PRACTICAL PHYSIOLOGY

PRACTICAL PHYSIOLOGY

BY

A. P. BEDDARD, M.A., M.D.
Assistant Physician, late Demonstrator of Physiology, Guy's Hospital

LEONARD HILL, M.B., F.R.S.
Lecturer on Physiology, The London Hospital

J. S. EDKINS, M.A., M.B.
Lecturer on Physiology, St. Bartholomew's Hospital

J. J. R. MACLEOD, M.B.
Professor of Physiology, Western Reserve University, Cleveland, U.S.A.

AND

M. S. PEMBREY, M.A., M.D.
Lecturer on Physiology, Guy's Hospital

ILLUSTRATED BY NUMEROUS DIAGRAMS AND TRACINGS

SECOND EDITION

LONDON
EDWARD ARNOLD
41 AND 43 MADDOX STREET, BOND STREET, W.
1905

PREFACE TO THE SECOND EDITION.

In the present edition considerable changes have been made in those portions of the work which deal with Physiological Chemistry. The new exercises have involved a slight increase in the total number of pages, and several new figures have been added.

July, 1905.

ERRATA.

Page 162, line 21, *for* "cupric hydrate" *read* "cupric sulphate."

,, 163, ,, 21, ,, ,, ,, ,,

,, 163, ,, 22, ,, ,, ,, ,,

,, 230, ,, 34, *for* "$C_5H_9(NH_2)COOH$" *read* "$C_5H_9(NH_2)_2COOH$."

,, 230, ,, 37, *for* "cystin" *read* "cystein."

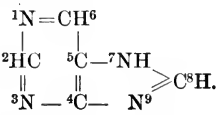
,, 231, ,, 4, ,, ,, ,,

,, 231, under formula, ,, ,, ,,

,, 236, line 30, *for* "invertine" *read* "invertase."

,, 250, ,, 2 of footnote, *for* "hypobromide" *read* "hypobromite."

,, 255, formula for Purin should be



Page 400, line 17, *for* "only about 0·2" *read* "that of the."

,, 424, ,, 31, *for* "glycogen with sugar" *read* "glycogen into sugar."

PREFACE TO THE FIRST EDITION.

PHYSIOLOGY is the basis of medicine, and the further advance of these sciences depends mainly upon the "experimental method." The medical student, the future physician, should undergo a training in practical physiology, for thereby he learns the most important of all lessons; he learns to observe, to draw conclusions from his observations, and to unravel the causes of his failures.

The importance of practical physiology is undoubted, but as to the nature and scope of the experimental work, which is most suitable for the medical student, there is considerable difference of opinion among teachers of physiology. In this country, perhaps, too much stress has been laid upon the physiology of muscle and nerve; for the hope that a study of the properties of these tissues will unfold the enigma of life is likely ever to remain without consummation.

An advance in the knowledge of the living organism as a whole, one organ reacting upon another, has been gained by experiments upon the living animal, treated as a unit and not as a collection of separate organs and tissues. Such practical physiology needs extension in the courses of instruction given to students. It should, as far as possible, have a direct relation to medicine.

The methods which are used in the investigation of the respiratory system, the circulation, the body heat, the nervous system and special senses; the chemistry of the blood, of digestion, and of urine—these are the subjects which are especially required by the clinician. These subjects, moreover, afford as excellent a mental training as the study of muscle and nerve.

In the present work the authors have attempted to give some extension to practical physiology along the lines just indicated.

The book has been divided into an elementary and an advanced portion. Part I. treats of elementary experimental physiology (the physiology of muscle and nerve, circulation, respiration, animal heat,

the central nervous system, and the special senses); Part II. of elementary physiological chemistry; Part III. of advanced experimental physiology; and Part IV. of advanced physiological chemistry.

The experiments upon the physiology of muscle and nerve are based upon the course given at Guy's Hospital—a course modelled on a reduced scale upon the excellent practical courses given at Oxford by Professor Burdon Sanderson and Professor Gotch. The experiments in this section have been limited as far as possible to those which can be conveniently performed with simple apparatus by a large class of students. For this reason the experiments with the galvanometer and capillary electrometer have been restricted to demonstrations, and very few details of such experiments are given.

There are some important experiments upon the circulation and respiration, which for various reasons cannot be properly performed by the student; these have been collected together as demonstrations in Parts I. and III.

The subject of vision is so important from a medical as well as a physiological and psychological point of view, that it has here received more extensive treatment than is usually the case in works on practical physiology.

In those portions of the book which treat of physiological chemistry, an attempt has been made to demonstrate, step by step, the chemical relationships which exist between the various substances, and to illustrate, by suitable experiments, the different properties of those bodies. The drawings of crystals were executed by Mr. W. R. M. Turtle, to whom the authors are deeply indebted.

Figures have been borrowed from *The Physiological Action of Drugs*, by M. S. Pembrey and C. D. F. Phillips. For the loan of numerous blocks illustrating physiological apparatus the authors are indebted to Messrs. Baird & Tatlock, of Hatton Garden, E.C. The sources of other diagrams and tracings, which have been borrowed, are indicated in the description of the figures. The initials of the author, who took the record of the original tracings, are appended to the respective curves.

