is nearer *b* or *a*.

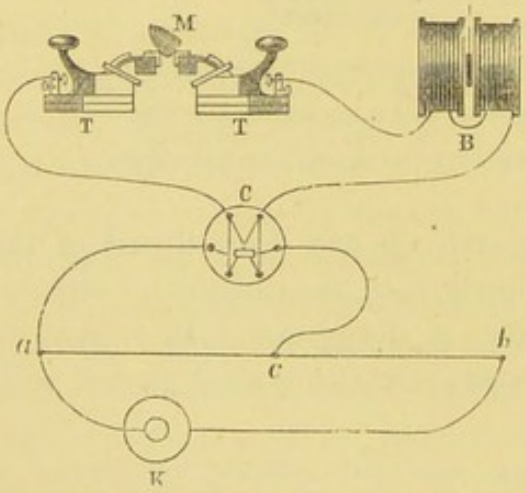


FIG. 297.—Arrangement for measuring the e. m. f. of a section of muscle.

The distance between *a* and *c* is read off by means of a millimetre scale placed beneath the platinum wire, and since the wire is of uniform thickness throughout, *a c* is a

[1] A very convenient form of long compensator, with resistance coils, is made by Messrs. Elliot Brothers, London.

measure of the resistance, and therefore a measure of the amount of current sent through the galvanometer. By means of the commutator the Daniell current can be sent through the galvanometer in one direction or another. Thus a current from the Daniell, whose amount can be measured, can be sent through the galvanometer equal in amount and opposite in direction to the current from the muscle, the one compensating for the other.

In experiments on the electromotive properties of muscle and nerve, it is necessary to have a convenient form of apparatus by which resistance can be introduced into electrical circuits. One of the most convenient instruments for physiological purposes is the *rheochord* of Du Bois-Reymond, shown in Fig. 298.

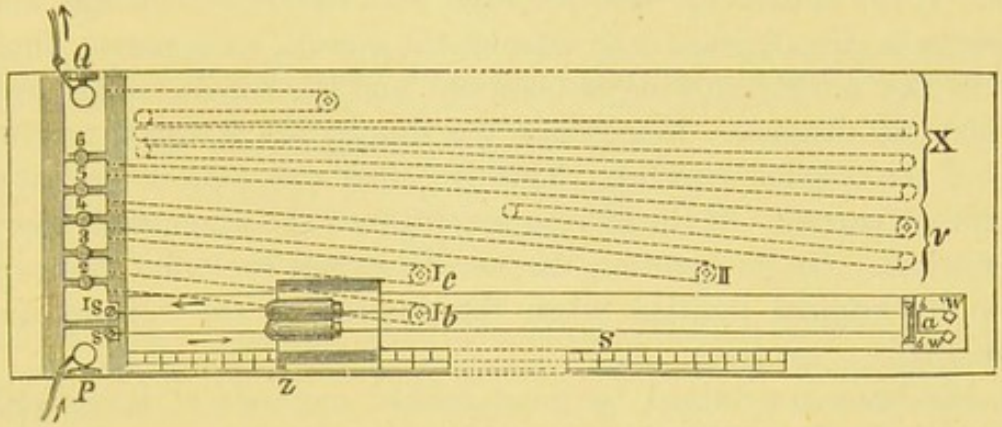


FIG. 298.—Rheochord of Du Bois-Reymond.

It consists of a long thin box containing lengths of German silver wire, I, I, II, *v*, and X, the ends of which are attached to brass plates, 1, 2, 3, 4, 5, 6, on one end of the upper surface of the lid of the box. Each of these brass plates may be brought into electrical contact with the next one, by inserting a cylindrical brass plug in the semi-circular holes between the plates. Further, from the first plate *s*, a platinum wire *b*, 1 metre long, runs to a peg *W*, passing over an ivory knife edge *a*. From the next brass plate 1*s*, another wire *c* runs to W. Running along the side of the instrument there is a platform *Z*, carrying two steel cylinders filled with mercury, and closed at the broad ends with small corks. The platinum wires pass through these cylinders, and, as the latter touch each other, a greater or less length of platinum wire can be thrown into the circuit, according to the position of the slider *Z*. The wires I and I, between 1 and 2 and 2 and 3, have the same resistance as the platinum wires, if *Z* be passed as far as *a*; the wire II has double, the wire *v* has five times, and the wire X ten times the resistance of the full length of the platinum wires. Suppose, now, a current entering at *P*, if *Z* be pushed up to the left as far as it will go, and if a brass plug have been introduced between each pair of brass plates, the current will meet with no resistance, and it will pass straight across the brass plates to Q, and thence back to the battery. It will be seen, however, that by pushing the slider *Z* to the right, when the current reaches *s*, it will pass up the first platinum wire to the steel bottl through which it passes, thence to the adjoining steel bottle, and thence back to the plate 1*s*, by the other platinum wire. Resistance has thus been introduced. This may be increased by taking out the plug between 1*s* and 2, when the current

must pass along the loop of silver wire I, then, by taking out the plug between 2 and 3, along the loop I, and so on, with reference to the other loops. If the steel bottles are pushed up to *a*, and if all the plugs are out, then the maximum amount of resistance has been introduced. In using the instrument practically, it is always introduced in short circuit—that is to say, two wires are connected with *P* and two with Q. One pair of wires is connected with the circuit of the battery, and the other pair, we will suppose, with the galvanometer circuit. By throwing resistance into the battery circuit, more current will pass to the galvanometer circuit, and, on the other hand, if the resistance in the battery circuit be very small, very little of the current, if any, will pass through the galvanometer.

The observations made by Du Bois-Reymond, already mentioned, are seen in *resting* muscle—that is to say, in living muscle in an inactive state. If the muscle has been prepared with the nerve attached, and if the nerve is stimulated so as to tetanize the muscle, an apparent diminution in the muscle current is observed, and the needle swings back a considerable distance. Sometimes the diminution is equal to 40 per cent. of the original current. This is called the *negative variation* of the muscle current. If the tetanus is continued the diminution becomes less marked. On its cessation the needle swings back, but never quite up to its original position when the current of the resting muscle affected it.

It has been ascertained by most refined methods of investigation, conducted by Von Helmholtz, that the commencement of negative variation is about the $\frac{1}{200}$ of a second after the moment of the excitation of the nerve. But Von Bezold showed that in the nerve itself there elapses about the $\frac{1}{200}$ of a second between the instant when the excitant is applied to the nerve and when the nerve is excited. Consequently, the commencement of the negative variation of the muscle current coincides exactly with the moment of the excitation of the muscle. The negative variation, which first increases and then decreases, lasts about the $\frac{1}{300}$ of a second, and it is entirely gone before the muscle begins to contract—that is to say, it occurs during a portion of the period of latent stimulation. (See p. 406.) Bernstein arrived at the conclusion that the negative variation travels with a rapidity of about 3 metres per second, which is the same rate as that of the wave of muscular contraction. This he was able to do by a mode of experiment suggested by the observations of Matteucci, Von Helmholtz, and others.

Matteucci was the first to observe that if the sciatic nerve of one prepared limb of a frog (Fig. 300), which we will call *A*, is placed over the muscles of a similar limb, which we will term *B*, when *B* is excited to contraction, *A* also immediately contracts; at all events, the contraction of *A* occurs apparently at the same time as the contraction of *B*. This phenomenon is termed *Matteucci's induced contraction*.

The arrangement is shown in Fig. 300, or the experiment may be demonstrated to a class by the method illustrated in Fig. 299.

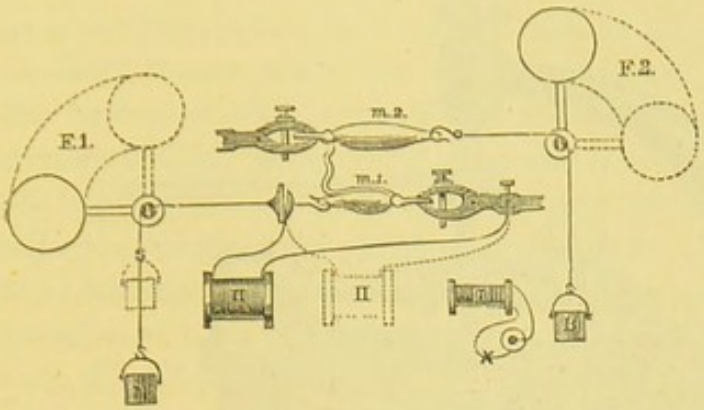


FIG. 299.—Arrangement to show Matteucci's induced contraction. F1, F2, muscle telegraphs; F1, connected with muscle *m*1 and F2 with *m*2. Current from inductorium sent through *m*1, over which the nerve of *m*2 has been laid. When *m*1 contracts, *m*2 also, and apparently simultaneously, contracts, and the two signals are moved. II, Secondary coil at a distance from I, the primary. In circuit of I is a voltaic cell, with a key at X. By opening and closing the key X, single shocks are obtained from II. II, with dotted outline, is the secondary coil brought nearer to I, the primary.

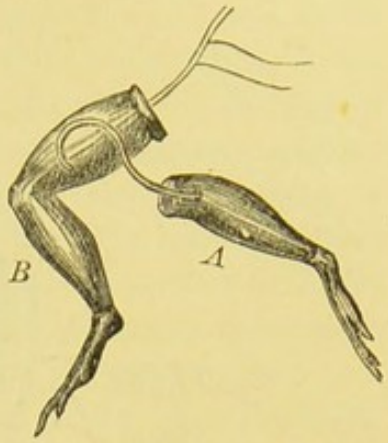


FIG. 300.—Arrangement of limbs to show Matteucci's induced contraction.

The explanation is that the negative variation current related to the contraction of *B* (Fig. 300) irritates the nerve of *A*, and consequently *A* also contracts.

Von Helmholtz, in his earlier studies of muscle, observed that the contraction of the second muscle (*A* in Fig. 300, and *m*2 in Fig. 299) occurred about the middle of the *latent period* of the first (*B* in Fig. 300, and *m*1 in Fig. 299). This observation was repeated by Von Bezold with the artifice of causing both muscles to record their curves on a quickly rotating cylinder, and he showed in this way that the contraction of *A* took place about the beginning of the latent period of *B*. Holmgren, by another method, observed fluctuations in the negative variation current. Then came a remarkable series of experiments by Bernstein, in which he succeeded in detecting and measuring the velocity of the wave of negative variation in a muscle or nerve. His method essentially consisted in irritating at regular intervals a muscle or nerve, and in observing, during these intervals, the effect of the electrical irritations on the electromotive properties of the muscle or nerve. Mechanical contrivances were invented for the purpose of throwing into the circuit of a galvanometer (during or immediately after the short periods of stimulation) the currents from the muscle or nerve. In other words, suppose a current flowed along a muscle, by Bernstein's arrangement this current could be tapped or drawn off into the galvanometer circuit at different points of its progress. This was accomplished by an instrument invented by Bernstein, called the *differential rheotome*, a form of which, simpler than that devised by Bernstein, is shown in Fig. 301.

This instrument serves the purpose of stimulating an organ, say a nerve or a muscle, with a series of induction shocks, and then, at short intervals of time after each stimulation, the length of which may be varied at pleasure, of switching the organ into a galvanometric circuit.

The rheotome has been of great service in electro-physiology, and merits here a short description.[1]

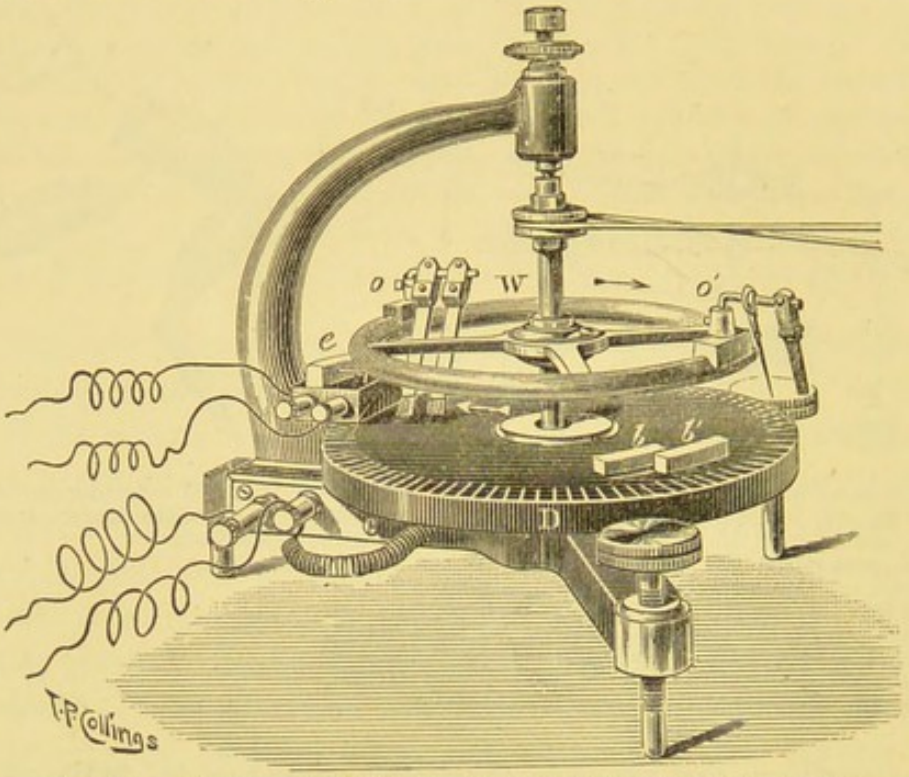


FIG. 301.—Rheotome of Hermann. From a photograph of the instrument in the Physiological Laboratory at Oxford. For description see text.

The wheel, W, makes say 10 revolutions per second. The primary circuit of the inductorium, of which the upper pair of coiled wires form part, is open, excepting when, as happens each time W revolves, the needle at *o'* strikes the wire at *e*, the brush being at the same time in contact with the block. The other two coiled wires are those of the galvanometric circuit. These end in the blocks *b* and *b'*, so that the galvanometer circuit is closed only when both brushes are in contact with them. As one of these blocks slides on the other, the duration of the contact can be varied. In using the instrument, the zero-position of the disk must be first determined, that is, the position in which it must be placed in order that the galvanometer circuit may be closed *at the same moment that the needle touches the wire*. This having been done, the shifting of the disk by one division, in the direction of the hands of a watch, *defers* the galvanometric contact *by one hundredth of a revolution period*, and so on. It will be understood that in an observation with the repeating rheotome the successive galvanometric effects sum together.

Theories of the Muscle Currents.—These may be briefly alluded to as three in number.

1. *The Electro-capillary Theory of Becquerel.*[2]—He showed that electro-chemical circuits may exist, as in the body, without the existence of a

[1] I have to thank Prof. Burdon-Sanderson for aiding me with regard to this instrument.

[2] Becquerel, *Comptes Rendus*, 1870.

metal. Two liquids of a different nature, separated by a narrow slit or series of pores, or by an organic membrane, may give rise to a current; the wall which is in contact with the liquid next the acid acts as the negative pole, while the opposite wall is the positive—the walls of the capillary spaces acting as solid conductors. According to Becquerel, the tissues of the body present an infinite number of electro-capillary couples, which give origin incessantly to electric currents. Thus, in the capillaries of the tissues, the face of the capillary wall in contact with the blood is the negative pole, whilst the face in contact with the fluid bathing the tissues is the positive pole. This theory rests on an insufficient basis of fact, and does not account for the phenomena.

2. *The Physical Theory of Du Bois-Reymond.*[1]—If we take a cylinder of zinc, having a bit of copper soldered on each side, and plunge it into water, there are formed an infinite number of isolated currents, which travel through the water from the zinc to the copper, and a part of which may be conveyed by conductors applied to the zinc and to the copper. If, then, a galvanometer be interposed in the circuit, it will be found that the zinc, forming the centre of the cylinder, is positive, and the copper, forming the sides, is negative, a result comparable to that obtained from a muscle. Du Bois-Reymond has suggested therefore that each muscular fibre is composed of an infinite number of small electromotive elements, analogous to the cylinder composed of zinc and copper above described. Each little element would have a positive equatorial zone, and two negative polar zones, and would be plunged into an intermediate conducting matter. The series of electromotive elements in a muscle might be represented thus—

— + — — + — — + — — + — — + — — + —

or, supposing each element to be divided into two dipolar molecules, having the positive poles turned in the same direction, thus—

— + + — — + + — — + + — — + + — — + + —

In the accompanying figure M shows a dipolar, and L a tripolar arrangement of such electromotive molecules—the darkly-shaded portions of the circles representing the negative parts (Fig. 302, p. 456.)

It is clearly to be understood that those *electromotive molecules*, or "carriers of electromotive forces," do not exist in any histological sense; they may be nothing more than "the foci of chemical change."

[1] Du Bois-Reymond, *Untersuchungen über Thierische Electricität*, 1848 to 1860. See also a translation by Mrs. Lauder Brunton of his paper "On the Secondary Electromotive Phenomena in Muscles, Nerves, and Electrical Organs," in *Translations of Foreign Biological Memoirs*, 1887, edited by Professor Burdon-Sanderson.

(Du Bois-Reymond.) The existence of molecules also enters into the conception of the physicist regarding the nature of electrolysis.

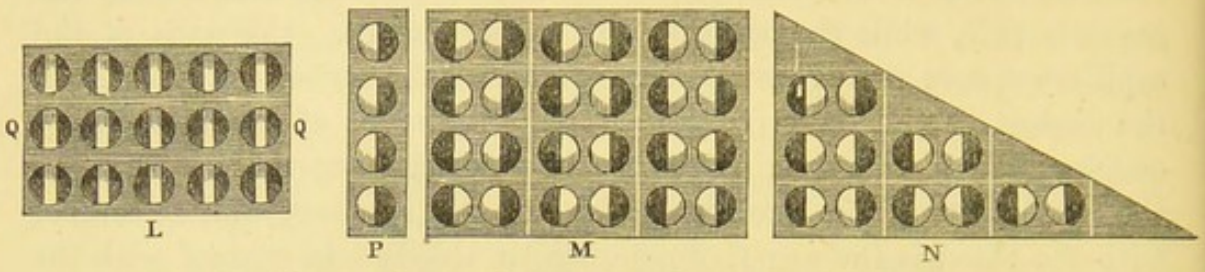


FIG. 302.—Diagram showing the hypothetical molecules of Du Bois-Reymond.

Du Bois-Reymond, in his earlier experiments, thought he obtained a current from an uninjured muscle—*i.e.* from one whose longitudinal and transverse sections were natural, and not artificially produced. Later, however, he discovered that, if special precautions had been taken not in the slightest degree to injure the muscle, no stream was obtained in the resting state, though on the production of tetanus the usual negative variation was observed. If a stream were obtained when the muscle was at rest it was in greatly diminished amount, and might even be in a direction quite contrary to the usual. Du Bois-Reymond explained this by supposing that, in the uninjured muscle, the tendinous end, which is the natural transverse surface, contains a layer of electromotive molecules, with their poles reversed, so that their positive surface is towards the transverse section. This layer he called the parelectronomic layer, and when one removes it by making an artificial cross section, the full muscle current is obtained.

This layer with reversed poles is represented in the figure by P, while N represents the arrangement in an oblique section, giving rise to the "inclination currents."