Sept., 1902.

CONTENTS.

PART I.

MUSCLE AND NERVE. CIRCULATION. RESPIRATION. ANIMAL HEAT. CENTRAL NERVOUS SYSTEM AND SPECIAL SENSES. (ELEMENTARY COURSE.)

PART II.

PHYSIOLOGICAL CHEMISTRY.

ELEMENTARY COURSE.

PART III.

MUSCLE AND NERVE. CIRCULATION. RESPIRATION. ANIMAL HEAT. CENTRAL NERVOUS SYSTEM AND SPECIAL SENSES. (ADVANCED COURSE.)

PART IV.

PHYSIOLOGICAL CHEMISTRY.

ADVANCED COURSE.

APPENDIX.

NOTE.—The tracings should be read from left to right, unless it is especially stated to the contrary.

WEIGHTS AND MEASURES.

Length.

Metric or Decimal.	English.
1 **Metre** (M.)	= 39·3701 inches.
1 Decimetre (dm.)	= 3·9370 ,,
1 Centimetre (cm.)	= 0·3937 ,,
1 Millimetre (mm.)	= 0·0393 ,,
1 Micromillimetre (mkm)	= 0·000039 ,,

The unit of the Metric System is the Metre, which represents one ten-millionth part of a quarter of the meridian of the earth. The multiples and subdivisions are obtained by the use of decimals; the former being designated by Greek prefixes, the latter by Latin prefixes.

1 Myriametre (Mm.)	= 6·2137 miles.
1 Kilometre (Km.)	= 0·6214 ,,
1 Hectometre (Hm.)	= 109·361 yards.
1 Dekametre (Dm.)	= 32·8084 feet.
1 Metre (M.)	= 39·3701 inches.

Weight.

Metric or Decimal.	English.
1 Kilogramme (Kgm.)	= 2·2046 pounds.
1 **Gramme** (Gm.)	= 15·4323 grains.
1 Decigramme (dgm.)	= 1·5432 ,,
1 Centigramme (cgm.)	= 0·1543 ,,
1 Milligramme (mgm.)	= 0·0154 ,,

The unit is the Gramme which represents the weight of a cubic centimetre of water at 4° C.

Apothecaries Weight.

437·5 grains (gr.)	= 1 ounce.
16 ounces (℥)	= 1 pound (lb.).
60 grains	= 1 drachm (ʒ).
20 grains	= 1 scruple (℈).
1 grain	= 0·0648 gramme.

Avoirdupois Weight.

16 drachms	= 1 ounce (oz.).
16 oz.	= 1 pound (lb.).
28 lbs.	= 1 quarter (qr.).
4 quarters	= 1 hundredweight (cwt.).
20 cwt.	= 1 ton.
1 pound	= 453·592 grammes.
1 ounce	= 28·35 grammes.

Capacity.

Metric or Decimal.	English.
1 Dekalitre (Dl.) - -	= 2·1998 Imperial gallons.
1 Litre (L.) - - -	=35·196 Imperial fluid ounces.
1 Decilitre (dl.) - - -	= 3·5196 ,, ,,
1 Cubic centimetre (c.c.) or 1 Millilitre (ml.) -	= 0·0352 ,, ,,

---

60 minims (♏) - - -	=1 fluid drachm (ʒ).
8 fluid drachms - - -	=1 fluid ounce (ȝ).
20 fluid ounces - - -	=1 pint (O).
8 pints - - - - -	=1 gallon (C).

---

1 cubic centimetre - -	= 16·9 minims.
1 fluid ounce - - -	= 28·42 cubic centimetres.
1 pint - - - - -	=568·34 cubic centimetres.
1 gallon - - - -	= 4·54 litres.

THERMOMETERS.

Fahrenheit and Centigrade Scales.

To convert degrees F. into degrees C., deduct 32, multiply by 5, and divide by 9.

To convert degrees C. into degrees F., multiply by 9, divide by 5, and add 32.

F.	C.	F.	C.
212°	100°0	80°	26°7
112	44·4	70	21·1
106	41·1	60	15·6
104	40·0	50	10·0
102	38·9	41	5·0
101	38·3	32	0·0
100	37·8	23	− 5·0
99	37·2	14	−10·0
98	36·7	5	−15·0
97	36·1		

AVERAGE WEIGHTS AND HEIGHTS.

Average weight of a healthy male child at birth - - -	= 6·8 lbs.
,, ,, ,, six months' old -	=12·4 ,,
,, ,, ,, twelve ,, -	=18·8 ,,

An adult man (dressed) 5 feet 8 inches in height, should weigh 11 st. 1 lb. and should have a chest circumference of 38½ inches.

PART I.

MUSCLE AND NERVE. CIRCULATION. RESPIRATION. ANIMAL HEAT. CENTRAL NERVOUS SYSTEM AND SPECIAL SENSES.

THE PHYSIOLOGY OF MUSCLE AND NERVE.