3. *The Contact Theory of Hermann.*[1]—These views of Du Bois-Reymond have found an opponent in Professor L. Hermann, of Zürich, who concludes, from many carefully-made observations, that in the absolutely uninjured frog's muscle there is no current. He believes that the current is the result of injury, causing death of a small part of the muscular fibre, and so producing difference of potential. In support of his views he adduces many arguments and observations. There are few muscles which can be prepared without some slight injury, since in the majority of cases connective tissue fibres pass out from the muscle

[1] L. Hermann. The results of his numerous investigations are summed up in *Human Physiology*, translated and edited by Dr. Arthur Gamgee, F.R.S., etc., chap. viii., and the most recent expression of his views is contained in a paper in Burdon-Sanderson's *Translations* (*op. cit.*), translated by Mr. Francis Gotch, entitled, "On so-called Secondary Electromotive Phenomena in Muscle and Nerve."

to the skin, and in stripping off the skin the fibres are torn, and an artificial cross section produced. Du Bois-Reymond, therefore, tried to obtain the current from the muscle in situ—*i.e.* without stripping off the skin—but found in the frog strong skin currents directed from without inwards, which interfered with the experiment. These skin currents are abolished by corroding the skin, *e.g.* with saturated salt solution, and, when this has been done, the ordinary muscle stream is obtained. Hermann objected that the corrosive agent not only set aside the skin current, but permeated to the muscle itself, and acted on it, producing chemically a transverse section, so that the current obtained was not from the uninjured but from the injured muscle. In fishes, which exhibit no skin current, and where, therefore, corrosion of the skin is unnecessary, no muscle current is obtained when the fish is curarized to render motion impossible. Hermann also points to the fact, shown by Engelmann, that the heart, which can be laid bare without injury to its fibres, shows no resting current, though it shows a negative variation. Hermann found that even the secretion of the skin of the frog applied to the muscle produced by its action a current, indicating injury of the fibres. Engelmann also showed that a section produced subcutaneously in a living muscle gave rise to a current, which, however, disappeared on the repair of the injury.

From such facts Hermann concludes that *striated muscle in a perfectly uninjured condition is absolutely streamless*, and that the muscle stream is connected with the existence of an artificial transverse section. According to him, a muscular fibre exhibits a current only when one part of it is dying and the other part is still living protoplasm, but, as soon as the whole fibre is dead, the stream disappears. In order to verify this view, Hermann devised an apparatus called the "Fall Rheotome."

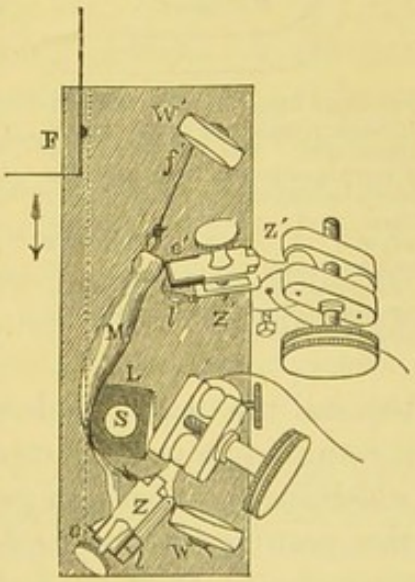


Fig. 303.—Fall-rheotome of Hermann.

By means of a falling weight, F (Fig. 303), shod with shagreen, part of the aponeurosis of the tendo Achilles of a gastrocnemius, M, is stripped off, and, almost instantaneously with this, the galvanometer circuit is closed, and, in a very short time thereafter, opened by the same weight (Fig. 304). By this means, and through a compensation arrangement, the amount of current obtained from the muscle immediately after injury is measured. A second similar observation is

immediately made on the same muscle now injured. Hermann found that there was a deflection of the needle obtained from the muscle immediately on its injury, but the second observation gave a very much greater deflection, showing that after the injury the current took a little time to develop. This time, in the gastrocnemius of the frog, Hermann gives as $\frac{1}{400}$ of a second, that is to say the electrical effect is not immediate, but takes $\frac{1}{400}$ of a second to develop after injury. Hermann admits the occurrence of negative variation in the *injured* muscle, but, since he denies the existence of a stream in an absolutely uninjured resting muscle, he cannot admit a negative variation in the *uninjured* muscle. Still, on stimulating an uninjured muscle which exhibits no current, a deflection of the needle is observed. This current, obtained in quite normal muscle by stimulation, Hermann calls the *action* current, which passes in the muscle from longitudinal to transverse surface. The time of occurrence of this action current or *negative variation* has been fixed by Von Helmholtz and Von Bezold as preceding the contraction of the muscle, that is to say, during the latent period.

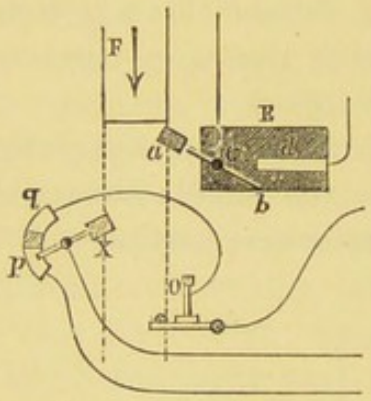


FIG. 304.—Diagram showing part of the arrangement of the "Fall Rheotome." The falling weight F having injured the muscle in the way shown in Fig. 303, is then made to strike against keys *a*, X, O, by which the galvanometer circuit can be closed and then opened, or other effects produced speedily, the one after the other.

By means of the rheotome Bernstein obtained by stimulation of a muscle, which gave no current while at rest, not only a single but a double deflection of the needle, the first in the negative, the second in the positive direction. This double variation was found to propagate itself through the muscle at the same rate as the wave of excitation. The "negative variation" then, or "action stream," is associated with the passage through the muscle of a wave of excitation. During the passage of the wave each irritated muscle point becomes negative to a point at rest, that is, when the wave reaches a point it becomes negative to another point beyond the wave, and when the wave has passed the first point it returns to a positive condition in relation to the part of the muscle to which the wave has now passed. The one point being first negative and then positive explains the double deflection. In a muscle with an artificial cross section, the second phase of the double deflection disappears, and thus the negative variation is accounted for. In a totally tetanized muscle, in which the stimuli pass so rapidly that the irritation wave is everywhere present, no "action stream" is found.

The Current in a Living Man.—Such is a general statement of the facts offered by Du Bois-Reymond and Hermann for and against the theory of an electrical current in normal living muscle. If Du Bois-Reymond were correct it was natural to suppose that the muscles of a living man would show a considerable current, and a negative variation on voluntary contraction. But Du Bois-Reymond completely failed to detect a current from the living muscles of man at rest. If, however, one dips both hands into vulcanite troughs filled with salt solution and connected with the galvanometer, and then voluntarily contracts the muscles of one arm, the needle is deflected to one side; if the muscles of the opposite arm are contracted, the needle swings in the opposite direction. This Du Bois-Reymond accounted a negative variation. But Hermann declares it to be no muscle current at all, but to be due to a secretion stream directed from without inwards on the irritated side—a skin current. In his experiments, stimulation produced sweating and the electric current, but when atropia was given both disappeared. "A man," he says, "paralyzed by curare would show the Du Bois-Reymond current in spite of the absence of contraction, and a man under the influence of atropia would not show the current in spite of the occurrence of contraction."[1] That is, in the former case, stimulation could not produce contraction on account of the curare, but it would produce sweating, and a current—a secretion stream—would appear; in the latter case it would produce contraction, but on account of the atropia there could be no sweating and there would be, therefore, no current.

To the Du Bois-Reymond theory of polar particles, or the pre-existence theory, Hermann, therefore, opposes what he calls a *difference theory* because it refers all electromotive effects of muscle to two kinds of physiological change. The first part of the theory is that *the dying portion of the substance behaves itself negatively to the living*, and the electromotive force has its seat in the demarcation zone between the living and the dying. To this he adds a rider that not only death but *irritation as well causes the affected substance to become negative to the unaffected portion.*

Hermann enunciates his views in the following four propositions—

(1) "Localized death in continuity of protoplasm, whether caused by injury or by metamorphosis (mucous, horny), renders the dead part negative electrically to the unaltered part; (2) Localized excitation in continuity of protoplasm renders the excited part negative electrically to the unaltered part; (3) Localized warming in continuity of protoplasm renders the warm part positive, localized cooling the cold part negative to the unaltered part; (4) protoplasm is strongly polarizable

[1] Hermann, *Handbuch der Phys.*, Bd. I. Th. I. *s.* 224 u. 225.

on its limiting surfaces (first shown as regards the protoplasm enclosed in tubes of muscles and nerves); the polarization constant decreases on excitation and on dying."[1]

Chap. XIX.—SUMMARY OF THE PHENOMENA OF A LIVING MUSCLE.

When the energy which, for want of a better term, is called nerve force reaches a muscle fibre, it acts on the motor end-plate, probably setting up molecular changes in it which are propagated to the contractile substance of the muscle. These molecular changes rapidly pass towards each end of the fibre and they occur simultaneously with, and are the source of, the action current or negative variation. According to Du Bois-Reymond, this wave of negative variation is due to the polarization of hypothetical electromotive molecules, while the rival theory of Hermann assumes that the result of the nerve stimulation is to produce negativity at the point stimulated, and that this negativity (as regards other points) passes from point to point along the muscle, thus producing a current, but it is important to notice that the *fact* of the propagation of a wave of electrical disturbance has been established and is independent of the theories offered as to its explanation. The short period during which the action current passes through the fibre does not exceed the $\frac{1}{300}$ of a second, and for the longer period of the $\frac{1}{100}$ of a second the fibre is apparently inactive. This is the period of latent stimulation, and during this period molecular phenomena are occurring which are antecedent to contraction. It is highly probable also that during the period of latent stimulation energy is liberated as heat, but a portion of the heat of muscular tissue is probably evolved during the succeeding phase of active contraction. At the end of the latent period, the fibre contracts, and during the contraction of a number of fibres existing together in the organ we term a muscle, a sound is produced—the muscle sound. The muscle fibre then relaxes and the katabolic products of the nervous stimulus are then quickly removed and the muscle substance is built up anew. Another consequence of the molecular disturbance excited by the nervous stimulus is the increased production of carbonic acid and probably of other waste products and the increased consumption of oxygen. This *resumé* embraces the principal phenomena, but others have been mentioned in the preceding pages. The student should make himself master of the physiological changes in muscle as they illustrate in a striking way the anabolic and katabolic processes occurring in living matter, and he will see that many of the processes

[1] Burdon-Sanderson's Translations, *op. cit.* p. 328.

happening in other tissues, such as in glands, in the sense organs, and even in the central nervous organs are analogous to those of muscular contraction.

Chap. XX.—THE PHENOMENA OF THE ELECTRIC FISHES.

The phenomena manifested by electrical fishes have in recent years attracted the attention of various physiologists as bearing on the mode of action of muscles, and especially of nerves. The indefatigable labours of Sachs, Fritsch, Ranvier, and many others, by which they have been able to describe the histological structure of electric organs, and the elaborate researches of Du Bois-Reymond and of Sachs, and more recently, in this country, of Burdon-Sanderson and Gotch, on the electrical phenomena manifested by these organs, have brought to light many details of one of the most remarkable structures in the whole realm of nature. To do justice to this subject would lead me beyond the space at my disposal, but I will attempt to give a brief account of the chief facts, because a general knowledge of these cannot fail in being both interesting and suggestive to the physiological student.

About fifty species of fishes are known or are believed to possess electrical organs, and to have the power of communicating electrical shocks. This may possibly be an over estimate of the number, and at all events, the electrical properties of not more than five or six have been investigated. From one point of view, it is remarkable that any animal should possess an electrical apparatus, but yet when one considers the value of such an organ to the animal, as a weapon of offence or of defence, it may appear strange that an electrical organ has been found only in a few fishes.

The electrical fishes best known are *Torpedo Galvani*, or *T. marmorata*, and some other species of torpedo found in the Adriatic and Mediterranean seas; *Gymnotus electricus*, an eel living in the lagoons in the region of the Orinoco in South America; and *Malapterurus electricus*, the ra"ăsh, or thunderer fish of the Arabs, a native of the Nile, the Niger, the Senegal, and other African rivers. In addition to these, we know of *Mormyrus longipinnis*, *M. dorsalis*, and *M. anguilloides*, fishes allied to the pike family, found in the Nile; *Rhinobatus electricus*, a ray from Brazilian seas; and *Trichiurus electricus*, a ribbon-like fish found in the Indian Ocean. The common skate of our own coasts, *Raia batis*, also possesses an electrical organ, first described by Stark in 1844, afterwards, in 1847, by Robin, and the electrical properties of which have been recently investigated by Burdon-Sanderson and Gotch. It will be

observed that the electrical fishes do not belong to one class or group, and that some are found in fresh water, while others inhabit the ocean.

Electrical fishes have been known from early times. Aristotle describes the benumbing shocks of the torpedo, as recognized by fishermen; and, in a recent work on the malapterurus, Fritsch figures an ancient Egyptian carving, executed 3,000 years before the Christian era, in which fishes, like malapteruri, are distinctly seen. It was not until 1773, however, that Walsh showed experimentally that the shocks of the torpedo are electrical in their nature. Since that period, many investigations have been made, both as to the structure and as to the functions of the electric organs.

1. *Torpedo.*—The electric organs, two in number, are large, flat, kidney-shaped bodies, placed on each side of the head and gills. The organ is composed of a number of hexagonal prisms, placed vertically between the dorsal and abdominal integuments, and each prism is divided by a series of delicate membranous plates or diaphragms attached by their edges to the aponeurotic sheaths separating the prisms (Fig. 305). The plates are separated from each other by a jelly-like albuminous fluid. About 30 plates occur in each mm., linear measurement, of a prism, and a prism of medium height contains about 615 plates. Each organ contains say 800 prisms, and thus there are roughly about 500,000 plates in each organ, or 1,000,000 in both organs. This powerful electric battery, thus divided into compartments is richly supplied with large nerves. These are (1) a large branch from the trigeminal, and (2) four branches from the vagus which spring from a large *lobus electricus* between the *corpora bigemina* and the *medulla oblongata*. According to Pacini, the nerves enter the laminæ at the points of their attachment to the prisms, and are distributed to their under surface, and in the fluid between that surface and the next lamina They ramify here in a very vascular nucleated tissue. Each

FIG. 305.—Torpedo Galvani, showing the prisms from the dorsal surface, and the great nerve trunks, *b*, ending in the smaller nerves *a*, distributed to the prisms.

prism presents a structure somewhat analogous to that of a voltaic pile. The piles are vertical and the plates horizontal. Savi, in 1844, observed that the nerves ended in the electric plates in the form of a rosette.

According to Ewald, one of the most recent writers on the subject, the electric nerves pass through the gills and enter the electric organ as four large branches and one smaller branch. They then divide freely, and run between the prismatic columns, always ending near the middle of the column. From these smaller branches still smaller ones originate, each consisting of three or four fibres, and these spread out obliquely over the lateral surface of the column. Each nerve fibre divides into from 12 to 25 delicate nerve filaments, called by Wagner, their discoverer (1847), nerve tufts (nervenbüschel) (Fig. 306). Each nerve tuft gives off as many fibrils as there are plates in the column, each plate being supplied with one fibril.

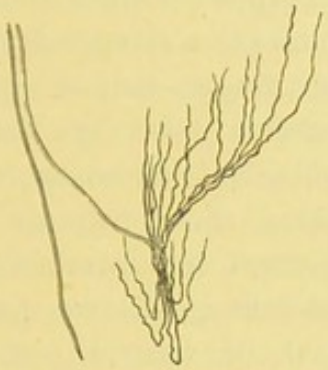


FIG. 306.—Nerve tuft in torpedo, showing numerous little branches coming off from the larger nerve.

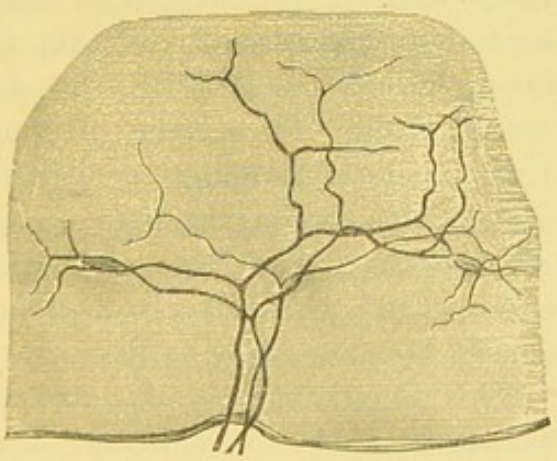


FIG. 307.—Terminations of nerve on the plate of the prism of a torpedo, seen after the action of osmic acid. × 800 d.

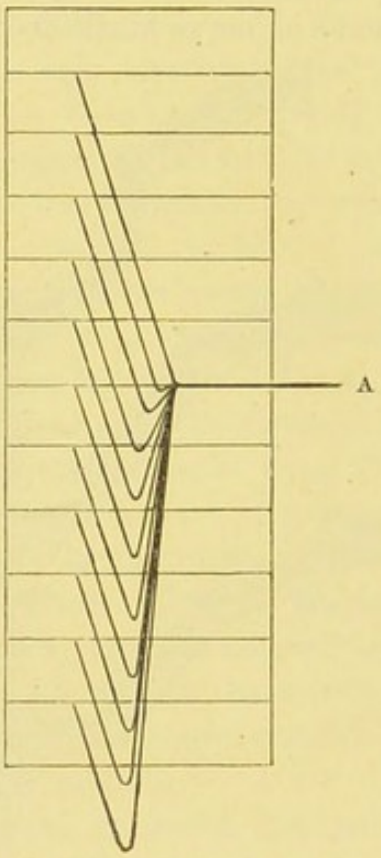


FIG. 308.—Diagram of prism of torpedo, showing 11 plates, each supplied by its nerve fibre, issuing from the chief nerve A, so as to form one of Wagner's tufts.

The main nerve reaches the centre of the column, and the branches given off from it to the individual plates lie almost in a straight line or plane parallel to the axis of the column. These fibrils do not pass to the plates by the shortest course, but only the shortest fibres go directly

to the plates, the majority making a hook-shaped bend, and then each reaches its appropriate plate after a course of greater or less length. This arrangement is seen in Fig. 308. When a fibril reaches a plate, it divides dichotomously again and again so as to form an expansion, as seen in Fig. 307, and it is remarkable that the arrangement of the divisions of the fibril on one plate corresponds almost exactly with that on neighbouring plates. The final little twigs of nerve end by forming an anastomosis with adjoining fibrils, and thus produce what may be called an electrical end-plate. The fact that a single nerve fibre, by splitting into fibrils supplies from 12 to 25 plates, shows that all those plates may be thrown into electrical activity at the same time by one nervous impulse.

Ranvier describes the structures between each pair of plates of the torpedo as consisting from below upwards of (1) a first layer, or *nervous* layer, formed of two portions—(*a*) a superficial portion containing the plexus of nerve fibrils above described, and (*b*) a deeper layer showing peculiar finger-shaped projections called *electric hairs* by Ranvier, and lying side by side so as to give the appearance of a palisade (Remak); (2) a second, or intermediate layer, also consisting of two parts—(*a*) a superficial or ventral portion, finely granular, next the palisade of electric hairs, and (*b*) a deeper portion, more coarsely granular than *a*, and containing nuclei; (3) a thin clear layer, called by Ranvier the *dorsal lamella;* and (4) a layer of connective tissue, forming a partition. Above this, we again find the first or nervous layer above described, and so on. These layers are shown in Fig. 309, according to Ranvier.

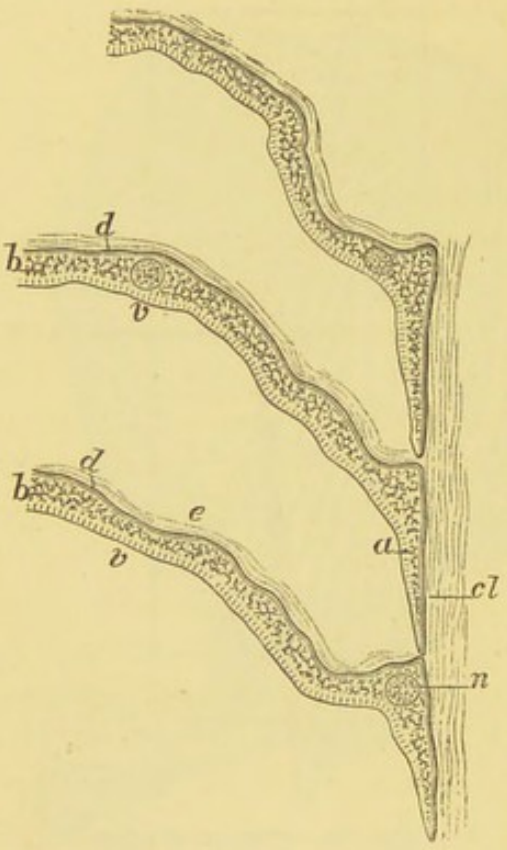


FIG. 309.—Attachment of the electric plates of torpedo to the sheath of the prism. *cl*, sheath of prism; *v*, ventral or nervous plate; *d*, dorsal plate; *e*, fine layer of connective tissue; *b*, intermediate layer; *n*, nuclei of this layer; *a*, a portion reflected from the plate.

This remarkable organ is now known to be related to muscular tissue. Fritsch, by studying the development of the torpedo at Naples and Villafranca, has found that, as previously shown by De Sanctis, the torpedo, in its ontological history, passes through a squaliform stage, a raiform stage and a torpedo stage; in the first resembling shark-embryos in

the second the common ray or skate, and in the third, by the formation of the electric organ, it becomes a true torpedo. At an early stage, the tissue of the electric organ is like that of embryonic muscle, showing numerous nuclei, and even a distinct longitudinal and a more faint transverse striation may be seen. Somewhat later, the striations, both longitudinal and transverse, disappear, the nuclei become larger and more numerous, and the disc-like arrangement of plates begins to appear. The process goes on until the slight resemblance to muscle is entirely lost. There can be no doubt that the electric organ takes the place of the outer gill muscles of the fifth gill arch. These muscles in ordinary rays and in sharks constitute powerful organs for moving the lower jaw; but in the torpedo these jaw muscles are absent, and in their place we find the electric organ.

In the electric lobe of torpedo, numerous large round ganglionic nerve cells exist, similar in appearance to those described and figured in treating of the nerve centres of Gymnotus (p. 468).

2. *Gymnotus electricus.*—This fish possesses four electric organs, two on each side, stretching from the pectoral fins to near the end of the tail. The proportional size of the electric organs to the body is much greater in the gymnotus than in the torpedo, the viscera of the animal occupying only a small portion of the anterior part of the body. Each electric organ consists of a series of horizontal membranes or plates arranged in the longitudinal axis of the body nearly parallel to each other. Each trough is divided by thin vertical laminæ or diaphragms. According to Pacini, each lamina is not simple as in the torpedo, but it may be regarded as consisting of two layers separated by a fluid. The posterior layer is a delicate fibrous structure, and in it alone the nerves ramify. The anterior is composed of vascular nucleated tissue. The fluid between the two layers of the laminæ differs in character from that in the interspace between the posterior and the anterior layer of the next lamina, but both fluids are of an albuminous character. In the gymnotus, the batteries are horizontal and the plates vertical—an opposite arrangement to that described in the torpedo.

A more careful examination than was possible to Pacini has enabled Fritsch to give a more detailed and accurate description. According to Fritsch, the electric organ consists of a series of partitions of connective tissue, between which lie the electric plates. These contain a peculiar yellowish albuminoid material. The anterior surface of the plate is covered with a number of elongated papillæ (anterior papillæ), having rounded ends (Fig. 310), and in these papillæ there may be seen granular amœboid cells. Between the papillæ and the anterior partition of connective tissue there is a transparent mucoid fluid, in

FIG 310.—Vertical section through one of the plates of the electric organ of gymnotus, about 1.800 diameters (?); semi-diagrammatic, to show all the structures. *P*, Pacini's line; *p*, anterior papillæ; *p'*, posterior papillæ; *st*, bacillary layer; *p''*, thorn papilla; *b*, amœboid cells in thorn papilla; observe the nerve fibre entering the apex of this papilla. *a*, nuclei of connective tissue; *el*, peripherical electrical nerve; *c*, *c*, nuclei of nerve fibres; *d*, blood corpuscles in vessel; *V*, posterior surface of lamella; *tm*, mucous tissue between the anterior papillæ and the next lamella; *s'*, aponeurosis between the plates; *v* blood-vessel.

which a few cells like those of connective tissue are interspersed. The middle of the plate is transparent and homogeneous in a fresh condition, but soon after removal from the body there appears a thin dark line, called *Pacini's line*, dividing the plate into two parts. The posterior surface of the plate is also thrown into papillæ (*posterior papillæ*) of very various sizes, constituting the *bacillary layer*, some being merely short rounded projections, while others called *thorn papillæ* are long, conical in shape, and reach nearly to the posterior partition of connective tissue. These have been supposed to be supporting pillars, but they are regarded by Fritsch as really parts of the electric plate, in which the electric nerves terminate. This view is clearly borne out by Fig. 310 copied from one of Fritsch's drawings.

The ultimate nerve fibrils pass along the anterior surface of the partitions and shortly before entering the papillæ, and on the posterior surface of the electric plate, they lose the white substance of Schwann (medullary sheath), the axis cylinder alone entering the electric plates and merging into their substance. The medullary sheath breaks up into fibres containing nuclei here and there, analogous to the nuclei of Ranvier's nodes in a nerve-fibre. These small fibrils pass to the posterior surface of the electric plate.

The microscopical examination of the electric organ of the gymnotus leads to the conclusion that the plates arise from embryonic muscle, each plate being equivalent to a number of primitive bundles cemented together. In some preparations, the plates may be found broken into parts corresponding to the individual bundles, or rather to the primitive cylinders or fibres of embryonic muscle, a longitudinal cleavage taking place like what may be seen in muscle. Sometimes, again, the plates cleave transversely, along Pacini's line, a division analogous to that of muscle into Bowman's discs (p. 309).

While the nerve cells in the central nervous system of the torpedo are located in one organ, the electric lobe, those of the gymnotus occur throughout the greater part of the length of the spinal cord. This organ in the gymnotus is not specially large, and it is of the usual shape. In the anterior part of the body of the fish it is somewhat flattened, it is rounder about the middle of the body, and is again flattened towards the tail. No special cells connected with the electric nerves occur in the encephalon. In the spinal cord, however, special strands or columns exist, one in each lateral half of the cord. These ganglion cells are so situated in the grey matter of the cord as to surround the central canal like a cylinder, open inferiorly, supposing the cord to be placed in the natural position of the fish, with the back upwards and the belly downwards. The cells are seen in Fig. 311, which represents one half of a transverse section of the cord. Broad axis cylinders arise from the poles of these cells and run out into the anterior roots of the spinal nerves, and pass on to the electric organ as electric nerves. The roots of these electric nerves run alongside the ordinary motor roots as far as the intervertebral foramina. The remaining poles of the ganglion cell—some of them larger than others—run into the neuroglia, and disappear in the direction of the lateral columns and of the commissure.

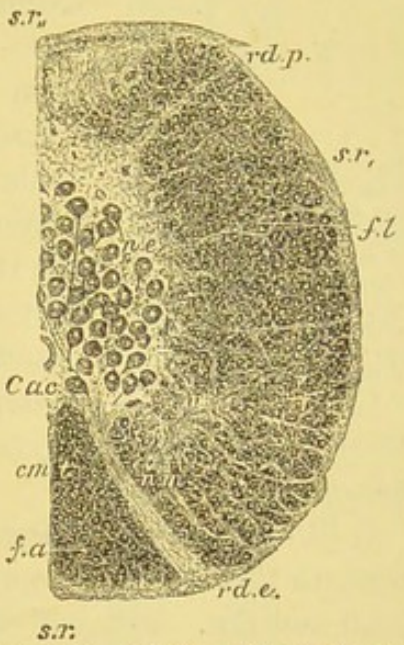


FIG. 311.—Right half of spinal cord of a small gymnotus, seen in transverse section, × 30 d. *sr*, substantia reticularis anterior; *sr'*, substantia reticularis lateralis; *sr''*, substantia reticularis posterior; *fa*, fasciculus anterior; *cmt*, commissura transversa; *ne*, a few small nerve cells; *rd.e*, anterior root of spinal nerve; *rd.p*, posterior root; *f.l*, fasciculus lateralis; *Cac*, Central canal of cord. Observe the large ganglionic cells in the grey matter, of which one is shown in Fig. 312.

The electric nerve-cells are of a roundish form (Fig. 312), and they consist of highly-granular protoplasm, from which very distinct poles (axis cylinders) emerge. The ordinary multipolar motor cells, characteristic of the spinal cord (Fig. 173, p. 313), become the fewer the more

numerous the electric cells, and the motor cells lie exclusively in the anterior cornua or horns of grey matter. The shape of these motor cells is irregularly polygonal, often with concave borders; there is less protoplasm, and the poles are not so distinct nor so broad as is the case with the electric cells. According to Fritsch, cells transitional from the motor to the electric cells may be observed. The more rounded the cell, the more granular the protoplasm, and the more distinct the poles, the more does the cell become of the electric and the less of the motor type. Such transitional cells occur in the inner group of the motor cells in the anterior cornua, and they are more especially present in a mass of grey matter in the cervical region, which, from its position, is analogous to the vesicular column of Lockhart Clarke, seen in the spinal cords of the highest vertebrates. The cells in this grey mass are few in number and small in size, but they show the characters of electric cells, and they send their poles into axis cylinders, passing out of the cord by the anterior roots. Towards the posterior end of the cord the electric cells become fewer in number, and gradually their characters merge into those of motor cells.

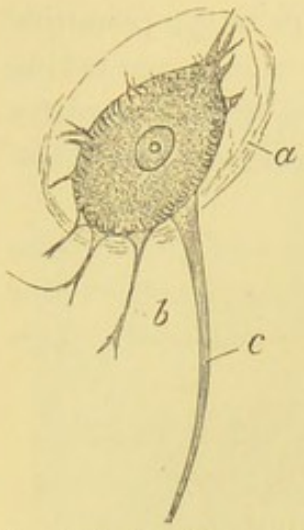


FIG. 312.—Electric cell from the middle region of the spinal cord of gymnotus, × 314 d. *a*, sheath of neuroglia; *b*, smaller nerve poles anastomizing with those of adjoining cells; *c*, chief nerve pole, passing into axis cylinder of an electric nerve.

3. *Malapterurus electricus.*—The electric organ in this fish forms a layer beneath the skin, enveloping the whole body, with the exception of the head and fins. A layer of fat separates it from the subjacent muscles. The ultimate structure shows numerous lozenge-shaped spaces or loculi filled with fluid, having a peculiar layer of electric tissue on two sides of the lozenge-shaped space, as shown in Fig. 313. The electric tissue consists of a layer of granular protoplasm, containing nuclei (often double), more especially towards each margin. Both borders, and more especially the inner border, show a delicate striation, which really consists of tubular pores, represented in the diagram. Around each nucleus there is a halo of clearer protoplasm. The broad firm tubulated zone is distinct from the more slimy inner one. Each space is surrounded by a well marked fibrous membrane. From the general appearance of the electric tissue, Fritsch regards it as consisting of a layer of modified epithelial cells forming giant cells. The inner free surface is bathed in a mucoid fluid. In the epidermis there are peculiar club-shaped cells, having double nuclei of a glandular character, which are forms transitional into those constituting true electric cells. In some

parts of the body, as towards the tail, the lozenge-shaped spaces may be found without any electric tissue, and transitional conditions also occur. Although quite different in character from the plates or discs in the torpedo and the malapterurus, being epithelial instead of muscular, the lozenge-shaped arrangements may also be called electric discs. The number of these discs is enormous. One cubic centimetre of the electric organ of a middle-sized malapterurus contains 14,000; a transverse section through the thickest part of the body exposes 3,000, in a row from head to tail 1,600 are found, and Fritsch computes that the total number in one fish amounts to 2,000,000.

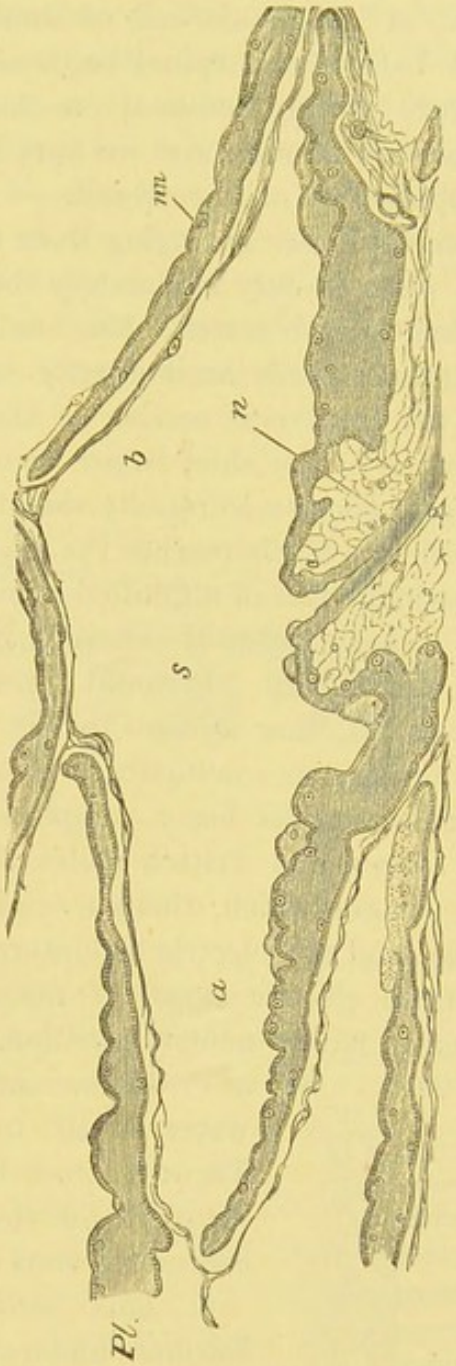


FIG. 313.—One of the lozenge-shaped spaces or loculi in the electric organ of Malapterurus, × 200 d. *s*, space filled with fluid. To the left of *a*, observe the electric tissue darkly tinted. Observe it *Pl* also on two sides of the lozenge-shaped space, and in four adjoining spaces. To the right of *b*, the connective tissue wall of the space. *nn*, nuclei in electric tissue. *n*, nerve fibre passing into electric tissue.

On tracing the nerve into the electric organ, it is found that only the axis cylinder enters at one side or angle of the space, and that it insensibly merges into the substance of the electric tissue. (See Fig. 313, *n*.) The finest nerve fibrils running between the discs have an extremely thin axis cylinder surrounded by a delicate medullary sheath (white substance), and they show the nodes of Ranvier. In larger nerves no longitudinal fibrillation of the axis cylinder can be seen under the action of osmic acid, and the apparent thickness of the nerve is due to the thickness of the medullary sheath. Not only is the medullary substance thicker, but the nerve fibril is surrounded by concentric layers of connective tissue, between which even delicate capillaries and nerve fibrils may run. When the nerves are traced inwards to the spinal cord, they are all found to spring from one single nerve, the electric nerve, the axis cylinder of which enters the cord and ends in a process or pole of a gigantic nerve cell. This nerve cell has numerous proto-

plasmic processes which coalesce here and there so as to form a perforated plate, in the meshes of which capillaries and nerve fibres may be seen. A ganglionic cell of similar size and appearance lies in the adjacent half of the spinal cord, and both cells are connected by a commissure passing across the median line. Each of these giant nerve cells measures ·21 mm., and we have here a unique example of an organ being supplied by one nerve cell.

The axis cylinder springing from one of the poles of the perforated nervous plate is only moderately thick, but as it ultimately supplies a nerve fibril to each electric disc, the sum of the transverse sections of these ultimate fibrils must greatly exceed that of the parent nerve. If we call the transverse section of the parent nerve 1, the total transverse section of the chief larger branches will be represented by 2, and the increase goes on so rapidly that the sum of the transverse sections of the ultimate fibrils reaches the enormous amount of 364,000, showing the remarkable fact of a gradual increase in the amount of matter forming the axis cylinder as we pass from the spinal nerve cell to the ultimate nerve fibril. It would appear that a similar increase is seen in the nerves of other animals, a fact not yet sufficiently recognized in theories of nervous conduction. (See p. 316.)

The malapterurus has a complete lateral nervous system, similar to that of *silurus*, and Fritsch states that the electric nerve is a portion of this system which, though originating from the *trigeminus* nerve, is in other and non-electric malapteruri connected with the vagus.

The fresh electric organ of malapterurus was found by Du Bois-Reymond to have a neutral reaction. It became acid on the third day after death. After immersion for five minutes in water at 40° to 50° C. it gave an acid reaction. Thrown into boiling water, pieces of electrical organ became acid, thus behaving like the tissue of the central nervous organs, and not like muscle.

FIG. 314.—Diagram showing the general arrangement in the skate (*Raia*) of the discs and *muscular* fibres as seen in a longitudinal section.

4. *Raia batis*, or Common Skate.—Professor Burdon-Sanderson and Francis Gotch have recently investigated the structure and functions of the electrical organ in the skate, and have supplied me with the following short sketch—"On each side of the spinal column from the caudal fin to about half way up the tail of this fish, partly in contact with the skin, and partly enveloped in the so-called sacro-lumbalis muscle (Goodsir) there is an elongated fusiform body, consisting of a number of longitudinal series of discs (Fig. 314). It is divided by septa of connective tissue into tubes, in which the discs are

suspended transversely at distances of about ·5 mm. One surface of the disc is directed forwards, and is flat; while the other looks backwards, is slightly concave, and shows a number of little alveolar depressions. One of these discs is shown in section in the sketch in Fig. 315. Upon the flat surface of the disc is the nervous membrane which in structure corresponds to a nerve-endplate in muscle. The alveoli on the posterior surface are occupied by capillary blood-vessels, but these do not penetrate the disc. The substance of the disc, underneath the nerve membrane (electric membrane), consists of fine laminæ, parallel to its surface, which are seen in section as striæ. But towards its posterior surface it consists apparently of structureless material, beset with numerous nuclei."

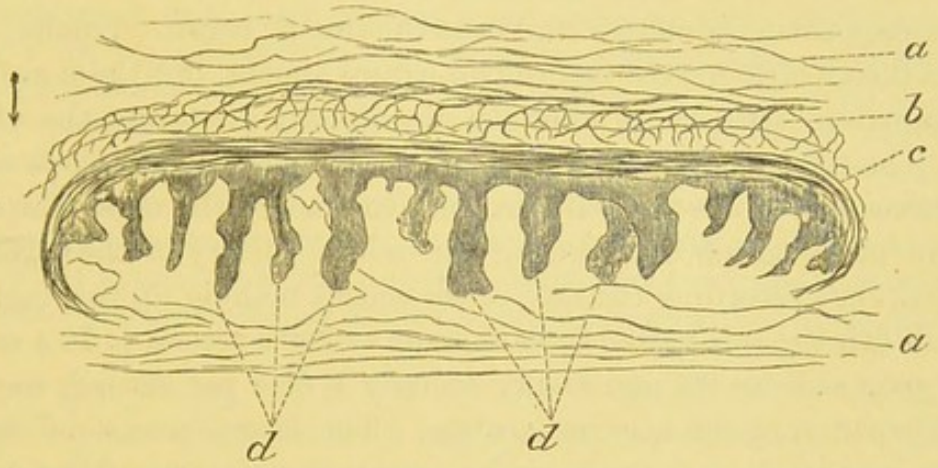


FIG. 315.—Semi-diagrammatic view of a disc from the electric organ of the skate. *a*, *a*, connective tissue with capillaries; *b*, nerve layer, nerve endings branching dichotomously, the terminal branches all tending in the direction of the arrow; *c*, striated layer, which corresponds to the bacillated substance of gymnotus; *d*, *d*, processes of transparent structureless material, containing numerous nuclei, corresponding to the thorn papillæ of a gymnotus.