Introduction.—Physiology, the study of the properties of living organisms, can be properly appreciated and learned only when it is approached from the practical and experimental side. The study of the simplest forms of life, the unicellular organisms, is as yet only in its infancy, and at the present moment experimental physiology deals almost entirely with the functions of the various tissues and organs which together make up a vertebrate animal.

The cold-blooded vertebrate, such as the frog, is the most suitable animal for elementary experiments; it is cheap, readily obtained, and, above all, its tissues under suitable conditions retain their vitality for many hours after they have been excised and cut off from their supply of blood. Moreover, the law relating to experiments on animals practically limits the experiments, which can be properly performed by a medical student, to observations upon frogs.

The muscular, nervous, and vascular systems of the frog are the most important in an experimental course of physiology, for although muscle and nerve are highly differentiated forms of protoplasm with correspondingly characteristic functions, yet they show only in an exaggerated way properties which are common to all living matter. Thus in muscle the power of contraction or movement is highly developed; in nerve the property of excitability or irritability, the response to a stimulus.

CHAPTER I.

ELECTRICAL APPARATUS FOR PHYSIOLOGICAL EXPERIMENTS.

In experimental physiology the stimulus most frequently used is an electrical one, for it is convenient, easily graduated, and less injurious to tissues than efficient thermal, chemical, or mechanical stimuli would be.

The **Daniell Cell**, which has an electromotive force (E.M.F.) of 1·1 volts, is the best source of electricity, for it yields an almost constant strength of current. It consists (Fig. 1) of (i) a plate of copper dipping into a solution of copper sulphate which is kept saturated by crystals of the salt, and (ii) a rod of amalgamated zinc placed in a porous pot filled with a 10 per cent. solution of sulphuric acid; the porous pot is surrounded by the solution of copper sulphate. The whole is generally placed for convenience in a glazed earthenware pot with a handle.

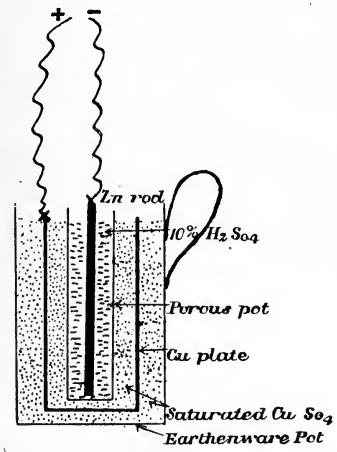


FIG. 1.—Diagram of a Daniell cell seen in section.

When the copper and zinc elements are connected by a wire the zinc dissolves in the sulphuric acid, forming $ZnSO_4 + H_2$. The H ions thus liberated become charged with the electricity originally stored in the zinc; they migrate through the porous cell into the copper sulphate and split it up into $H_2SO_4 + Cu$, and their charge of electricity is transferred to the Cu ions. These in turn deliver up their charge of electricity to the copper plate and, as they discharge, become deposited on the plate as metallic copper.

Thus inside the cell electricity passes from the zinc, or positive

element, to the copper or negative element; outside the cell the current passes from the copper binding-screw, the positive pole or anode of the battery, to the zinc binding-screw, the negative pole or kathode.

If plates of copper and zinc were simply immersed in 10 per cent. sulphuric acid, the chemical action set up would soon cause the copper plate to be covered with bubbles of hydrogen gas. This would cause a resistance to the flow of current inside the cell, and further, hydrogen being electro-positive to zinc, a polarisation current in the opposite direction to the original battery current would be set up in the cell and rapidly reduce its E.M.F. Daniell, by placing the copper plate in a solution of copper sulphate, which the hydrogen splits up, prevented polarisation from taking place within the battery.[1] Therefore as long as there is free sulphuric acid present and the copper sulphate is saturated, the current produced by the cell remains constant. Provided too that the porous pot, which is to prevent the deposition of copper on the zinc rod, remains permeable to the H ions.

The zinc rod has to be amalgamated because commercial zinc contains iron and other metallic impurities; these in the presence of the sulphuric acid would, with the zinc, constitute a number of minute batteries. By covering the impurities with zinc amalgam their disturbing action is removed, and as the zinc is dissolved away, the mercury combines with fresh zinc so that the electromotive properties of the zinc rod remain constant.

[1] A more accurate description of the chemistry of a Daniell cell is as follows: The cell consists of two metals, zinc and copper, dipping into an electrolyte containing various ions in solution; these are H, SO_4, OH, Cu and SO_4, of which Cu and H, being positive ions, will work their way towards the negative element, the copper plate and the OH and SO_4 being negative ions towards the zinc. When in use chemical changes take place around both metallic plates. The zinc is attacked by the SO_4 ions discharging, forming $ZnSO_4$, and energy is liberated, which is conducted across the electrolyte by the ions in solution. Around the copper plate the copper sulphate is being split up into SO_4 and Cu ions, in which process energy is stored up. But the energy liberated at the zinc plate is greater than that stored in the neighbourhood of the copper plate, therefore the cell, when working, is always liberating a balance of energy which appears as an electric current. The SO_4 ions, constantly being liberated in the copper sulphate solution and charged with electricity, migrate through the porous pot towards the zinc, discharge forming $ZnSO_4$ and a liberation of energy as explained. Towards the copper plate both H and Cu ions charged with electricity are constantly streaming. That it is the Cu ions and not the H ions which discharge and become precipitated on the plate depends simply upon the fact that it requires a less energy and a lower E.M.F. to separate Cu than H ions. Therefore as long as there are sufficient Cu ions present to conduct the current, Cu ions and not H ions will discharge and be precipitated on the copper plate.