The electric organ in the skate thus resembles that of the gymnotus. The organs are supplied by nerves coming from the ventral or motor roots of the spinal nerves, and these run along with the motor nerves going to the muscles of the tail. I am not aware of the existence of special nerve cells in the spinal cord.

5. *The General Electrical Properties of the Electric Fishes.*—The general phenomena of the electric fishes may be illustrated by considering in the first place some of those manifested by the *torpedo*. The shock emitted by the torpedo is weak compared with that of the malapterurus, but as pointed out by Du Bois-Reymond the strength of the shocks of the torpedo is much diminished by the good conductivity of the sea water by which the fish is surrounded. The shock of the torpedo is weaker because less current passes to the person touching it. When a living

malapterurus was placed in salt solution its shocks became much weaker, indeed Du Bois-Reymond states "it apparently lost its electrical properties." When the living torpedo is touched it may or it may not emit a shock, and it soon becomes evident that the discharge is to a certain extent under the control of the will. If the brain be removed without injuring the electric lobes, irritation of one or other of these lobes will cause an electric discharge limited to the side corresponding to the organ irritated. The electric lobes are thus undoubtedly the electric centres. These may be also excited by reflex action, that is to say, an irritation of the skin may cause a reflex discharge, the impression being carried to the nerve centres from the part of the skin irritated by the sensory nerves supplying that part, and on reaching the electric lobe charges are set up therein which result in the transmission of an excitatory change along the electric nerves to the electric organ. Finally, irritation of the electric nerves themselves causes shocks, but these are weak in comparison with those obtained by directly irritating the electric lobe. The reflex "discharge" of the fish was found by Marey to consist of a succession of shocks. He led the current of the organ through a delicate chronograph, and during the reflex activity of the organ this vibrated, thus recording the rate of the shocks making up the discharge. In fresh fish the rate varied from as many as 200 a second to 70 a second; but it soon sank to 20, and finally to only 1 or 2 per second, owing to the exhaustion of the nervous centres. The latent period of the discharge is at least $\frac{4}{100}''$, as shown by recent experiments of Gotch upon the discharge in curarized torpedoes.

Each individual excitatory state of the organ, the repetition of which makes up the discharge, may be evoked in a cut-out portion of organ by mechanical, chemical, or electrical stimulation of its nerve. There is a distinct interval of time between such nerve stimulation and the electromotive response of the organ. This is partly accounted for by the slow transmission of the excitatory process from the point of stimulation down the nerve trunk, the rate of transmission being only 5 to 7 metres per second. Besides this, however, there is a true latent period, the duration of which is dependent upon the temperature, etc., of the organ, but which, under the most favourable conditions, is not less than $\frac{5}{1000}''$. The response after this interval begins very abruptly, developing a high electromotive force, and then subsides at first rapidly, and subsequently more slowly, its total duration amounting to about $\frac{5}{100}''$. The discharge is therefore analogous to tetanus of a muscle and the individual shocks composing it to the individual short 'twitches' of a muscle. Physical conditions affect the organ: thus, heating to 22° C. stimulates the organ while cooling makes it less active. The administration of strychnia

to a torpedo causes the fish to emit a rapid series of shocks, by which it soon becomes exhausted. It is remarkable that the response of the organ to excitation of its nerve is *not* in any way affected by curare. This was shown by Moreau in 1862, and the recent experiments of Gotch (*Proceedings of Physiological Society*, 1888) fully confirm his conclusions. The reaction of the organ at rest is neutral but it becomes acid by activity. Moreau has succeeded in charging a Leyden jar by the shocks of a torpedo.

In a recent research on the electrical organ of torpedo,[1] Mr. Francis Gotch arrives at the following conclusions—

In the active state, the ventral surface of each plate becomes negative to the dorsal surface, and the effect is summed up so that the dorsal end of a column becomes positive to the ventral end. The electromotive activity of the organ may be produced in at least three different ways, and then shows itself (*a*) as the response of the organ to excitation of its nerves, whether reflex or direct, this nerve-organ response being characterized by a short period of delay, a very rapid development, and a less rapid decline; (*b*) as a prolonged after-effect following the passage of a strong current through the substance of the organ, and which therefore follows a powerful response; and (*c*) as a prolonged electromotive change following mechanical or thermal injury along the length of the columns of the organ, this latter being the electromotive expression of a prolonged local excitatory process occurring in the immediate neighbourhood of the injury.

According to Pacini, the nerves are always distributed to the side of the electric plate which becomes negative in the discharge. This law has been confirmed by all later researches. In the torpedo, therefore, as the nerves are in the lower plate, the shocks pass from the belly to the back; in the gymnotus the posterior surface of the plate is negative and therefore the discharge is from the tail to the head, and in the malapterurus the negative side of the organ is anterior and thus the current passes from the head to the tail.

An electric shock may be obtained from the *gymnotus* by touching the fish with one finger or with a conducting substance interposing a resistance similar to that of animal tissue, such as a bit of wet wood or wet leather. Such a shock is slight and the full effect is obtained only when the body forms part of a complete circuit through which the greater part of the current passes. The wider apart the points of contact and the better the conducting medium, the more severe is the shock. Dr. Sachs having one naked foot in contact with the head and the other foot with the tail of a gymnotus received a series of shocks which caused him to shriek with pain and stand erect as if petrified, and sometimes a man may be thrown on the ground by a single shock. Fishes and frogs

[1] *Phil. Trans.* 1887, p. 478.

in the water along with a gymnotus are killed by the shock, and the electric eel, in attacking its enemies, throws itself into an arch and touches them with head and tail, and thus gives them the strongest possible shocks at its command. If the current is sent through a man's head, he sees a bright flash of light. As already stated, the current passes in the fish from the tail to the head and any point of the surface of the fish is positive to any posterior, and negative to any anterior point. The fish cannot alter the direction of the current, but it can limit the amount of the electric organ thrown into action. If a metallic circuit is made, a spark may be obtained on *breaking* the circuit while a current is passing, but a spark is rarely obtained between the two poles of an open circuit. The current will not illuminate a vacuum tube. It will magnetize a needle. It is remarkable that the discharge of a gymnotus will not pass through flame, and that it can scarcely be perceived with dry copper handles.

If a section of the electric organ of a gymnotus be laid on a pair of non-polarizable electrodes connected with a galvanometer, a slight current is obtained. The organ may be excited to action by mechanical, chemical, or thermal stimuli. The organ at rest is alkaline in reaction; after electrical tetanus or that produced by strychnia, it becomes neutral, and after exposure to the air for an hour or two, it gives an acid reaction, from the formation of lactic acid. The time of the electric discharge is about equal to that of a muscular contraction.

The gymnotus does not become so readily fatigued as the torpedo. Sachs mentions that an eel which in the course of an hour had given about 150 discharges, was still able to send a strong shock through a circuit or chain of eight persons, the first of whom touched the head and the last the tail.

Du Bois-Reymond gives a very interesting account of the *malapterurus*. Generally the fish gives one or more discharges on being touched, but occasionally it may not give a shock, even in escaping from the hand. He has no doubt that some of the discharges are voluntary and others reflex. Malapteruri quickly kill other fishes by electrical discharges. A frog brought into contact with the skin is tetanized. As regards the strength of the shock, Du Bois-Reymond writes—[1]

"In comparison with its size, the shock of the malapterurus is surprisingly violent. If the head and tail of a powerful fish are touched with the fore-fingers in the water, the shock does not extend beyond the knuckles. If it is seized with

[1] Du Bois-Reymond. "Observations and Experiments on Living Malapterurus." Burdon-Sanderson's *Biological Memoirs*. Translation by Miss Edith Prance, p. 387.

hands thoroughly wetted, a severe shock is felt up to the elbow. If it is touched with one hand, a pricking sensation is experienced in the skin, a burning one in wounds, and a painful shock is felt in all the joints of the submerged parts. The best way to take the shock is to hold with wet hands ordinary metal handles, which are connected with the linings of a leading-off cover, and to let an assistant put this on the fish. As one is accustomed to test electric shocks in this way and is not disturbed by anxiety to get proper hold of the fish without hurting it, or by the feeling of repulsion at laying hold of it, one can better judge of the sensation caused by the shock. The shock does not seem so sharp as that of a Leyden jar, but has a somewhat swelling character. Several maxima may be frequently distinguished in it. By sending opening shocks of an induction apparatus, with two Grove's in the primary circuit, through the water of an experimental tub by means of copper plates plunged into it, having first approximated the secondary coil to the primary as closely as possible, the shock which I experienced when my hands were immersed in the water was certainly not stronger than that of a vigorous fish. An opening shock with the coil quite pushed in, and one Grove in the circuit, taken directly through the handles, has about the same strength as such a shock."

Mr. Francis Gotch, in a communication to the Physiological Society,[1] states "that a malapterurus gave to the fingers a smart shock which became unpleasantly severe when both the head and tail were touched, and these seemed comparable with the break shock of a Du Bois-Reymond coil, with three Daniell cells in the primary circuit and with the secondary coil pushed quite up. A shock could also be obtained by placing the fingers in the water surrounding the fish and then exciting the animal mechanically."

The discharge of the malapterurus effects the electrolysis of iodide of potassium but it does not decompose water. No sparks could be obtained from the discharge although the interval between the metallic conductors were as small as ·01 mm. Du Bois-Reymond also made slits in tinfoil not wider than ·0033 to ·005 mm., and no spark across the slit was noticed. On the other hand the secondary current of the induction coil passed over this slit when the secondary was 90 mm. from the primary coil, and yet these induction shocks could not be perceived by the tongue. The difference between the behaviour of the fish and of the inductorium is due to the fact that, in the case of the fish, most of the current is short-circuited, and the shock is only obtained by derivation. Du Bois-Reymond[2] puts the matter thus: Suppose two equal currents, Ia and Ib, flowing into two conductors, A and B, with resistance λ. Suppose also the end of conductor B be connected to a side conductor λ_1, while A forms part of an unbranched circuit. It can then be shown that if the resistance λ of the two conductors be increased by the same amount, Ib will lose more in strength than Ia; or to state it in another form, as the actual resistance in both circuits increases, the difference in favour of the current in the conductor A (that is, the un-

[1] *Proceedings of the Physiological Society*, 12th December, 1885.

[2] Du Bois-Reymond, *op. cit.* p. 394.

branched circuit) increases. Hence, in experimenting with the fish, it is important to have the resistance of the external or experimental circuit as small as possible. To obtain sparks, the circuit must be suddenly opened when the shock is strongest. Du Bois-Reymond succeeded in passing the discharge of the fish through the primary coil of a Ruhmkorff's inductorium, so that a shock was emitted by the secondary coil. Three shocks of a malapterurus magnetized needles 37 mm. long by ·7 mm. thick, when placed in the central cavity, 8 mm. wide, of a coil of 735 turns of copper wire ·4 mm. thick.

Any point of the organ nearer the tail is positive to any point nearer the head, and the intensity of the shocks increases according to the distance at which electrodes leading off a current are applied.

It is remarkable that the shocks emitted by the electrical organ pass through the body of the fish, and that the accumulated discharges strike the central cerebro-spinal system vertically to its axis. This of course leads to the question of how far the nervous organs of the fish are sensitive to electric shocks. Du Bois-Reymond found that induction shocks from an inductorium, having two Grove's elements in the primary circuit, killed or rendered unconscious such fishes as tench, perch, chub, pike, and silurus, and the same current tetanized frogs, but a malapterurus was apparently unaffected. When the induction shocks were made much stronger, the fish noticed them, but,

> "If it came in the neighbourhood of the electrodes, where the current density was greatest, it withdrew hastily, gave a shock or two, and sought with correct instinct that position in which its axis of length cut perpendicularly the least dense current curves, as if it knew the laws of the distribution of current in non-prismatic conductors." [1]

A constant current from 30 Grove's cells did not appear seriously to inconvenience the fish, but here also "it sought that position which is theoretically the most protected." These experiments clearly show that malapteruri have a certain immunity from electric shocks, and that they do not suffer from their own discharges. Now, if we consider that sensory nerves require a certain strength of stimulus to excite them, and that electrical currents produce peculiar effects on nerves, it is clear that the lower limit at which excitation occurs is higher for the nerves of malapterurus than for the nerves of other animals.

When the electric nerve is directly irritated, a number of rapid shocks are emitted, so that if the nerve of a nerve-muscle preparation (Fig. 261, p. 401) is laid on the organ on the side corresponding to the electric nerve irritated, the muscle of the preparation is tetanized.

[1] Du Bois-Reymond, *op. cit.* p. 406.

Du Bois-Reymond failed to get evidence of a current from the electric nerve when it was placed on the cushions of the galvanometer (Fig. 294, p. 447), nor did he observe any negative variation when it was irritated, but the nerve with which the experiment was made was in a very exhausted condition (removed $2\frac{3}{4}$ hours after the circulation had ceased), and he states that "it is conceivable that a more efficient electrical nerve of the malapterurus might show traces on the galvanometer of the nerve-current and of its negative variation."

Mr. Francis Gotch,[1] in his observations on a malapterurus, in which he used both the galvanometer and the capillary electrometer, made the important discovery that the discharge on electrical excitation of the skin is not of a reflex character, but is the result of a direct excitation of the electrical organ at the point irritated by a break induction shock with 1 Daniell's element in the primary circuit and the secondary coil 80 mm. or less from the primary. In these circumstances the latent period is extremely short. Further, the molecular disturbance set up at the point irritated is propagated through the electrical organ at the rate of about 2·5 metres per second. When the fish was irritated by a single induction shock on "the dorsal surface between the eyes," a long discharge took place, causing tetanus of a nerve-muscle preparation in contact with it, the individual contractions of which were at the rate of 10 per second.

As regards the electrical phenomena of the skate, Dr. Burdon-Sanderson has communicated to me the following facts—

The direction of the discharge is towards the tail, that is to say, it is such that at the moment of activity the ends of the electric nerves become positive to the trunks, in accordance with the law of Pacini. The discharge may be induced in the living animal by mechanical stimulation of the surface of the body, and then consists of a rapid succession of similar instantaneous effects. In the separate organ the anterior end is negative to the posterior, that is, there is an "organ current" in the same direction as the discharge. This difference of potential is increased enormously by increase of temperature or by injuring the surface. The electromotive force of the discharge relatively is far inferior to that of torpedo in its most active state. The discharge can be heard by the telephone, but cannot be felt by the fingers.[2]

6. *Special Electrical Phenomena of the Electric Fishes.*—Du Bois-Reymond and Sachs, and more especially the former, have discovered a number of phenomena which are of profound interest, but some of

[1] Francis Gotch, *Proceedings of Physiological Society*, *op. cit.* xxviii.

[2] Goodsir communicated to Du Bois-Reymond the suggestion that the electrical organ of the skate is active only during sexual excitement.

which, from their very nature, are difficult of explanation. Thus Du Bois-Reymond has investigated the distribution of the current in the torpedo, and has shown that the shock penetrates the body of the fish and attains a greater density in the brain and spinal cord than elsewhere. The currents flowing in the back from the borders of the organ to the mesial line, and in the belly from the mesial line to the borders, pass through the brain and cord, as this "is the shortest path between the most active portions of both organs." The greater the length of a column in torpedo (the number of plates being constant in a unit of length) the greater is its electromotive force. As the columns are longest in the median region of the organ, the electromotive force of these columns is greater than that of the lateral columns. Hence, in air, a current flows in the back from the middle of the organ to the borders, and in the belly in the reverse direction. In salt water, however, the currents of the higher columns have nearly the same strength as those of the shorter ones. If all the columns in both organs were of the same length, and if the organs were united in the middle plane, then the most positive part would be "the middle of the median line" on the back, and the most negative the corresponding part on the ventral aspect. When the organs are separated, then the most positive part is nearer the inner than the outer border, in consequence of the thinning of the organ towards the sides. Hence the inner border is most positive dorsally, and most negative ventrally, and therefore a current flows in the back from the outer border to the middle line, and in the belly in the reverse direction. Further Du Bois-Reymond has shown that the curves of the direction of the currents passing out from the dorsal surface through the water and back to the ventral surface are inclined outwards, and also that, in consequence of the greater inclination of the median columns, by making the current curves still more oblique, the dorsal surface of the fish when it lies half buried in mud at the bottom of the sea is more protected electrically than the ventral surface which does not require protection.

By the *organ current* is understood a current existing in the organ during rest and passing in the direction of the shock. Such a current exists in all electrical fishes. Thus, in the torpedo, on applying the cushions of the galvanometer to the dorsal and ventral sides, after the electric lobe of the brain had been destroyed, so as to remove from the animal the power of giving voluntary electric shocks, a current was readily passed through the coils of the galvanometer. By another method, Du Bois-Reymond was able to examine bundles of columns of the length of 29 mm., and when these were placed on the electrodes so that one electrode touched the end and the other the side of the column,

a current was obtained. The current thus obtained from bits of the organ of torpedo was remarkably small, "considerably less than the force of the nerve-current in frogs."[1] Sachs obtained from bits of the organ of a gymnotus 40 mm. long, an e. m. f. of from ·015 to ·030 (mean, ·0225) of that of a Daniell's element. With strips of the organ of torpedo 29 to 12 mm. (mean, 20 mm.), Du Bois-Reymond obtained ·0085 of a Daniell's element. Double this would have been ·017, thus a little less than that of a gymnotus. He also shows that the e. m. f. of a single plate of gymnotus is about ·00006 of a Daniell's element, while that of a single plate of torpedo is only ·0000117 of a Daniell's element, or about 3·3 times less than that of gymnotus.[2] The torpedo is a sea-water fish, while the gymnotus inhabits fresh water, and it is remarkable that the ratio of the e. m. f. in the two fishes is almost the same as that of the resistance of fresh water to salt water. (Christiani.) Each plate of the fresh-water fish has a larger e. m. f. than that of the sea-water fish because it has a greater resistance to overcome.

The organ current decreases as the organ dies, but in the cold it may remain for 24 or 48 hours after the death of the fish. Strips of the electric organ of a malapterurus have, in the hands of Du Bois-Reymond, yielded no organ current, but, as he suggests, this may be owing to the weakness of the animals under investigation.

Du Bois-Reymond has made a number of remarkable observations on the polarizing effects produced by passing currents through the organ of the torpedo. In this way polarization currents are produced. If the polarizing currents are in the direction of the natural shock-current of torpedo, as it runs in the body of the animal, that is from belly to back, they are termed *homodromous*, and if in the opposite direction to that of the natural shock-current, that is from back to belly, he calls them *heterodromous*. "Polarization effects from belly to back are absolutely positive, and, according as they follow an absolutely positive or negative polarizing current, they may be considered relatively positive or negative." The character of the polarization effect seems to depend on the length of the time of closing. Thus, with homodromous currents, a long closing time gives a polarization relatively negative, while a short closing time gives a polarization relatively positive. As functional activity diminishes, positive polarization disappears and then

[1] Du Bois-Reymond—Burdon-Sanderson's Translations, *op. cit.* p. 441.