Keys are instruments for making or breaking electrical circuits and for short-circuiting currents.

The **Mercury Key** consists of a small cup hollowed out of a piece of vulcanite (Fig. 2). From the cup, which is nearly filled with clean mercury. pass in opposite directions two stout copper wires with

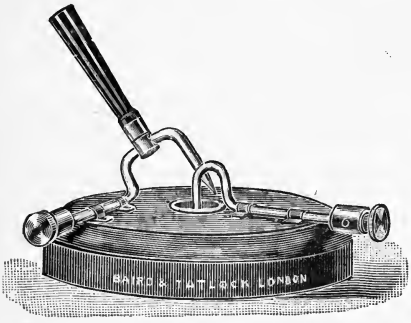


FIG. 2.—The mercury key.

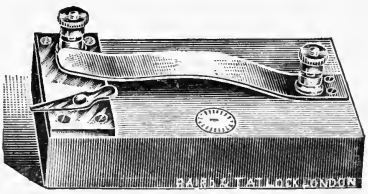


FIG. 3.—The spring key.

binding-screws; one wire and binding-screw are fixed to the vulcanite base, the other wire can be raised out of or lowered into the mercury by an insulated handle. In some forms of mercury key the wires connecting the binding-screws to the mercury cup run through the vulcanite; the ends of these wires are liable to become oxidised and dirty, and in consequence they make bad contact with the mercury. In order to avoid this it is only necessary to fix the insulated wires from the battery to the binding-screws and to turn the naked ends of these wires over into the mercury.

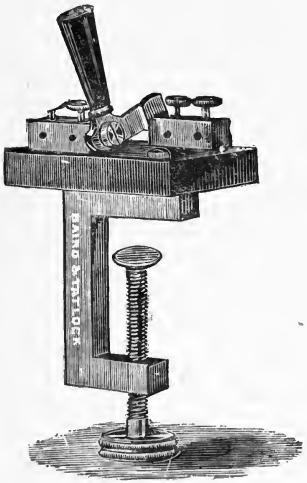


FIG. 4.—The Du Bois key.

The **Spring Key** is made of a block of lacquered wood, to one end of which is attached a broad brass spring with a binding-screw, and to the other end a plate of brass with a binding-screw (Fig. 3). When the spring is depressed by the finger its free end touches the brass plate and connects together the two binding-screws. The brass plate carries a clip which can clamp the spring in contact with the plate.

The **Du Bois Key** consists of two metal blocks each carrying two binding-screws and attached to a vulcanite base (Fig. 4). The

metal blocks can be connected by a thick brass bar attached to an insulated movable handle. This key, like the mercury and spring key, may be used as a simple make and break key (Fig. 5); but its

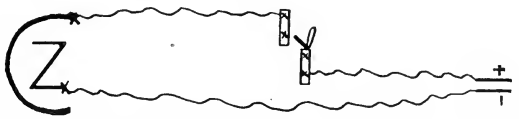


FIG. 5.—Plan of the use of a Du Bois key, as a simple make and break key.

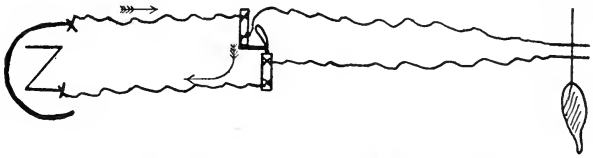


FIG. 6.—Arranged as a short-circuiting key: key shut.

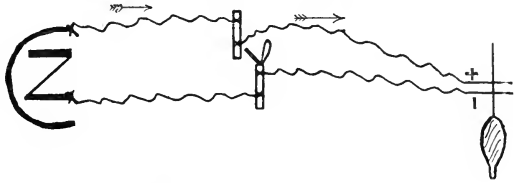


FIG. 7.—Arranged as a short-circuiting key: key open.

proper use is as a short-circuiting key (Figs. 6 and 7); and when a Du Bois key is directed to be used, it must be inserted into the circuit as a short-circuiting and not as a simple key.

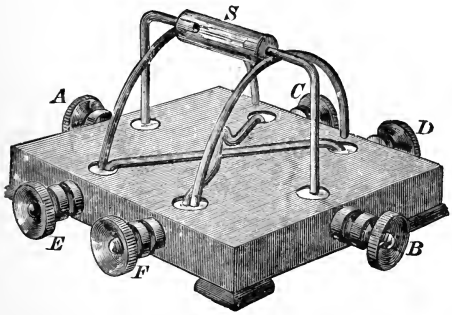


FIG. 8A.—The Pohl's reverser. *A* and *B* the two side cups; *C*, *D*, *E* and *F* the four corner cups; *S* the handle made of glass or vulcanite.