[2] The e. m. f. between the longitudinal and transverse section of the gastrocnemius of a frog is about ·03 to ·08 Daniell, and that between the longitudinal and transverse section of a sciatic nerve of a frog about ·02 Daniell. Burdon-Sanderson found, on an average, an e. m. f. between the lobe of the leaf of *Dionæa muscipula* and the mid rib of ·006 Daniell.

only negative polarization remains. With homodromous currents polarization may be in two directions, first relatively negative and then relatively positive. Since shocks of very short duration polarize *positively*, the strength of the normal shock of the organ may be itself increased. From these and other observations Du Bois-Reymond infers that "the strength of the homodromous current is so much greater than that of the heterodromous that the relatively positive heterodromous polarization is constantly masked by the relatively negative." As early as 1857, Du Bois-Reymond observed this law in experimenting with fresh strips of the organ of malapterurus "in which positive polarization appeared in full force in the direction of the shock, the descending current (homodromous in malapterurus) was always greater than the ascending (heterodromous) in the relation of 100 to 112, 116, or 125, and he inferred from this the existence of a polarization current which was added to the current of the battery passed through the organ." In some of his more recent experiments with torpedo he found the strength of the homodromous current of 30 Grove's elements to be more than twice that of the heterodromous current. The e. m. f. of this additional polarization current must be assumed to be high when the difference between the effects of homodromous and heterodromous currents is so great. But a difference of current strength depends not only on unequal electromotive forces, but on unequal resistances, and hence the explanation of these facts may be, not the generation of a new e. m. f. by the homodromous current, but that the organ conducts better in the natural direction of the shock current than in the reverse direction. That is to say, the resistance is greater, in the organ of torpedo, in the downward than in the upward direction, or the resistances are *irreciprocal*. This irreciprocity, or inequality of resistance in the two directions, may depend on some peculiarity of the organic molecules during life as it gradually disappears as the organ slowly dies, or it may at once be removed by plunging the organ into boiling water. In later researches, Du Bois-Reymond found that the irreciprocity increases, but not proportionally, with the current density, or, in other words, when the distance of the secondary coil from the primary of the inductorium, by which shocks were transmitted through the organ, was diminished to zero, the irreciprocity became greater than when the secondary stood 100 mm. from the primary. At 100 mm. the resistance to a homodromous current to that of a heterodromous current was as 1·33 to 27·66, giving an index of irreciprocity of ·0481 (that is 1·33 ÷ 27·66), while at zero the ratio was as 264·7 : 477, or an index of irreciprocity of ·5549, while if the irreciprocity had been in direct proportion to the current density, the index would have been ·8293.

This apparent irreciprocity exists in each transverse lamella of the organ. A careful inquiry into the conductivity of the organ of torpedo, enables Du Bois-Reymond to state that—

"Even in the homodromous direction, in which the organ conducts best, it conducts scarcely half as well as frog muscle parallel to the fibre, and 7·5 to 12 times worse than the sea water of the aquarium. The ratio would be still more unfavourable with sea-water from the Mediterranean, which conducts nearly 150 times better than tap water. But in the heterodromous direction the organ conducts even 20 to 58 times worse than sea water."

In summing up the results of his investigations on the torpedo, Du Bois-Reymond endeavours to show the important part played by irreciprocal conduction, and his opinion is that it takes the place of insulating septa, which were assumed by the earlier physiological

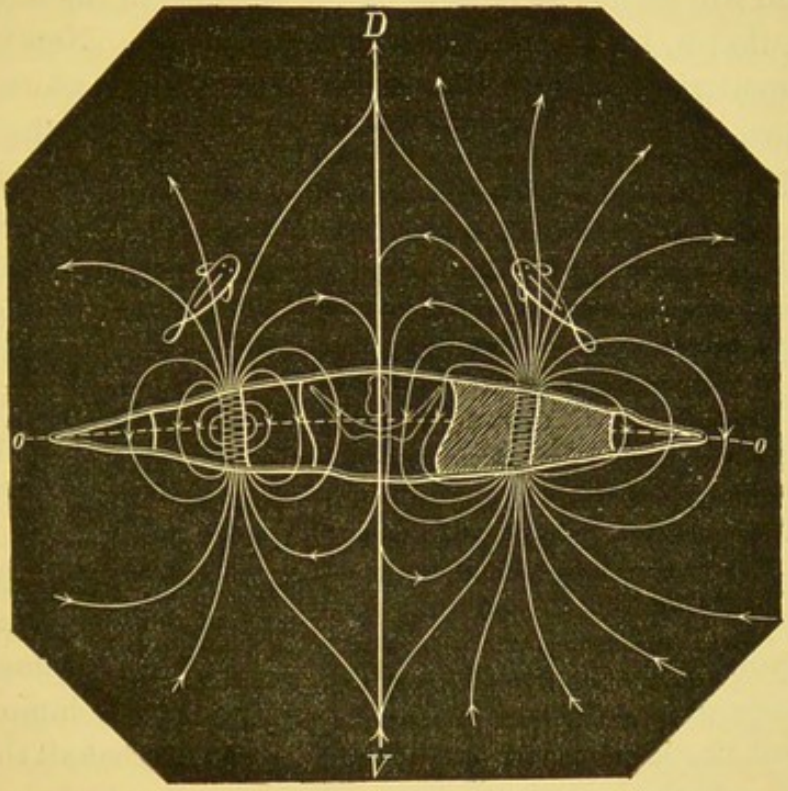


Fig. 316.—Diagram showing the current curves in the electrical discharge of torpedo.

electricians either to exist permanently around the organ or to be called into existence at the instant of the discharge. His view will be understood with the aid of the diagram in Fig. 316. Suppose a single column of the organ of a torpedo surrounded by a conducting mass. The current curves will issue from the upper end and pass through the conducting mass to the lower end, and these curves will be symmetrically arranged around the axis of the column. Suppose also the column divided into an upper and lower portion by a transverse plane. The part above the transverse plane, O – O, Fig. 316, will be the positive dorsal

end of the column, and the part below O – O will be the negative ventral end. All the curves issuing from the dorsal end will pass perpendicularly through the transverse plane, so that the tension of this plane will be zero. In the column, currents will flow from belly to back, those near the axis of the column nearly parallel to it, while those nearer the lateral surfaces are more divergent, and some of the most divergent will cut the lateral surface. On the left, the figure (Fig. 316) shows the outermost system of curves around one column cutting the transverse plane. If now a second column be placed alongside of this one, the current curves on the two halves of the columns in apposition will be compounded, and so with a third, a fourth, or any number of columns placed side by side. Thus the density of the current in 1 will be doubled by that of 2, trebled by that of 3, and increased n-times by the addition of n^{th} column. Each column will be met as it were by the currents of the others, homodromously, that is, in the direction of its own current. Now the torpedo has two symmetrical organs, as shown in Fig. 316, in which D V, the sagittal plane, may be regarded as insulated. Looking at the left of the figure, imagine the strip with transverse marks to represent one column, it is clear that the currents from this column pass heterodromously through all the other columns. The currents issuing from the axis of the column pass out and meet the small fish seen in the diagram. The little fish will receive a shock which would be stronger if the column under consideration were insulated. This is shown in the right of the diagram, when the shaded portion indicates an imaginary arrangement for insulation. It will be seen that a larger number of current curves strike the little fish on the right side than on the left. The insulation also compels currents which, on the left side, pass through part of the organ, to pass on the right side, round the edges of the organ. Thus insulation would increase the action of an electromotive column on any part of external space. In torpedo, however, the columns are not insulated, but the same effect is obtained by the fact that all the currents passing through the organ are heterodromous, in which, as we have seen, the resistance is much greater, and consequently the same effect is produced as if the columns were insulated. That is to say, by increasing the resistance in the downward direction, the density of the total current into external space is increased. Each column conducts its own homodromous current but offers resistance to the heterodromous currents of all the others, and consequently the heterodromous currents are forced to pass round the edges of the organ as if it were composed of a non-conducting substance. The part of the animal, however, between the median borders of its two electric organs, that is its cerebro-spinal axis, is traversed by the strongest flow of current, and, with

reference to this, the importance of the fact that the torpedo and electric fishes in general have a kind of immunity from the effects of electric shocks is obvious.

These remarkable results of Du Bois-Reymond, to explain which he has advanced the beautiful theory above set forth, have not been unquestioned, and in a communication made to the Royal Society by Mr. Francis Gotch,[1] the fact of the existence of irreciprocal conduction in the torpedo has apparently been disproved. Du Bois-Reymond's method depended on observing the deflections of a galvanometer when the polarizing homodromous or heterodromous currents were sent through a strip of the organ and also through the circuit of the galvanometer.

Gotch discovered that intense currents of short duration, such as were used by Du Bois-Reymond, always cause "an excitatory response" in the tissue of the electric organ; that the intense current due to this response is added to the homodromous current, and the result is that the latter must be stronger than the heterodromous. Thus, calling the homodromous current a, and the heterodromous b, and the "excitatory response current" of Gotch c, when a short strong current passes upwards (homodromous), the result is $a+c$; but if the short strong current passes downwards (heterodromous), the result is $b-c$. By means of a fast moving rheotome, Gotch was enabled to "switch in" the induction currrent only, and then, on passing it in either direction, upwards or downwards, the resistance is found to be the same, that is to say, there is no irreciprocity. The apparent irreciprocity, therefore, is an excitatory phenomenon, not due to a difference in resistance, but to the adding or subtracting of an additional electromotive force.

Mr. Gotch has also made the remarkable discovery that the organ may be excited in a secondary way by its own discharge. Strong healthy fish, in the summer, when excited by *one* irritation, gave not only a first discharge, but also in quick succession a second, third, fourth, and so on at intervals of about $\frac{1}{100}$ of a second, each successive shock becoming weaker than the one before it. Thus, as clearly stated by Mr. Gotch—

"The response of the isolated organ to nerve excitation is multiple; a primary, secondary, tertiary response following the application to the nerve of a single stimulus. Since all these responses produce currents similarly directed through the columns of the organ, each column during

[1] Francis Gotch, M.A. Oxon., B.A., B.Sc. Lond. *Further observations on the electromotive properties of the electrical organ of torpedo marmorata.* Read before the Royal Society, March 9th, 1888.

its activity must reinforce by its echoes the force of the primary explosion, both in its own substance and also in that of its neighbours."

The brief account I have given of the electric organs indicates that such investigations will probably throw light on the obscure relations that exist between nerves and muscle and other terminal organs. In the case of torpedo, gymnotus, and raia, the nerve termination is evidently analogous to a motorial end-plate in muscle (Fig. 317), and in malapterurus to the terminations of nerves in the cells of glands (Fig. 318). The nerve stimulus, or, as it may be put, the molecular disturbance transmitted along a nerve causes changes in its end-organ, and these are propagated to the surrounding substance. These changes are associated with a change of potential, and the part becomes negative. A wave of negativity passes through the organ, and then there is probably a physiological result according to the kind of organ in which the changes may take place. If it be a muscle, the chief expression of the change is a variation in form or

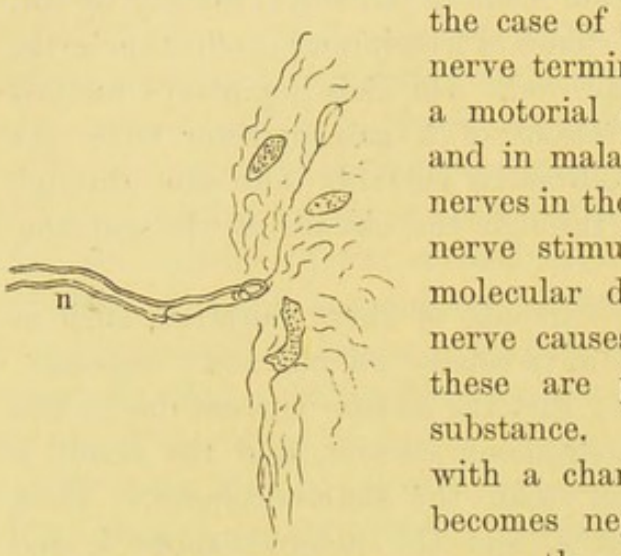


FIG. 317.—Motorial end-plate in muscle. *n*, nerve.

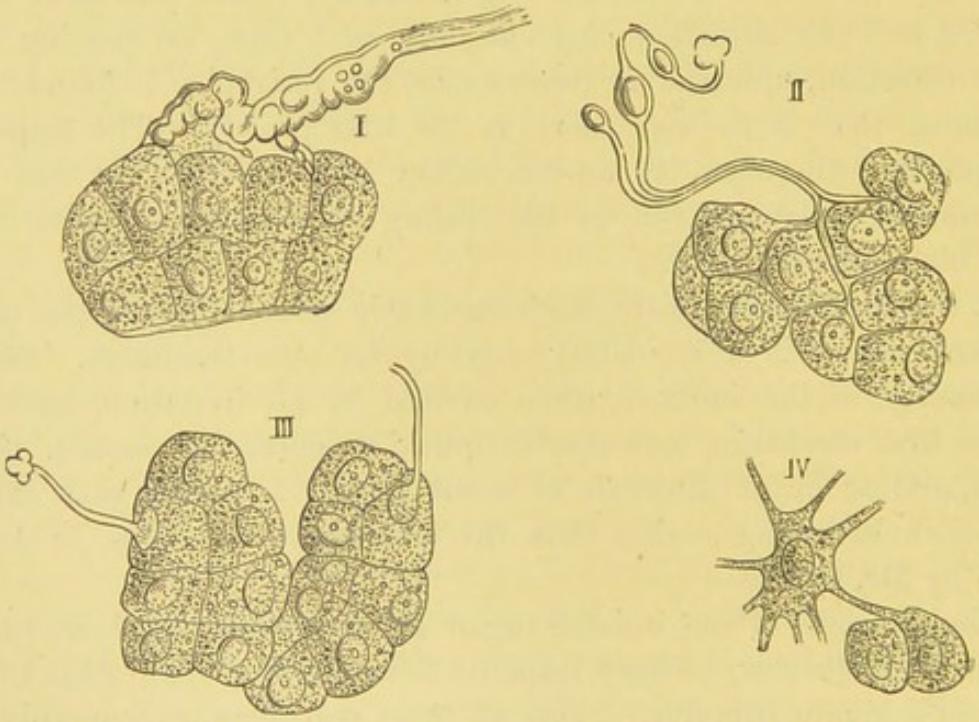


FIG. 318.—Mode of nerve-terminations in glands, according to Pflüger. I. Nerve ending in a group of cells, but not apparently uniting with any of them. II. Nerve sending branches between cells. III. Nerve filaments apparently continuous with substance (nucleus?) of cells. IV. Ganglionic nerve cell, with process passing to secreting cell. Pflüger's observations have not been confirmed. Kupffer describes a similar arrangement in the salivary gland of insects, and Macallum believes he has traced nerve-endings into the cells of the liver.

contraction; if it be a gland cell, the chief expression of the change is the formation of certain matters or metabolism; and if it be an electrical organ, it is an electrical discharge. In all these, however, similar phenomena occur, but to varying amounts. Thus, call contraction *a*, electromotive phenomena *b*, and metabolic changes *c*. In a muscle, *a* is large, and *b* and *c* relatively small; in a gland, *a* probably does not occur as an active contraction, although the cell may slowly change in form and bulk; *b* is also small, but *c* is relatively large; and in an electrical organ, there is no evidence that *a* occurs, *b* is largely shown, and *c* is relatively small. Thus, all these phenomena are linked together, and as our knowledge advances into the molecular processes of the one, it is likely soon to shed light on the other two.

Finally, I cannot help remarking that in few departments of physiological science can more striking examples of internal adaptation be found than in the construction and place in nature of the electric fishes. As written by Du Bois-Reymond, we have in them "surprising instances of that organic adaptiveness, which is an ever-new source of wonder even to the strict adherent of mechanical casuality." It seems to me,—again using a phrase of the distinguished Berlin physiologist,—that if any human imitation of the electrical mechanism of a torpedo or gymnotus could have been the outcome only of the "profoundest reflection of a clever brain," even so we can account for such adaptations only by assuming an intelligent consciousness acting behind natural phenomena.

Consult as to the older views of the nature of electric organs:—John Goodsir's *Anatomical Memoirs*, vol. II. p. 289 *et seq.* As to the more modern histology:—Ranvier, *Histologie du Système Nerveux*, vol. II. p. 157 *et seq.*; Carl Sachs, *Untersuchungen am Zitteraal* (Gymnotus electricus), 1881; Gustav Fritsch, *Das Gehirn und Rückenmark des Gymnotus electricus*, 1881; Gustav Fritsch, *Die elektrischen Fische, erste Abth. Malapterurus electricus*, 1887; August Ewald, *über den Modus der Nervenverbreitung im elektrischen Organ von Torpedo*, 1881. As to electro-physiology, see the Translations of Du Bois-Reymond's *Memoirs* in Burdon-Sanderson's *Biological Memoirs*, 1887; also, Paper by Du Bois-Reymond in Carl Sach's volume above mentioned; Papers by Burdon-Sanderson and Gotch, communicated to the Royal Society, already referred to, 1887-88; and Paper by Gotch, at the end of which is an excellent bibliography, on the electromotive properties of *torpedo marmorata*, *Phil. Trans.* vol. 178 (1887) B, pp. 487-537.

APPENDIX I.

Regarding Reagents, see p. 260; Modes of Isolation, p. 265; Modes of Fixing, p. 266; Hardening, p. 268; Decalcification, p. 268; Staining, p. 270; and Investigation of Fresh Objects, p. 276. By the following methods preparations may be made similar in character to those represented in the page or pages referred to under each method—

SPECIAL METHODS OF MAKING HISTOLOGICAL PREPARATIONS.

1. Nuclei, Method No. 1, p. 206. The reticulum in nuclei can be readily seen in the cells in the skin of the larvæ or tadpoles of amphibia. The larvæ of the triton or newt are easily procured in the months of June or July. [Dr. Macallum, of University College, Toronto, informs me that the phenomena may be readily studied in the epithelial cells of the skin of *Necturus*.] Fresh specimens, 3 to 4 cm. long, are thrown into 20 c.cm. of chromo-osmium-acetic acid (p. 261), in which they quickly die. After one or two days, cut from the tail a portion, 1 c.cm. long, or peel off a bit of skin from the tail with the forceps. Scrape off with a scalpel some of the epithelium; the remaining thin portion of skin is hardened in 50 c.cm. of alcohols of gradually increasing strengths (p. 267), colour in saffranin (p. 263), and mount in dammar varnish. Striated muscles of the tail, and involuntary muscle from the intestine, also give beautiful results by the same process. See also p. 271, par. 3.

2. Karyokinesis, Method No. 2, p. 213. Place for two days portions of the anterior segment of the eyes of young newts in chromo-osmium-acetic acid (p. 261), wash thoroughly in water, and harden in alcohol of gradually increasing strengths (p. 267). Two days later, cut out the cornea, and pull off with the forceps a thin lamina of corneal tissue. Colour in saffranin (p. 263), and mount in dammar varnish, so that the convex side of the cornea is directed upwards. The nuclei are stained red, and with high powers karyokinetic forms will be seen.

3. Testis, Method No. 3, p. 215. Divide into two equal portions by a transverse cut the testis of a young human subject, and fix both portions in 50 c.cm. of Kleinenberg's sulpho-picric acid (p. 261), then harden in 30 c.cm. of alcohols of gradually increasing strengths (p. 267). Colour thick transverse sections with carmine (p. 262) and with hæmatoxylin (23, p. 262), and mount in dammar varnish.

4. Tubuli Seminiferi, Method No. 4, p. 216. Fix portions, 2 cm. broad, of fresh testis of ox in 20 c.cm. of Müller's fluid (p. 261), and at the end of fourteen

days transfer them to alcohol of gradually increasing strengths (p. 267). Colour very thin sections with hæmatoxylin (p. 262), and mount in dammar. The smaller canals, containing developing spermatozoids, are coloured intensely blue, and the nuclei of the peripheral cells are often stained more deeply than the nuclei of the cells lying near the lumen.

5. Spermatoblasts, Method No. 5, p. 218. Fix small portions, 5 mm. broad, of testis of ox newly killed in 10 c.cm. of chromo-osmium-acetic acid (p. 261). At the end of one or two days, wash the preparations under the water tap for one hour, and then harden in 20 c.cm. of alcohol of gradually increasing strengths (p. 267). Colour thin sections with saffranin, and mount in dammar.

6. Elements of testis, Method No. 6, p. 218. Place a portion, 1 c.cm. in size, of fresh testis of ox in 20 c.cm. of Ranvier's alcohol (p. 260), and at the end of five hours teaze out in a drop of the alcohol. Colour while under the cover-glass (p. 277) with picrocarmine (p. 262), and preserve in diluted glycerine.

(*a*) To see *living spermatozoids*, place on a clean slide a drop of the milky fluid issuing from the cut surface of a fresh testis, add a drop of salt solution (p. 260), put on a cover-glass, and examine with a high power. After a little time, allow a drop of distilled water to pass under the cover-glass (p. 277), and the movements of the spermatozoids will cease. Observe the different forms, oval or round, of the head. Immature spermatozoids carry a little behind the head small bits of protoplasm. To preserve spermatozoids, allow semen diluted with water to dry on a slide, place a cover-glass on it, and fix with cement.

(*b*) To detect semen *stains* on linen, cut a bit of the stained portion, 5 to 10 mm. broad, moisten in a watch-glass with distilled water, for from five to ten minutes, and then teaze out a small portion. Then mount the teazed fragment in water, put on cover-glass, and examine with a magnifying power of 500 diameters. Examine the edges of the linen fibres, as the spermatozoids adhere to these. Sometimes the heads of the spermatozoids break off. The isolated heads may be recognized by their peculiar brilliancy, and in the semen of man, by their very small size, about 4 μ, or the $\frac{1}{6000}$ of an inch.

(*c*) Spermatozoids of the *frog*. The male frog is recognized by well-developed warts on the fore feet. Kill the animal; open the abdomen. The testes are two small kidney-shaped bodies which lie at the sides of the vertebral column. Cut one testicle by a transverse incision, squeeze out a drop of fluid on a slide, dilute with a drop of salt solution (p. 260), put on cover-glass, and examine. The spermatozoids are then seen to have very long slender heads, and the tails so thin as almost to escape notice. Immature spermatozoids lie in clusters.

7. Ovary, Method No. 7, p. 220. Ovaries of small animals are to be fixed as a whole. The ovary of one of the higher animals, or of a woman, should have a number of transverse and longitudinal cuts made in it before throwing it into from 100 to 200 c.cm. of Kleinenberg's sulpho-picric acid (p. 261). It is afterwards hardened in 100 c.cm. of alcohol of gradually increasing strength (p. 267). Ova often fall out of the larger Graafian vesicles, and many sections may have to be made to secure a good specimen. Colour with hæmatoxylin (p. 262) or with borax carmine (p. 262), and mount in dammar.

8. Ovary, Method No. 8, p. 221. See Method No. 7.

9. Ovary, Method No. 9, p. 222. See Method No. 7.

10. Ovary, Method No. 10, p. 223. See Method No. 7.

(*a*) *Fresh ova*. Obtain the fresh ovaries of a cow from the slaughter house. The large Graafian vesicles are about the size of a pea. Cut one or more out of

the ovary by means of scissors. Lay the isolated vesicle on a slide, and prick it with a needle, making the puncture as near the slide as possible. The liquor folliculi flows out, and among cells of the cumulus ovigerus, the ovum must be sought for with the aid of a magnifying glass. On finding it, place it in the middle of a slide in a drop of salt solution, surround it with a little ring of paper, and then lay the cover-glass gently on, so as to avoid pressure. This is an operation much easier to describe than to perform.

(*b*) *Ova of frog.* Lay on a slide a bit of fresh ovary of a frog, and puncture all the black ova, so that the contents flow out. Place the remainder in a watch-glass, in distilled water, and wash by moving the contents to and fro with needles. Place the watch-glass on a black background, so as to see distinctly the unpigmented follicles. Remove a bit of the unpigmented portion to a slide, cover with a drop of salt solution, put on a cover-glass, and examine. Such ova have a very large germinal vesicle, the germinal spot disappears early, and usually cannot be seen. The dark patch seen is the yolk nucleus. Often on the circumference of the ovum a delicately striated membrane may be seen. This is the theca folliculi with a single layer of epithelial cells.

11. Leucocytes, Method No. 11, p. 295. Clean a slide and cover-glass carefully with alcohol. Place on the slide a small drop of frog's blood, lay on the cover-glass, and fix it with a layer of paraffin (p. 277). Coloured and colourless corpuscles are seen. Examine the more granular colourless corpuscles carefully with high power. The movements occur slowly, and the most effective way of being convinced that they do occur is to make drawings of the same leucocyte at intervals of one or two minutes.

12. Secreting Epithelial Cells, Method No. 12, p. 297. To see the changes connected with secretion, use the stomach of a dog or cat that has received no food for a day or so. The stomach of the rabbit is not suitable, as the chief cells are small. Portions of mucous membrane, 1 cm. broad, are placed in 10 c.cm. of absolute alcohol, and the alcohol is changed in half an hour. Then examine. The human stomach, if obtained soon after death, shows the gland structure even better than that of the dog or cat, as the gland tubes are wider apart. Cut very thin sections. For further details, see Stomach, Vol. II.

13. Blood Corpuscles, Method No. 13, p. 300. Carefully clean with alcohol a slide and a small cover-glass. Then puncture with a clean needle the tip of the finger. The drop of blood first coming to the surface is wiped away and the second drop is caught on the cover-glass and the cover-glass is placed on the slide. Seal up the preparation with a little paraffin. Examine with high power, observe rouleaux and coloured and colourless blood corpuscles. The jagged edges of many corpuscles are produced by evaporation. After removing the paraffin from the rim of the cover-glass on the one side, place a drop of water on the edge of the cover-glass, thus discolouration of the blood corpuscles is noticed, while the water becomes yellowish, the blood corpuscles also become globular, and they appear as pale spheres which disappear entirely at the close of the experiment. Then study carefully *one* corpuscle.

(*a*) *Small blood plates* or *plaques* are obtained if, *previous* to the puncture of the finger, we lay on the tip a drop of a filtered mixture of 5 drops of an aqueous solution of methyl violet (p. 263) along with 5 c.cm. of a solution of common salt (p. 260), and then prick the finger through the drop. The blood issuing out of it mixes with the methyl violet; a drop is laid on the under surface of the cover-glass and examined with high powers. The plaques are coloured intensely

blue, of peculiar brilliancy, disc-shaped, and not to be confounded with the white corpuscles likewise coloured. Their quantity varies in individual cases. Avoid confounding the blood plaques with granulated impurities which may be even in the filtered colour solution.

(*b*) *Coloured blood corpuscles of animals* (frog, etc.) should be taken from the newly-killed animal and treated according to Method No. 13.

(*c*) *Blood stains.* Moisten small portions of the stain in 35 per cent. solution of caustic potash on the slide, teaze small portions of blood-stained linen in a drop of the caustic potash. Although the coloured blood corpuscles of our domestic mammalia are smaller than those of man, it is nevertheless impossible to decide, from the size of the blood corpuscles, the question whether the blood originally belonged to man or to some other mammal. It is, on the other hand, easy to distinguish the oval blood corpuscles of the other vertebrates from disc-shaped ones of the mammalia.

(*d*) *Blood crystals* (pp. 119 and 129). (α) The production of the hæmin crystals is easy (p. 129, Fig. 53). Cut a small flap, 3 mm. broad, of blood-soaked, dry linen, and place it along with a morsel of common salt, the size of a pin's head, on a clean slide. Add a large drop of glacial acetic acid and pound, with a blunt glass rod, the salt and the linen until such time as the acetic acid assumes a brownish hue. Do this rapidly otherwise the acetic acid evaporates. Then gently heat the slide over the flame until the fluid boils up *once*. The small bit of linen is now removed and the dry brown spaces investigated on the slide with high powers. We occasionally perceive, even without a cover-glass and without any preserving fluid, *brown* crystals of hæmin near numerous fragments of white crystals of common salt. For preservation cover the slide at once with a large drop of dammar varnish and a cover-glass. The form and size of the hæmin crystals vary. We may obtain from the same blood complete crystals, partly isolated, partly lying crosswise under each other, partly united into stars, along with forms resembling that of a whetstone, and the smallest particles may scarcely exhibit the crystal form. The discovery of hæmin crystals is of great importance for medico-legal purposes. It is often thus possible to discover the crystals on portions of clothing, but it is often difficult to furnish proof from small stains, especially on rusty iron, that they have their origin in blood. The instruments and reagents to be employed in such investigations must be absolutely clean.

(*e*) *Hæmatoidin* crystals (Fig. 52, p. 129) are found by teazing out old extravasations of blood, which to the naked eye are recognized by their rusty-brown colour. Hæmatoidin crystals occur in apoplectic cysts, in a corpus luteum, etc.

(*f*) *Hæmoglobin crystals* (Fig. 48, p. 119) are produced from human blood (best from the splenic vein) only with difficulty. We sometimes obtain them if we convey a drop of the blood of the vein of the spleen to the slide, and after five minutes add a drop of water, cover the whole with a cover-glass and allow it to stand in the light. After some time the crystals may appear on the margin of the preparation.

14. Epithelial Cells, Method No. 14, p. 301. Scrape off from the upper surface of the tip of the tongue a little mucus and place it on a slide with a drop of salt solution (p. 260), then put on a cover-glass. Then observe the flat squamous epithelium (Fig. 147, 1, p. 301) and the salivary corpuscles (Fig. 143, *b*, p. 296). Sometimes also one may find a dark mass of fungus threads (*Leptothrix buccalis*). Stain under cover-glass with picrocarmine (p. 277), and after adding a little glycerine the preparation may be kept.

15. Bristle Cells, Method No. 15, p. 302. Cut from the bill of a newly-killed duck or goose the yellowish skin passing over the margin of the mandible and place small portions, 1 to 2 mm. thick and 1 cm. long, into 3 c.cm. of a two per cent. solution of osmic acid along with 3 c.cm. of distilled water. Leave this for twenty-four hours in the dark. Then wash small portions for one hour under the tap and transfer to 20 c.cm. of 90 per cent. alcohol. After six hours cut vertical sections, mount in dammar. Such preparations are better unstained. (See Touch, Vol. II.)

16. Pigment Epithelium of Retina, Method No. 16, p. 302. Open eyeball of sheep or ox in water, allow retina to float off, and on the surface of the choroid behind it the cells will be found. The light spots in the cells are nuclei. They are not readily preserved, but sometimes they keep well in a drop of glycerine.

17. Simple Cylindrical Epithelium, Method No, 17, p. 302. See Method No. 12.

18. Stratified Pavement Epithelium, Method No. 18, p. 303. Remove from the dead body of a cat the larynx, trachea, etc. Place the preparation for a period of from two to six weeks in from 200 to 400 c.cm. of Müller's fluid (p. 261), wash under water tap for one hour, harden in 200 c.cm. alcohol of increasing strengths (p. 267). At the end of eight days cut transverse and longitudinal sections, through vocal cords, through trachea, and through the thyroid gland ; stain for five minutes in hæmatoxylin (p. 262), and mount in dammar.

19. Stratified Ciliated Epithelium, Method No. 19, p. 304. Found in *regio respiratoria* of nose. Cut small portions, 5 to 10 c.cm. broad, from lower portion of *septum narium*, fix and harden in 20 c.cm. of absolute alcohol. For fine sections, use nasal mucous membrane from rabbit. Stain with hæmatoxylin (p. 262) and mount in dammar.

Ciliary motion, p. 321. Kill a frog, lay it on its back, and remove lower jaw, by using scissors ; the roof of the mouth is thus exposed. Cut from the roof of the mouth a small strip of membrane, 5 mm. long, and place it in a few drops of solution of common salt (p. 260) on a slide, and cover with a cover-glass. With a low power a beginner can scarcely perceive anything, unless currents, in which large blood corpuscles swim, direct him to the right spot. Put on, therefore, the high power and search the margins of the preparation. At first the motion of the cilia is still so lively that the observer is unable to distinguish *individual* cilia, the entire ciliary margin shows a wavy motion like that of a cornfield moved by the wind. After a few minutes this rapid motion abates and the cilia become visible. If the motion is stopped it may be started anew by leading through one drop of concentrated caustic potash ; the effect is, however, short-lived, so that the eye of the observer must not, during the process of leading through, leave the ocular. The addition of water immediately arrests the ciliary movement.

20. Fat Cells, Method No. 20, p. 305. Take from the axilla of a human subject a small fragment of the reddish yellow gelatinous fat, teaze out with needles on a dry slide, quickly add a drop of salt solution (p. 260), and put on a cover-glass. Thin places show fat cells. Colour under cover-glass (p. 277) with picrocarmine (p. 262), and mount in dilute glycerine. Always examine fat cells in a solution of common salt.

21. Involuntary Muscle, Method No. 21, p. 306. Place a small bit of stomach or intestine of newly killed frog into 20 c.cm. of caustic potash and treat as in Method No. 22 *b*.

22. Striated Muscle, Method No. 22, p. 307. (*a*) *Transversely striated muscle fibres of the frog.*—Cut with the scissors in the direction of the course of the fibres a strip of muscle 1 cm. long out of the adductors of a newly killed frog; teaze a small part, taken from the inner surface of the strip, in a drop of salt solution (p. 260); then add a second larger drop of the same fluid, and lay on a cover-glass without pressure; with a low power (50 d.) observe the cylindrical shape, the various thickness, occasionally even the transverse striation of the isolated muscle fibres; with high powers (240 d.) notice the distinct transverse striation, sometimes pale nuclei and glittering granules. Very numerous granules in muscle fibres are pathological. In cases in which the muscle fibres are cut transversely, we may perceive the muscle substance squeezed out of the tube of the sarcolemma. (*b*) *Of man.*—Beautiful transverse striation is seen on human muscles taken from a fresh subject. For preservation, colour under the cover-glass (p. 277) with picrocarmine (p. 262), and after complete colouring, in five minutes, introduce diluted glycerine, and seal up. (*c*) *Sarcolemma.*—Allow a couple of drops of water to flow on preparation for Method No. 25 *a*. After two to five minutes, observe with low power (50 d.) how the sarcolemma is raised up in the form of transparent vesicles; in other places, where the torn muscle substance has contracted, the sarcolemma appears as a fine striation. (*d*) *Nuclei.*—Take a bit of preparation for Method No. 25 *a*, and allow a drop of acetic acid to flow on it. Even with low powers the shrivelled or sharply outlined nuclei appear as dark or spindle-shaped lines. (*e*) *Fibrils.*—Place a fresh muscle of a frog in 20 c.cm. of chromic acid ·1 per cent. (p. 261). After twenty-four hours, teaze in a drop of water, and thus obtain fibres whose ends split up into fibrils. If a permanent preparation is desiderated, place the muscle in water for one hour, then into 20 c.cm. of .33 per cent. alcohol for ten to twenty hours; or preserve it in 70 per cent. alcohol. If the chromic acid is exhausted, protracted immersion for several weeks in alcohol frequently changed will suit. Allow picrocarmine to act on the preparation, and, after complete colouration, replace it by dilute glycerine. (*f*) *Terminations of the muscle fibres.*—Place the fresh gastrocnemius of a frog in 20 c.cm. of concentrated caustic potash. After thirty to sixty minutes the muscle disintegrates on its fibres being pressed slightly with a glass rod. If this does not occur, the potash has been too weak. Transfer a number of fibres into a drop of the same potash on the slide (the fibres cannot be investigated in water or glycerine, for the caustic potash thus diluted immediately destroys the fibres), and cover cautiously with a cover-glass. With low powers, we perceive the terminations of the muscle fibres and numerous glittering nuclei, some of which may be vesicular.

23. Ramified Muscular Fibres, Method No. 23, p. 307. Cut out the tongue from a newly killed frog and place it in 20 c.cm. of pure nitric acid, to which 5 grm. of chloride of potassium are added. Undissolved KCl must still remain at the bottom of the vessel. After fifteen hours, raise cautiously with glass rods the tongue out of the water, and place it in 30 c.cm. of distilled water, which is to be often changed. The tongue may continue in this up to eight days, but it may be operated upon after twenty-four hours. Place it in a small test-tube half filled with water, and shake it for a few minutes; thereupon the tongue disintegrates. Now pour the whole into a small dish, and, after one hour or later, place a little of the precipitate formed in a drop of water on to a slide. Teaze with needles so as to isolate fibres. Stain with picrocarmine under the cover-glass and preserve in dilute glycerine.

24. Striated Muscle, Method No. 24, p. 311. See Method No. 22 *e*.

25. Isolated Muscle Fibres, Method No. 25, p. 313. See Method No. 22 *f*.

26. Muscle Fibre of Heart, Method No. 26, p. 313. Cut out a papillary muscle from human heart, and place in 40 c.cm. of absolute alcohol. After from twenty-four to forty-eight hours, it is sufficiently hard for cutting. Stain with hæmatoxylin (p. 262), and mount in glycerine.

27. Ganglionic Nerve Cells, Method No. 27, p. 314. Teaze out a small portion of the gasserian ganglion of a sheep in a drop of salt solution, and colour with picrocarmine (p. 262).

28. Nerve Cells, Method No. 28, p. 314. Take some grey matter from the fresh spinal cord of an ox and place a portion, 1 to 2 cm. long, in 50 c.cm. of very dilute solution of chromic acid (5 c.cm. of ·5 per cent. solution in 45 c.cm. of distilled water). After changing the fluid often during from three to eight days, the grey matter will be found to be macerated to a soft pulp. Transfer this carefully with a spatula into the undiluted carmine solution (p. 262), and leave it there for from twelve to twenty hours. Then transfer into 50 c.cm. of distilled water, so as to wash out part of the colour, and, after five minutes, spread it out in a thin layer on a dry slide. The multipolar nerve cells will then be seen, but the processes are not visible. Allow the layer to dry, cover with dammar, and place a cover-glass gently over it.

29. Nerve Cells of Cerebrum and Cerebellum, Method No. 29, p. 314. Same process as detailed in Method No. 28.

30. Medullated Nerve Fibres, Method No. 30, p. 315. Remove the sciatic nerve of a newly killed frog, cutting out a portion about 1 cm. in length. Teaze a small bit in a drop of a solution of common salt. It is better to teaze on a dry slide. Apply the needle to the lower end of the nerve, so as to pull asunder the fibrils for about half their length, with a drop of salt solution and a cover-glass. The thin film of nerve matter contains numerous isolated nerve fibres. Do this very quickly (say in fifteen seconds), so that the nerve fibres do not dry.

31. Changes of the Marrow Sheath, Method No. 31, p. 315. Let a drop of water flow from the rim of the cover-glass into the preparation. Even in one minute, the changes shown in Fig. 176, 4, p. 315, may be observed.

32. Axis Cylinder, Method No. 32, p. 315. Teaze when dry (as in No. 30), and, instead of a solution of common salt, add a drop of absolute alcohol. The discovery of the axis cylinder requires practice. (See Fig. 176, 5, p. 315.)