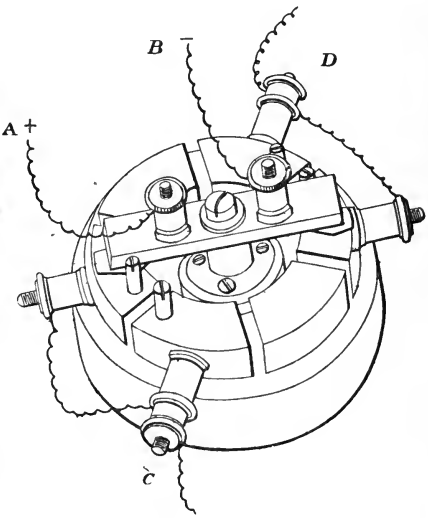


FIG. 8B.—Universal key (Gotch). The key is used by rotating the arm containing the screws connected with the wires *A* and *B*, which come from the battery. In the position shown the current flows from the wire of *C* to that of *D*; if rotated through 45° there is a complete double break of the battery-circuit; if rotated through 90° then the current is remade and the current flows from the wire of *D* to that of *C*.

The **Pohl's Reverser** consists of six mercury cups hollowed out in a block of vulcanite, each cup being connected to a binding-screw (Fig. 8A). The four corner cups are connected diagonally by stout copper wires which do not touch each other. The two side cups are joined by stout copper wires to a non-conducting cross-piece, which acts as a handle. Each end of the handle also carries a semicircle of copper wire which is connected to the wire going into the side cup, and is of such a length that it will dip into the cup at either end by turning the handle over towards that end. If the handle is in such a position that a current, entering the reverser by one of the side cups, emerges by an end cup of the same side, then, by turning the handle over, the cross-wires come into use, and the current will now emerge by the end cup of the opposite side.

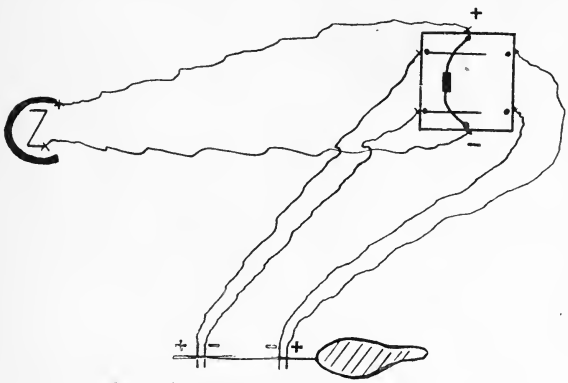
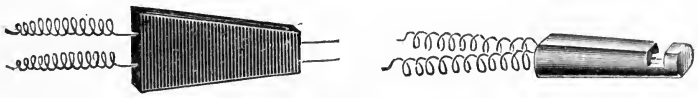


FIG. 9.—Plan of the arrangement of the two alternative circuits.

The instrument may also be used to send a current into either of two circuits. The cross-wires are removed, the wires from the battery are connected to the two side binding-screws, and to each pair of end cups the wires of the two alternative circuits (Fig. 9). Then by turning the handle over the current may be sent into either of these two circuits.

A much more efficient instrument is the universal key (Fig. 8B), which has recently been introduced by Gotch. It can be used as a double break-key, a reverser and a shunt.

The term **Electrodes** is applied to the free ends of the two wires which conduct the current to the tissue to be stimulated. They consist



FIGS. 10 AND 11.—Two forms of electrodes.

of two insulated wires, the ends of which are clean and free from insulating material, carried in some form of holder; this is generally made by running the wires through a piece of vulcanite, cork, or model-

ling wax (Figs. 10 and 11). A form of electrode sometimes very useful is made by soldering the free end of each wire to the head of a needle.

The **Rheochord** is used to alter the strength of a constant current to be sent through a muscle or nerve. In its simplest form it consists of

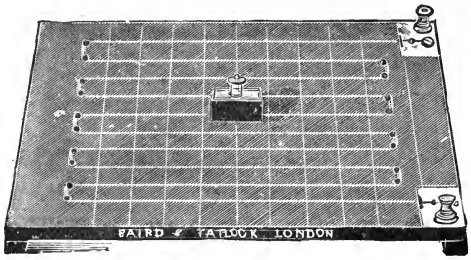


FIG. 12.—Simple form of monochord.

a single straight or zig-zagged wire with a binding-screw at either end and a movable contact between them (Fig. 12). If a Daniell cell be

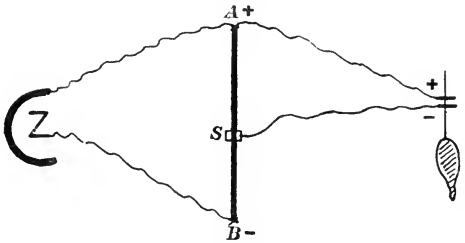


FIG. 13.—To illustrate the principle of the monochord.

connected to the two ends of the monochord A and B (Fig. 13), there will be a fall of potential in it from A to B. If from A and the

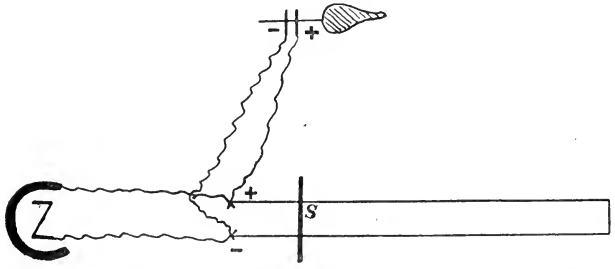


FIG. 14.—The rheochord arranged to vary the strength of a current passing through a nerve. It consists of two parallel wires connected by a movable metal slider S. By moving the slider S to the right the resistance of the rheochord in circuit and therefore the amount of battery current passing through the nerve would be increased.

movable contact S two electrodes pass to a nerve, the current from the battery has two circuits open to it and can pass either through the nerve or along the monochord back to the battery. The

amount of current which will pass through the nerve will be directly proportional to the difference in potential between A and S, *i.e.* if the fall in potential in the monochord is uniform, proportional to the distance between A and S, being greater as S is moved away from A; it is also inversely proportional to the resistance of the circuit through the nerve. But the resistance of this circuit may be considered constant for all positions of S, since the resistance in the nerve itself is enormously greater than that caused by any change in the length of the monochord wire in the circuit.

Although the Daniell cell is the most convenient source of current, and its strength can be regulated by a rheochord, and although the

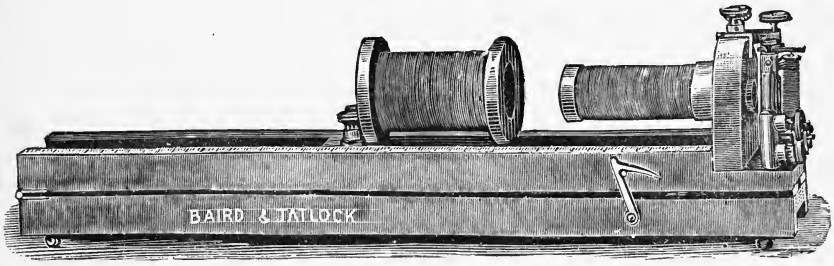


Fig. 15.—The induction-coil.

make and break of a constant current do act as a stimulus to muscle and nerve, it is often more convenient to use induced currents. These are obtained by connecting a Daniell cell to an induction coil, and their advantages are: (1) That being of extremely brief duration as compared with the make of a constant current, they set up practically no polarisation in the tissues (see page 312). (2) Having a comparatively large E.M.F. and rapid development, as compared with the galvanic current, they constitute a much more effective stimulus. For, the law of excitation states that the effectiveness of a current as a stimulus depends not only upon the total variation in its intensity, but also upon the amount of such variation in the unit of time, *i.e.* the greater the rapidity of the total variation, the more effective is the current as a stimulus.

The **Induction-coil** (Fig. 15) consists of two coils, of which the primary is made up of a few turns of insulated thick copper wire with only a small resistance. This is wound round a core of iron wire to increase the number of lines of magnetic induction which pass through it. The ends of the wire forming the primary coil are connected with the top binding-screws 1 and 2 (Fig. 16).

The secondary coil is made up of a large number of turns of insulated

fine copper wire. The large number of turns of wire in the secondary as compared with the primary coil, transforms the low E.M.F. of the current in the primary circuit into a high E.M.F. in the secondary circuit; for each turn in the primary coil induces an effect in every turn of the secondary coil, so that the sum of all these effects is a single one of greatly increased intensity.

The long fine wire of the secondary coil gives it a great resistance, but when the induced currents are passed through the relatively enormous resistances of animal tissues this is unimportant.[1]

The ends of the wire of the secondary coil are connected to the binding-screws 3 and 4 (Fig. 16).

The E.M.F. of the induced current varies with the following factors: (1) It varies directly with the intensity of the change of current in the primary circuit, so that if no current or a current of constant strength be running through the primary coil no induction occurs; but if the strength of the current in the primary circuit does change, whether it be an increase or decrease, the greater the change the stronger will be the induction. (2) It varies directly as the rate of change in the

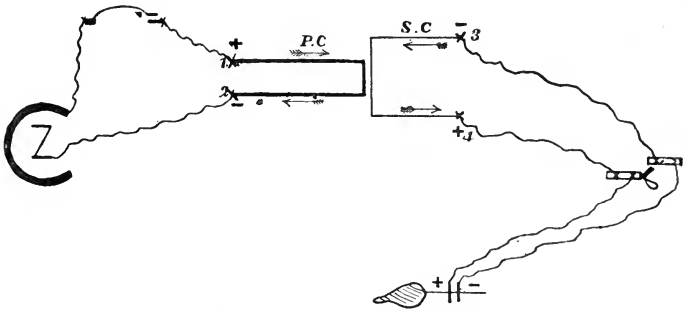


FIG. 16.—Diagram of an induction-coil and its connections.