33. Nerve of Rabbit, Method No. 33, p. 315. Remove the sciatic nerve of a newly killed rabbit, by the following process, without touching it with the fingers. Push a long splinter of wood below the nerve and tie the nerve to the ends of the splinter above and below. Then divide the nerve and lift out the splinter with the nerve attached, and place it in 100 c.cm. of a 1 per cent. solution of chromic acid. *Axis Cylinder.* After twenty-four hours, the ligatures are cut through ; a small portion of nerve, ·5 to 1 cm. long, is cut off, and teazed into fine bundles, not into fibrils. The bundles separate in the solution of chromic acid. After twenty-four hours more, they are transferred into 50 c.cm. of distilled water ; and, after two to three hours, they are hardened in alcohol of increasing strengths (p. 267). It is well to leave the bundles a longer time, say from one to eight weeks, in 90 per cent. alcohol, because they then stain easily. After complete hardening, the bundles are minutely teazed in a drop of picrocarmine, and after completed colouration, which invariably, after the duration of the preceding hardening in alcohol, takes place in from half a day to three days. Preserve in

acidulated glycerine. The nodes are not so distinct as in fresh preparations or in that of osmic acid, but are to be recognized as fine transverse lines. The somewhat shrivelled axis cylinder and nuclei are coloured red. The axis cylinder not unfrequently moves out of its place, so that the biconical excrescence does not any longer lie on the constrictions, but above or beneath them.

34. Nitrate of Silver on Nerves, Method No. 34, p. 316. Make the following—Mix 10 c.cm. of 1 per cent. solution of nitrate of silver with 20 c.cm of distilled water. Kill a frog, open the abdominal cavity, remove the entrails, so as to expose the nerves lying on the side of the vertebral column. Rinse out the abdominal cavity by pouring distilled water over it, and, after the water has run off, pour about one third of the solution of nitrate of silver upon the nerves. After two minutes, cut out the delicate nerves carefully, and place them for half an hour in the remainder of the nitrate of silver solution. Transfer them into 10 c.cm. of distilled water, in which they may lie from one to twenty-four hours. If we then examine the nerve in a drop of water, we recognize with low powers the endothelial sheath (*Henle's sheath*), and numerous pigment cells. A blood-vessel often lies near the nerve. Teaze a bit of the nerve, cover it with a cover-glass, and place on the margin of the cover-glass a small drop of dilute glycerine. If we now examine with high powers, we will notice little coloured constrictions and axis-cylinders. Allow the preparation to lie a few hours exposed to daylight (only a few minutes in sunlight), and the blackening of the parts becomes more marked. It is at first difficult for the beginner to recognize the swellings on the nerve, which, on account of teazing, are often widely separated from the constrictions. After a little practice, the forms are easily observed.

35. Non-Medullated Nerve Fibres, Method No. 35, p. 316. The vagus nerve of a rabbit is teazed when dry, then covered with a few drops of a 1 per cent. solution of osmic acid. After five to ten minutes, the medullated nerves are blackened. Now, let the osmic acid solution run off, and add a few drops of distilled water; the water is renewed after five minutes. After other five minutes, pour the water off, add a few drops of picrocarmine to the preparation, cover it with a cover-glass, and leave it from twenty-four to forty-eight hours in a moist chamber, then replace the picrocarmine by acidulated glycerine. With high powers we perceive the medullated nerve fibres delicately striated longitudinally. They show a blue-black hue. The non-medullated fibres are similarly striated, but show a pale grey tint. The pale nerve fibres not infrequently appear to run parallel for a long distance. Similar treatment of the sympathetic nerve yields more numerous non-medullated nerve fibres, only this nerve is more difficult to discover. Cut through the great cornua of the hyoid bone, then the nerve and the hypoglossus nerve will be seen. Behind the vagus the sympathetic lies, and it is recognized by the large, oval, yellowish-translucent ganglion, the superior cervical ganglion. Teaze out a bit of the firm part of the nerve below the ganglion. The preparation will also show double-nucleated ganglion cells, but it is very difficult to isolate the cells so as to show their processes or poles.

36. Connective Tissue, Method No. 36, p. 323. *Connective tissue bundles.* Intermuscular connective tissue. The thin lamina lying between the serratus magnus and the intercostal muscles is cut into small strips, 1 to 2 cm. long. A very small portion is quickly spread out with needles on a dry slide, and covered with a drop of salt solution and with a cover-glass. We observe the bundles of connective tissue pale and undulating.

37. Fine Elastic Tissue, Method No. 37, p. 323. To the last preparation

(Method No. 36) add a few drops of acetic acid. The bundles of connective tissue swell and become gelatinous, and the elastic fibres remain unaltered, and appear as sharply-defined dark curling fibres.

38. Coarse Elastic Tissue, Method No. 38, p. 323. Larger elastic fibres are obtained by teazing the fibres of a small portion (1 cm. long, and of the thickness of a pin), of the fresh ligamentum nuchæ of an ox in a drop of salt solution. Stain with picrocarmine, and preserve in diluted glycerine.

39. Transverse Sections of Coarse Elastic Fibres, Method No. 39, p. 323, are obtained by drying a piece of the ligamentum nuchæ, 10 cm. long, 1 to 2 cm. thick, and then treating it by Method No. 45.

40. Fenestrated Membranes, Method No. 40, p. 324, are obtained if we bring a small portion, 5 mm. broad, of the endocardium on a slide in a drop of water, and allow 1 to 2 drops of caustic potash to flow under the cover-glass. Examine the margins of the preparation. The basilar artery also furnishes fenestrated membranes. Cut from the artery a portion, 1 cm. long, lay it on to a slide, open it longitudinally with scissors, add a drop of water, endeavour, by scraping with a scalpel, to separate the wall of the artery into lamellæ. Place on it a cover-glass, then add caustic potash, and the small holes of the membrane appear like glittering nuclei.

41. Mucous Connective Tissue, Method No. 41, p. 325. Fix for three to four weeks the umbilical cord of a human embryo of three to four months (or of the embryo of a pig, 3 to 6 cm. long), in 100 c.cm. of Müller's fluid. Then harden in 30 c.cm. of alcohol, strengthened by degrees. The cord will still be very soft; to obtain transverse sections of it, embed it in liver, and on being cut it must be somewhat compressed by the fingers. Stain the sections in picrocarmine for two hours, or with hæmatoxylin for five minutes. Examine in a drop of distilled water; the delicate processes of the cells and the bundles of connective tissue are invisible in glycerine or dammar varnish. Examine pieces remote from vessels. The older the embryo the greater is the number of the connective tissue bundles. It may be preserved in dilute glycerine.

42. Connective Tissue, Method No. 42, p. 326. Connective tissue cells are made visible by addition of a drop of picrocarmine to preparation No. 36 under the cover-glass. In most cases we perceive the nucleus of the cell, particularly where the cell lies on connective tissue bundles. We may also perceive the pale yellow variously-shaped body of the cell.

43. Spiral Cells in Connective Tissue, Method No 43, p. 326. Remove from connective tissue surrounding the vessels of the circle of Willis, at the base of the brain, a fragment, 1 sq. cm. in size, wash it in a watch-glass with salt solution, and spread it out with needles in a drop of this solution. Put on cover-glass. With low powers, we find outside of numerous fine blood-vessels the usual connective tissue bundles, but also more sharply-outlined shining bundles, which are distinctly higher than the other connective tissue bundles. With high powers, we can distinguish the more sharply outlined glittering elastic fibres, and in favourable places also the nuclei of the connective tissue. Place such a bundle in the field, and then add a few drops of acetic acid under the cover-glass. As soon as the acid reaches the bundle, the latter swells, the fibrillar character disappears, and there appear elongated nuclei. The swelling up, however, is not regular, but divided by constrictions into sections of various lengths. With weak illumination, we perceive the fibres (cell processes) causing the constrictions. To demonstrate

the cells themselves take a similar preparation from a newly-born child. The manipulation is the same as in the case of an adult.

44. Eyelid to show Plasma Cells, Method No. 44, p. 326. Fix a human upper eyelid in 100 c.cm. of 0·5 per cent. solution of chromic acid for one to three days, and harden, after two hours' washing under the tap, in 50 c.cm. of alcohols of increasing strengths. Cut thin sections; stain with hæmatoxylin. This is difficult with fresh sections, but succeeds after several months' hardening in alcohol. Mount in dammar.

45. Tendon, Method No. 45, p. 327. Cut a portion, 5 to 10 cm. long, of a tendon, and let it dry in the air (not in the sun). Thin tendons (*e.g.* those of the flexor digitalis pedes) are sufficiently dry after twenty-four hours' exposure to the temperature of a room; thicker ones require several days. Next make with the scalpel (*not with the razor*) a smooth transverse section, and then cut small thin slices from the tendon. The smallest bits are thrown into water, and after the lapse of two minutes stained during five minutes in 3 c.cm. of picrocarmine, and mounted in dilute glycerine. Very frequently we see on the transverse section a striation produced by the mode of guiding the knife. Place a second section unstained in a drop of water on the slide, and then allow a drop of acetic acid to flow to it under the cover-glass. The marginal parts of the transverse section immediately become much swollen.

Fibrils in tendon.—Place a portion (2 cm. long) of a tendon in 100 c.cm. of saturated watery solution of picric acid. Next day tear up with forceps the tendon in the direction of its length; take out from the interior a bundle 5 mm. long, and pull it asunder on a *dry* slide; cover it with a drop of distilled water, lay on a cover glass, and examine with a high power. The fibrils appear most delicate, pale, minute.

46. Tendon, Method No. 46, p. 327. In order to study the more delicate tissue of the tendon, the cells, and their processes, place portions of a fresh thin tendon (*e.g.* of palmaris longus), 3 cm. long, in 200 c.cm. of ·5 per cent. solution of chromic acid for at least four weeks, changing the chromic acid solution frequently. The portions are then washed from one to two hours under the tap, and hardened in 40 c.cm. of alcohols of increasing strengths. Transverse sections are cut with a very sharp knife, for tendons are often friable, and tend to exfoliate. The sections must not be too thin. Mount uncoloured in dilute glycerine. Even low powers show delicate forms well seen when light falls obliquely on them (see Fig. 184, p. 327, *B*). The black jagged hollow cavities (*z*) enclose tendon cells.

47. Lymphoid or Adenoid Tissue, Method No. 47, p. 327. Human lymphatic glands are with difficulty recognizable, as the entire cortex is a mass of tissue hanging together, in which lymphoid tissue is irregularly dispersed. By shaking, the lymph sinuses of the gland come indistinctly into view, the protoplasmic matter falls out, and what remains appears at this stage as round gaps. On the other hand, the mesenteric glands are well adapted for showing the network of *trabeculæ*. Place portions of it, 2 cm. long, into 200 c.cm. of concentrated aqueous solution of picric acid, and, after twenty-four hours, make delicate sections with a *sharp* knife, moistened with water. This plan may not succeed so well as after fixation with alcohol, but thick sections are useful. The sections are, after one hour, placed in 100 c.cm. of distilled water, frequently changed, and then stained with hæmatoxylin and eosin. Mount in dammar. The trabeculæ are red; the lymphoid tissue blue. The leucocytes, formerly found in

the meshes, are loosened by the picric acid treatment, and they may be almost removed by shaking.

48. Hyaline Cartilage, Method No. 48, p. 329. Cut out with scissors the thin ensiform cartilage of a frog, lay it on a dry slide, cover it with a cover-glass, and investigate quickly with high powers. The cartilage cells completely fill up the cartilage spaces. For longer observation, add a drop of salt solution.

49. Hyaline Cartilage of Ribs, Method No. 49, p. 329. Without further preparation, make fine sections with a dry razor; place these in a drop of water under a cover-glass. Find glittering places in the cartilage. These contain solid fibres, due to calcification. Preserve in glycerine.

Fresh cartilages are not readily susceptible of colouring. Place them in absolute alcohol or in Müller's fluid, and then in alcohol, and lastly stain with hæmatoxylin. Mount in glycerine. Dammar illuminates too strongly, and causes the finer details to disappear.

50. Elastic Cartilage, Method No. 50, p. 330. Take a cartilage from the larynx of an ox. The yellowish colour of the *processus vocalis* shows elastic cartilage. Cut in such a manner that the boundary between elastic and hyaline cartilage falls in the section, and examine the sections in water. Preserve in glycerine. The development of elastic fibres may also be studied in the cartilages of adult persons, especially in that of the epiglottis and in the processus vocalis of the arytenoid cartilages. This kind of cartilage stains well with picrocarmine, the cells being stained red or pink and the elastic substance yellow. Mount stained sections in dammar.

51. Connective Tissue Cartilage, Method No. 51, p. 330. An intervertebral disc of an adult man is cut into portions of 1 to 2 cm. broad, fixed for twenty-four hours in 100 c.cm. of Kleinenberg's sulpho-picric acid, and hardened in 50 c.cm. of alcohols of increasing strengths. After three days' immersion in 90 per cent. alcohol, the bits are coloured strongly with borax carmine (p. 262), again hardened in alcohol and then cut. Preserve in dammar. Sections through parts of the margin also show hyaline cartilage; sections of the central parts of the disc show groups of cartilage cells.

52. Bone, Method No. 52, p. 332. Bones to be used for sections should not be dried previous to maceration, but must be immersed fresh for several months in water, which is several times changed. After they have been dried, a portion is fixed in a screw-vice between two pieces of cork or layers of cloth, and a transverse lamina, 1 to 2 mm. thick, is cut off with a thin leaf saw. The lamina is firmly attached, by means of sealing wax, to the lower surface of a cork stopper (the sealing wax must entirely cover the lamina), the whole is dipped one moment into water, and then filed quite even with a fine flat file; then, by heating the sealing wax, detach the lamina of bone and stick the smoothed side on to the stopper. The lamina is again operated upon by the file, until it has become so thin that the sealing wax shines through. Then lay the whole in 90 per cent. alcohol, where, in a few minutes, the lamina of bone is easily detached. Now take a coarse whetstone, moisten it with water; produce by means of rubbing with a second whetstone a little paste. Place the bone lamina in it, and rub it on both sides with a circular motion. When the lamina has attained the requisite thinness —the operator may satisfy himself as to this by drying it between filter paper and then inspecting it with a low power—smooth it on both sides upon a fine whetstone (the method is exactly similar to that of rubbing on the coarse whetstone); next dry it with filter paper and polish it on the sides. To do this, nail

a small portion of deerskin (wash-leather) smoothly upon a board, chalk the leather, and rub the lamina up and down with a little saliva on the tip of the finger. The dull lamina soon acquires a glittering upper surface. Finally, remove the adhering chalk by rubbing on fine wash-leather. The lamina is dried and placed under a cover-glass, which is then sealed round with cement.

53. Bone, see Method No. 52.

54. Haversian Canals and Bone Lamellæ. Make longitudinal and transverse sections through the bone, which has been decalcified according to the fixation and hardening method, in 3 to 9 per cent. nitric acid, and again hardened. For this purpose select a metacarpal bone of a growing individual. Compact portions of larger bones (*e.g.* the femur) require too long a time (several weeks) for decalcification. Let the periosteum remain on the bone. For longitudinal sections of the Haversian canals very thick sections (·5 mm. and more) are prepared, which must be preserved in dilute glycerine. For transverse sections and systems of lamellæ do not use very thin sections; the lamellæ are best perceived if we examine the section in a few drops of distilled water, and turn the mirror in such a manner that the object is only half illuminated. We then also perceive the delicate striæ caused by the small bone canals, which run perpendicularly to the lamellæ. Preserve in dilute glycerine, which, however, renders the lamellæ systems partly indistinct. It is not every part of the bone which displays entire systems of lamellæ; thus the outer and also the inner ground lamellæ are frequently absent. If sections are made near the epiphyses we perceive how the compact substance is continued into the small trabeculæ of the substantia spongiosa. We not seldom find that the concentric rings of the Haversian lamellæ are divided by an irregular line. The bone already formed has been again re-absorbed up to this line. All that lies within the line is newly added bone. These formations are called *Haversian spaces.*

55. Bone, Method No. 55, see Methods No. 52 and 54.

56. Bone Marrow. Procure from the slaughter house the vertebra (cut in halves) of a newly killed calf; scrape off with a scalpel the spongy bone substance, and take a little of the red bone marrow from the deeper part of the spongiosa. Take only a little marrow, as much as covers the point of the knife. Place a little in a drop of salt solution on a slide, stir round with a needle, and after a small piece of hair has been placed in the preparation, cover with a cover-glass.

Usually there lie in the preparation a few small bone trabeculæ of the spongiosa, which prevent the cover-glass from lying smoothly. The larger trabeculæ or spicules must be removed with a needle previous to covering the preparation. If we examine with a high power, we perceive over and above the small trabeculæ, fat cells and red blood corpuscles, marrow cells of various sizes and giant cells, but not, or only seldom, any nuclei. Now let a few drops of picrocarmine flow into the preparation; the nuclei become red after 1 to 2 minutes, but they are still pale. If we replace the picrocarmine, first by means of salt solution and next by dilute acidulated glycerine, the nuclei become dark, and sharply outlined. A hair placed below the cover-glass prevents many cells from floating away.

57. Sharpey's Fibres. Decalcify a hardened humerus, with periosteum adherent, of a new-born human subject, in 3 per cent. nitric acid (6 c.cm. of nitric acid in 200 of distilled water), and make *delicate* transverse sections through the diaphyses. These may be teazed in a drop of salt solution. With a high power we perceive in certain places the pale fibres standing out freely. The fibres may also be seen on the surface of unteazed sections. The fibres are very pale and

rise but little above the level of the section. One may also see, after some practice, intersected fibres on both longitudinal and transverse sections. Staining does not help much in identifying the fibres, as fibres and bones take the colour almost equally. Try picrocarmine and preserve in dilute (not acidulated) glycerine.

58. Ossification, Method No. 58, p. 338. For preparations showing bone development, human embryoes of four to five months, and embryoes of animals, sheep, sow, or ox, from 10 to 14 cm. long, are suitable. The latter can be without difficulty procured from slaughter houses. Procure the entire uterus, place all the embryoes (2 to 3 portions in 1 litre) for four weeks in Müller's fluid. Change frequently. Then wash for one hour under water tap, and harden them in 200 to 400 c.cm. of alcohol of increasing strengths. After the embryoes have lain one week or longer in 90 per cent. alcohol, cut off the head, and the extremities close to the trunk, and place them for decalcification in 200 c.cm. of distilled water, to which 2 to 4 c.cm. of pure nitric acid have been added. After two to five days, during which the decalcifying fluid has been changed about three times, the extremities are taken out (the head is not yet completely decalcified, and must remain a few days longer in 2 per cent. nitric acid), washed for one to two hours under water tap, and once more hardened in alcohol of increasing strengths. After they have been about five days in 90 per cent. alcohol, cut the extremities into portions 1 cm. long, and place these, if they should be still too soft, for one to two days in 30 c.cm. of absolute alcohol. For preparations showing the early stages of bone development, make longitudinal sections directed through the phalanges and metacarpal bones. Good sections should pass through the axes of the extremities; marginal sections are not so good. For the more advanced stages, make special transverse sections through the humerus and the femur. Sections through the diaphyses show perichondral, and sections through the epiphyses show enchondral, bone formation. The most beautiful *osteoblasts* are found in transverse sections of the lower jaw. This bone is also adapted for preparations illustrating development of tooth. For still later stages, small portions of the skeleton of newly born animals must be employed, the phalanges still exhibit early processes. Such bones require longer time for decalcification (eight days). For the formation of bone in connective tissue membrane, cut surface sections through the parietal and frontal human bones. The sections are placed for ten minutes in 4 c.cm. hæmatoxylin; transferred for ten minutes into 10 c.cm. of distilled water; next stained for 10 minutes in 4 c.cm. picrocarmine; then brought for a quarter of an hour into 20 c.cm. of distilled water, and lastly placed for preservation in absolute alcohol, lavender oil, and dammar. If the staining process has succeeded, cartilage will appear blue and bone red. Sometimes the cartilage may not have acquired the vivid blue tint. In that event, place the sections in 5 c.cm. of distilled water, to which have been added 5 drops of filtered solution of hæmatoxylin. The cartilage will become blue if left in this solution for six to fourteen hours. Picrocarmine staining is sometimes not very uniform in the case of bone.