strength of the inducing current, so that, if the constant current be increased or decreased greatly in strength, but sufficiently gradually, no induction takes place; on the other hand, for a given change in the constant current the more rapid the change the greater the induction. (3) It varies with the angle between the primary and secondary coils in such a way that when the two coils are accurately at right angles there is no induced current; but the strength of the induction increases as the angle between the coils is altered until the maximum is reached, when the wires are parallel to each other. If the secondary coil be

[1] The resistance of a piece of a frog's sciatic nerve 1 cm. long is about 100,000 ohms.

movable horizontally on a central point, the strength of the induced current can be graduated by altering the angle between the two coils. (4) It varies inversely as the distance between the two coils, being greatest when the secondary is completely over the primary coil, and becomes less and less as the coils are separated. The strength of the induced current is usually regulated by varying the linear distance between the coils, and most induction-coils are graduated by a millimetre scale fastened to the side of the carrier, so that the pointer in the secondary coil is at the zero of the scale when the one coil is exactly covered by the other. This graduation, however, is purely arbitrary, for the absolute decrease in the strength of the induced current becomes less and less for every centimetre that the coils are separated. An exact graduation can be obtained by a scale corresponding to equal galvanometric deflections.

The direction of the induced current in the secondary coil is, at make of the battery-circuit, in the opposite direction, and at break of the battery-circuit, in the same direction as the battery-circuit in the primary coil. Most coils are so wound that when at make the battery current enters the primary coil by one top binding-screw, the induced current leaves the secondary coil by the binding-screw of the opposite side (Fig. 16).

The Use of Make- and Break-Induction Shocks as Stimuli.—Two wires are connected with the poles of a Daniell cell; the free end of one wire is fastened to one binding-screw of a spring-key, and to the other screw of the key is fixed a third wire. The clean free ends of the wires are placed on the tongue, and the key is opened and closed; no shock is produced, but only a sensation of taste; the intensity of the current is insufficient to produce a marked excitation.

The free ends of the wires are now connected with the screws, or terminals, 1 and 2 of the induction-coil and a Du Bois key is placed in the secondary circuit (Fig. 16). The secondary coil is pushed far apart from the primary, and the Du Bois key is opened; make and break of the primary circuit produces no excitation, for the induction-currents are too weak. The secondary coil is gradually moved towards the primary, and the spring-key is opened and closed from time to time, until a point is reached at which a shock is felt at break, but not at make of the constant current. The position of the secondary coil on the scale is noted. As the secondary coil is moved up further, the break-shock becomes greater, and a slight shock is also felt at make; in a similar way the two shocks can be further increased, but *the break-shock remains greater than the make-shock.*

It is especially to be noted that there is no induction-shock if the

primary circuit remains closed by the spring-key. *An induction shock is produced only at the make or the break of the constant current.*

Closure of the Du Bois key short-circuits the electrodes, and no shock will be felt on make or break of the constant current. By means of this key the make- or break-induction shock, or both, can be shut off from the electrodes.

The secondary coil is now removed from the grooves of the carrier, and is placed close to, but at right angles to, the primary coil: no shock is produced when the primary circuit is closed or broken. The secondary coil is gradually turned on its vertical axis, and the spring-key is opened and closed from time to time. A shock will be felt first at break, then at make, and these will increase until the maxima are reached when the secondary coil is parallel to the primary.

These simple experiments show that the make and break of a galvanic current can act as weak stimuli; that on connecting the Daniell cell with the induction-coil induced currents are produced in the secondary coil only at make and break of the battery-current and not when it is running with constant strength through the primary coil; that the induced currents are very effective stimuli, can be easily graduated in strength and short-circuited by a key. It has further been shown that the break induction-shock is stronger than the make. The cause of this difference lies in the primary coil, and needs explanation.

When the battery-current enters the primary coil, it induces a current in it as well as in the secondary coil. This "self-induced" or make extra current, like that induced in the secondary coil, is a momentary current in the opposite direction to the battery-current; hence it delays the rapidity with which the battery-current reaches its maximal intensity in the primary coil and weakens the effect which change in current in the primary coil will induce in the secondary coil. On the other hand, when the battery-current is broken, the current in the primary coil suddenly runs down to nothing; and although a break extra current, running momentarily in the same direction as the battery-current, is induced in the primary coil, it cannot delay the rapidity of the fall of the battery-current, because a primary circuit no longer exists in which the extra current could run.

Demonstration of the Break Extra Current.—Connect a cell with binding-screws 1 and 2 of the induction-coil, placing a spring-key in the circuit. Fasten to the same binding-screws of the primary coil two wires, the free ends of which are placed on the tongue. On closing the spring-key no shock is felt, but, on opening it, the shock of the break extra current.

A purely physical proof of the break extra current can be obtained by connecting one pole of a battery to the primary coil, and by touching with the other wire from the battery the milled head of the other binding-screw of the primary coil. Every time that the battery circuit is broken, the break extra current will pass across from the screw to the wire as a minute spark; no spark, or a very feeble one, is seen on touching the first terminal, for in this case there is no current in the primary coil.