59. Ossification. See Method No. 58.

60. Ossification. See Method No. 58.

61. Ossification. See Method No. 58.

62. Ossification. See Method No. 58.

63. Ossification. See Method No. 58.

64. Ossification. See Method No. 58.

65. Bundles of Transversely Striated Muscle, Method No. 65, p. 356.

Make with a sharp razor in a parallel-fibred muscle (*e.g.* an adductor of the rabbit) a deep incision directed transversely to the course of the fibres, and, at 2 to 3 cm. downwards from this, a second incision. Connect both by longitudinal sections, and prepare without tearing the portion so circumscribed. Fix in 100 c.cm. 1 per cent. solution of chromic acid. After the lapse of fourteen days, wash for two or three hours under water tap, and then harden in 50 c.cm. of alcohol of increasing strengths. Transverse sections are to be examined unstained in dilute glycerine. We perceive muscle fibres of different degrees of thickness. Although the muscle fibres are cylindrical, and consequently round when in transverse section, they here appear irregularly polygonal on account of reciprocal pressure. The colour of the transverse sections is very various, some being entirely dark, others quite clear. The perimysium of individual muscle fibres is better seen under high powers.

66. Muscle and Tendon, Method No. 66, p. 357. Remove from a newly killed frog the skin of the lower part of the thigh, cut off with a pair of scissors the bone above the knee joint (the origin of the gastrocnemius), and fix the lower part of the thigh and the foot in 50 c.cm. of Kleinenberg's sulpho-picric acid. After twenty-four hours, place it in 50 c.cm. of 70 per cent. alcohol, with a view to gradual hardening. After six days, cut off the gastrocnemius along with a portion of the tendo Achillis, place it for staining in borax carmine, and then harden repeatedly with 90 per cent. alcohol. In cutting (sagittal or longitudinal sections), place the razor first of all on the tendinous surface on the posterior surface of the muscle. Mount in dammar. The transverse striation on the muscle fibres often disappears.

67. Muscular Fibre of Intestine, Method No. 67, p. 358. Remove the stomach and duodenum of a cat about one hour after death, open both lengthways, remove the contents by means of gentle movement in salt solution, and immerse the pyloric portion and the upper half of the duodenum (a portion 5 to 6 cm. long) for three to six days, in 100 to 150 c.cm. of 0·5 per cent. solution of chromic acid. Make longitudinal sections, stain with hæmatoxylin, preserve in glycerine or dammar. (As to further details in treating sections of portions of intestinal canal, see Appendix, Vol. II.)

68. Motor Nerve Terminations, Method No. 68, p. 358. (*a*) *End Plates.* Cut out a thin portion, 1 cm. long, of the muscles of a lizard or the short muscles (intercostal or ocular muscles) of small quadrupeds. Prepare a mixture of chloride of gold and formic acid, boil it and let it cool. Place the bit of muscle in it for one hour in the dark. Transfer the small portions at the end of that time into 10 c.cm. of distilled water, and after a few minutes into a fresh portion of water to which a few drops of formic acid have been added, then expose to daylight (not sunlight) for twenty-four to forty-eight hours. The portions of muscle will now have a dark violet tint. Then transfer them for hardening into 30 c.cm. of alcohol of increasing strengths. After the dark violet portions of muscle have lain three to six days in alcohol, teaze the bundles, 2 mm. thick, of muscle fibres in a drop of dilute glycerine, to which a small drop of formic acid has been added. On account of the brittleness of the muscle fibres this process may not be successful. A light pressure on the cover-glass is often of advantage. Low powers show muscle fibres from a delicate rose-red hue up to a deep purple tint, others again of red-violet up to light blue-violet; in the latter we may notice the end plates most distinctly. In order to find these out, follow the deep black nerve fibres already discernible with low powers. (*b*) *Nuclei of the Motor Plate.* Place the

anterior halves of the muscles of the eye of a freshly killed rabbit in 97 c.cm. of distilled water + 3 c.cm. of acetic acid. After six hours transfer the muscles into distilled water, cut off a flat small piece with the scissors and spread it out on a slide. We may now perceive with the naked eye the ramifications of the white looking nerve; with low power (50 d.) we notice the anastamoses of the nerve fibres. The blood-vessels are also easily discernible by their transverse nuclei (belonging to the smooth muscle fibres in their walls). The discovery of the end-plates is not easy on account of the great number of sharply outlined nuclei, which belong to the muscles, the intermuscular connective tissue, etc. Follow a nerve fibre and one may notice that its medullated sheath suddenly ceases and is apparently lost in a group of nuclei. These are the nuclei of the motor end-plate.

69. END-PLATE, Method No. 69, p. 358. See Method No. 68.

APPENDIX II.

CHEMISTRY OF MUSCLE.

The account of the chemical composition of muscle given at p. 360 is founded chiefly on the researches of Kühne on the plasma obtained from the muscles of frogs. Dr. W. D. Halliburton, in an elaborate research, has investigated the chemical composition of the muscles of warm-blooded animals[1] (rabbit, horse), and he has largely extended our knowledge of the subject. He succeeded in obtaining plasma from the muscles of the rabbit by washing out the blood from the vessels by a stream of cold salt solution (·6 per cent. salt solution at 5° C.), then placing pieces of the muscles in a freezing mixture of ice and salt having a temperature of −12° C., and then subjecting frozen slices to pressure. A yellowish, somewhat viscid fluid, of a faintly alkaline reaction, was thus obtained, which "set into a solid jelly in the course of from one to two hours; at a temperature of 40° C. coagulation takes considerably less time, viz., twenty to thirty minutes: simultaneously an acid reaction is developed." Solutions of neutral salts prevent coagulation of the plasma. Dilution of "salted muscle plasma causes coagulation. Saline extracts of rigid muscle differ from salted muscle plasma in being acid, but resemble it very closely in the way in which myosin can be made to separate from it; myosin, in fact, undergoes a recoagulation." The separation of the plasma into clot and "salted" muscle serum "does not take place at 0° C.; it occurs most readily at the temperature of the body, and is hastened by the addition of a ferment prepared from muscle in the same way as Schmidt's ferment is prepared from blood."[2]

According to Dr. Halliburton, the proteids of muscle plasma are—

1. Paramyosinogen, coagulated by heat at 47° C.
2. Myosinogen, coagulated at 56° C.
3. Myoglobulin, differing from serum globulin in its coagulation temperature (63° C.).
4. Albumin, apparently identical with serum albumin α, coagulating at 73° C.

[1] W. D. Halliburton, M.D., B.Sc., Assist. Professor of Physiology, University College, London: "On Muscle Plasma," *Journal of Physiology*, vol. viii. No. 3.

[2] Halliburton, "On Muscle Plasma," *Proceedings of Royal Society*, vol. xlii.

5. Myo-albumose, having the properties of deutero-albumose, and identical with, or closely related to, the myosin ferment.

The two first bodies form myosin clot and the other three remain in the muscle serum. The three first bodies are globulins, "completely precipitated by saturation with magnesium sulphate or sodium chloride, or by dialyzing out the salts from their solutions." Dr. Halliburton has also made the remarkable observation that " when muscle turns acid (by the formation of lactic acid), as it does during *rigor mortis*, the pepsin which it contains is enabled to act, and at a suitable temperature (35° to 40° C.) albumoses and peptones are formed by a process of self-digestion. It is possible that the passing off of *rigor mortis*, which is apparently due to the reconversion of myosin into myosinogen, may be the first stage in the self-digestion of muscle."

Dr. Halliburton has constructed the following table showing how these proteids may be separated from muscle plasma, which he has given me his kind permission to copy—

SALTED MUSCLE PLASMA.

Dilute to six times its volume, and expose this to a temperature of 35° C. for one or two hours. It separates into clot and salted muscle serum. Filter.

Clot : consists of MYOSIN. Wash with water, redissolve in 5 per cent. magnesium sulphate solution. Heat to 47° C. A precipitate is produced. Filter.	*Salted muscle serum* : contains myoglobulin, albumin, and myo-albumose. Saturate with magnesium sulphate or sodium chloride. A precipitate is produced. Filter.

Precipitate : consists of PARAMYOSINOGEN.	*Filtrate* : contains MYOSINOGEN, which is precipitated at 56° C.	*Precipitate*: consists of MYOGLOBULIN.	*Filtrate* : contains albumin and myo-albumose. Heat to 73° C. A precipitate is produced. Filter.

Precipitate: ALBUMIN.	*Filtrate*: contains MYO-ALBUMOSE.

As regards the muscle-ferment, Dr. Halliburton's conclusions are as follows :—

"1. By keeping muscle under alcohol for some months, most of the proteids are coagulated. Water will, however, extract from the alcoholic precipitate a proteid which has the characters of an albumose.

"2. This albumose has the properties of a ferment in causing the coagulation of muscle plasma, or it may be that the ferment is in very close combination with the albumose.

"3. This myosin ferment, as it may be termed, does not hasten the coagulation of blood plasma ; nor does fibrin ferment hasten the coagulation of muscle plasma ; the two are therefore not identical.

"4. The juice expressed from muscle, however, hastens very markedly the coagulation of salted muscle plasma. This is not due to its containing fibrin

ferment, but it is due to the proteid substance myosinogen, which enters into the condition of heat coagulum at 56° C. Fibrin ferment is absent, or only present in exceedingly small quantities.

"5. The activity of fibrin ferment is destroyed at 75° to 80° C. ; the activity of myosin ferment is not destroyed till the temperature of 100° C. is reached."[1]

APPENDIX III.

LATENT PERIOD OF MUSCLE.

Professors Gerald F. Yeo (King's College, London) and Theodore Cash (Aberdeen), after an elaborate research on the muscles both of cold-blooded and of warm-blooded animals, in which they made use of the pendulum myograph, have arrived at the following conclusions—[2]

"I. Increase in the *strength* of the *stimulus* is accompanied by (α) a steady and gradual shortening of the latent period ; (β) a sudden prolongation of the actual contraction when a certain degree of stimulation is reached ; (γ) an elevation of the altitude of the curve with the early and the final parts of the increase ; (δ) and a removal of the summit to a later part of the curve as soon as the elongation of the curve is established.

"II. Increase in the *weight* used as a burden for the muscle is accompanied by (α) elongation of the latent period ; (β) commonly a slight shortening of the duration of the contraction ; (γ) depression of the height of the curve ; and (δ) no marked change in the position of the summit except in extreme cases.

"III. The application of *heat* causes (α) very marked and continuous shortening of the latent period ; (β) a gradual and distinct increase in the height of the curve ; and (γ) a more rapid arrival at the summit, followed by a sudden fall of the lever, which usually passes considerably below the abscissa. (Extreme warmth has, however, an opposite effect ; when above 90° F., the altitude gets lower and the muscle remains contracted.)

"IV. Cooling causes (α) the latent period rapidly to increase ; (β) a great increase in the duration of the contraction ; (γ) at first a slight elevation in the altitude (extreme cold, however, lowers it) ; (δ) the initial part of the curve is flattened, and the summit is delayed until a later period.

"V. Gentle *activity* seems to increase the rate and power of contraction. Very weak interrupted currents have an effect like that produced by gentle heat. If extreme *fatigue* be induced, (α) the latent period becomes much longer ; (β) the duration of the contraction is increased ; (γ) the height of the curve is considerably lessened ; (δ) and its summit is moved away from the beginning of the contraction towards the end of the curve."

[1] *Journal of Physiology*, vol. viii. No. 3, p. 182.

[2] Yeo and Cash—On the relation between the active phases of contraction and the latent period of skeletal muscle. *Journal of Physiology*, vol. iv. No. 2.

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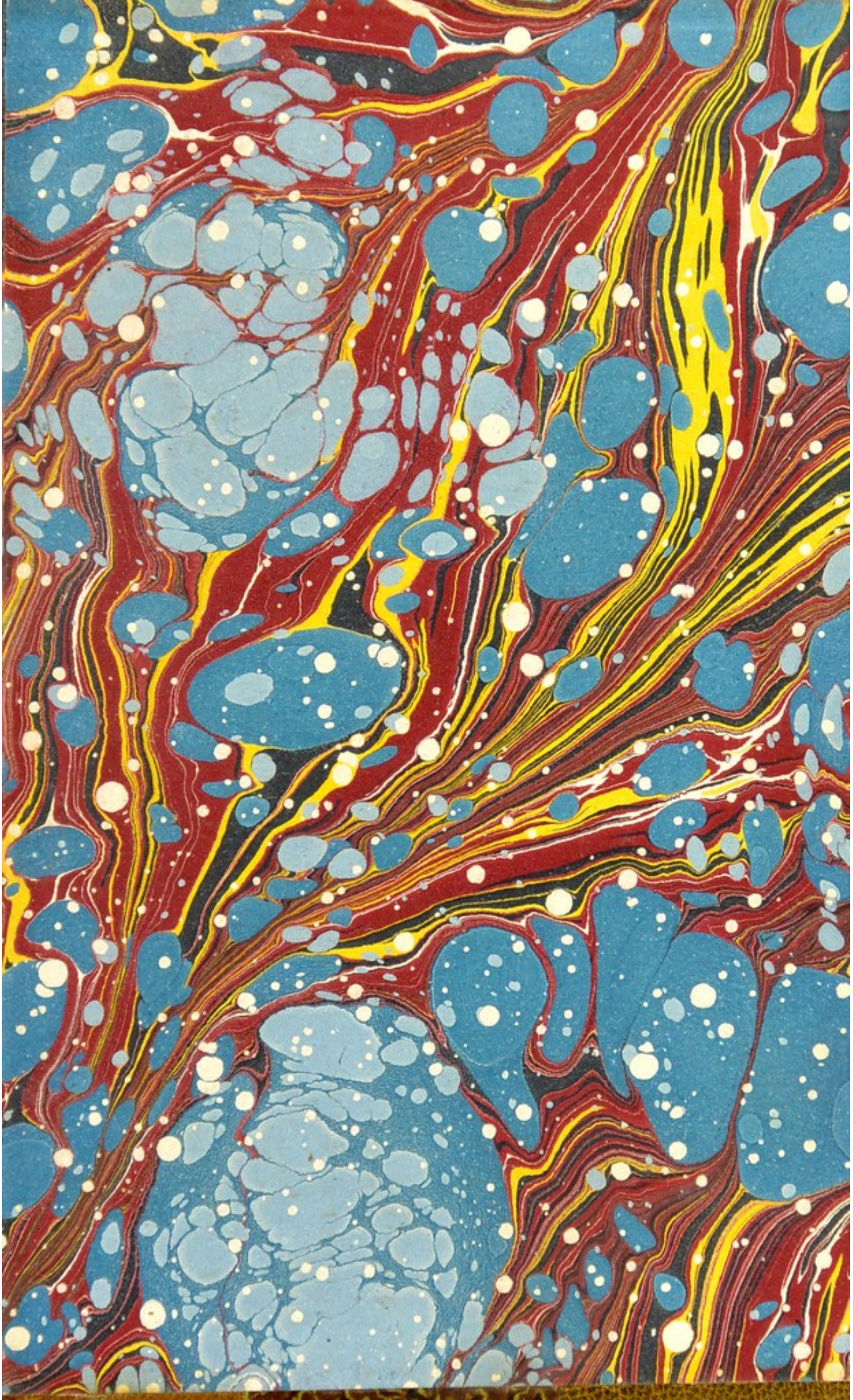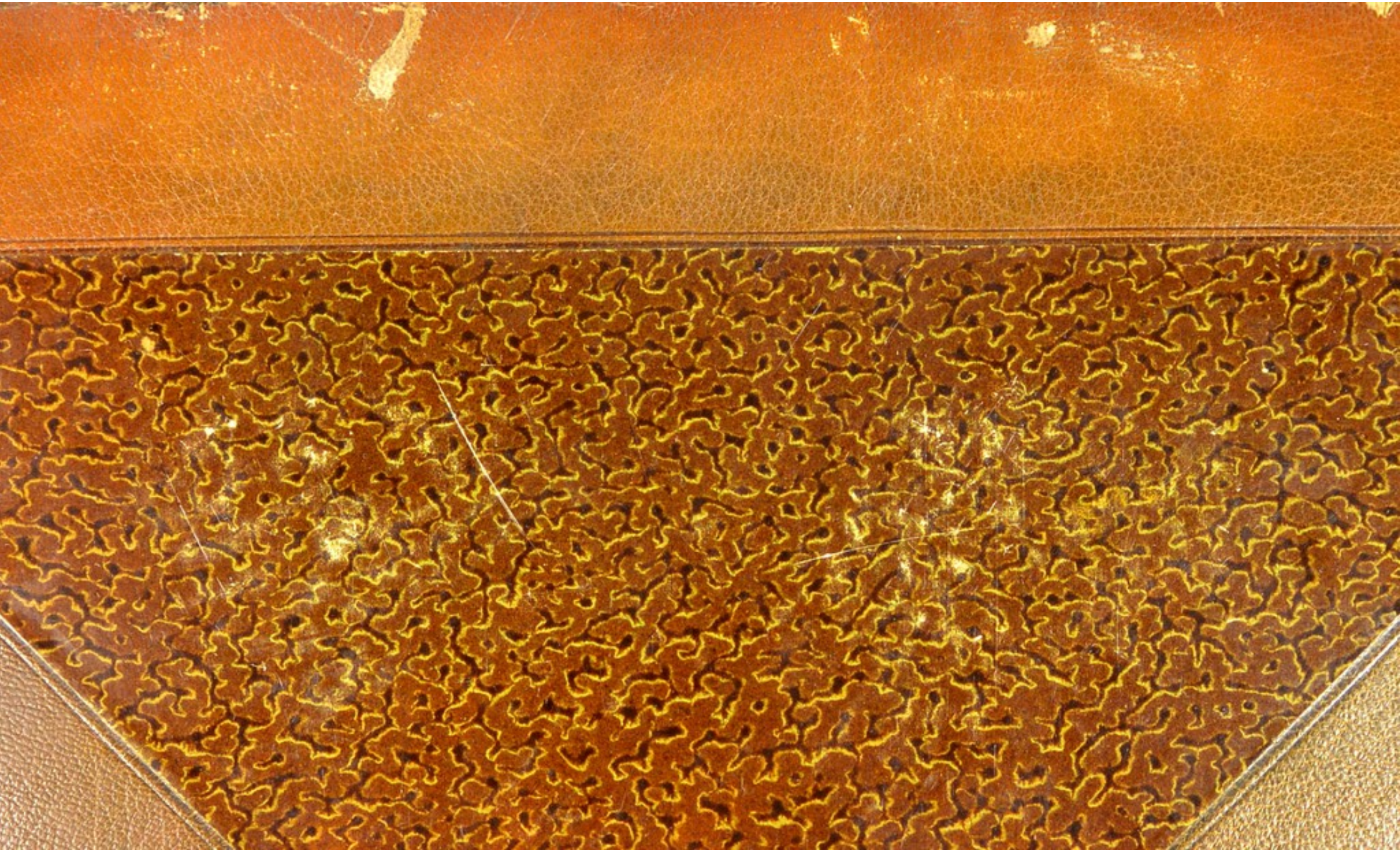
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