Equalisation of Make and Break Induced Currents.—From what has been said it is clear that, if the break extra current were provided with a circuit to run in, the strength of the current induced in the secondary coil at break would be reduced to that of the current induced at make; and so they would be equalised. In order to effect this the battery-circuit is not broken, but is nearly completely short-circuited out of the primary coil by a Du Bois key (Fig. 17). Now again test the relative strengths of the make and break induced currents.

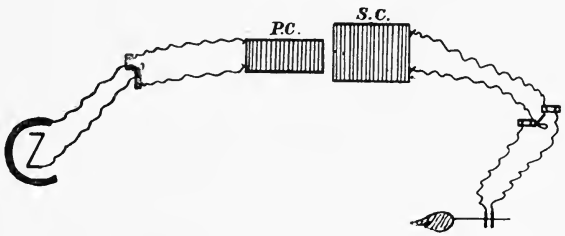


Fig. 17.—Arrangement of apparatus for equalising the make and break induced currents.

They may be approximately equal, but the original difference is not infrequently overcorrected, and now the break-shock is the weaker. This is caused by the make and break extra currents running in circuits of different resistance. At make the extra current runs not only through the primary coil but also through the resistance of the Daniell cell; but at break the extra current has to run only through the resistance of the primary coil, hence it is the more effective current of the two, and reduces the effect induced in the secondary coil at break more than the make extra current does on closing the primary circuit.

Faradic or Tetanising Shocks.—Induction-coils are provided with an automatic arrangement for rapidly making and breaking the primary circuit by means of Wagner's hammer. Connect up the battery to screws 5 and 6 of the coil, interposing a spring-key, and follow out the primary circuit (Fig. 18). The current passes up the pillar A along the spring H to the screw S_1, through the primary

coil to the electro-magnet E, and so to the pillar B. When the circuit is thus made, E becomes an electro-magnet, pulls down the spring H from its contact with S_1 and breaks the circuit; consequently E ceases to be a magnet, the spring flies up into contact with S_1, and again

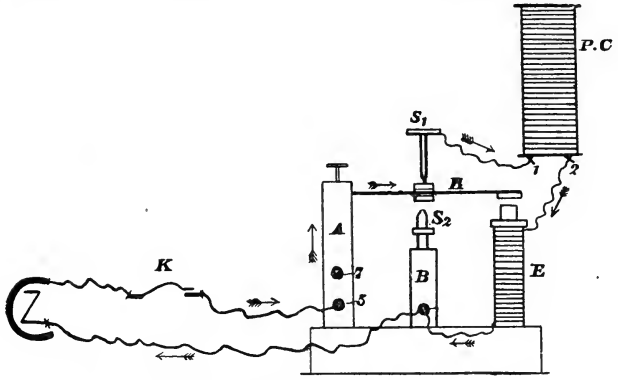


FIG. 18.—Diagram to show the action of Wagner's hammer.

completes the circuit. The number of times the circuit will be thus made and broken per second depends upon the length of the spring H; in most coils it is of such a length as to give 50 complete vibrations per second. At each make and break of the circuit a current is induced in the secondary coil, just as when the circuit was broken by hand;

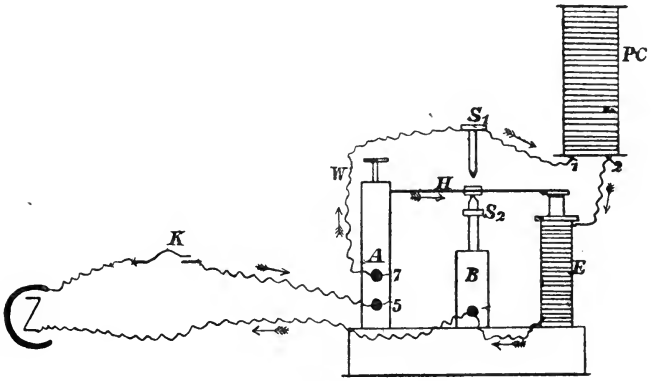


FIG. 19.—Diagram to show the action of the Helmholtz side-wire.

further, the break-shock is stronger than the make-shock, and for the same reason as before.

Determine the distance necessary between the two coils for the shocks just to be felt on the tongue.

Helmholtz showed that it is possible to equalise these Faradic shocks by short-circuiting, instead of completely breaking, the battery-current, and for the reason already explained. For this purpose (Fig. 19) a stout wire, W, connects the binding-screws 7 and 1, S_1 is screwed up out of reach of the spring, and S_2 is screwed up. Follow the circuit of the current, which passes from binding-screws 7 to 1 by the side-wire, and so to the primary coil, back to the electro-magnet E, to binding-screw 6 and to the battery. When, however, the current reaches E, it becomes a magnet, and pulls down the spring into contact with S_2. This short-circuits the battery-current out of the coil, for the current will now pass from the pillar A, by way of H, to the pillar B, and so back to the battery. There is still left the circuit 7 W, 1, PC, E, H, A, 7, in which the break extra current can run and reduce the strength of the current induced in the secondary coil at break.

Determine the distance between the coils at which the shocks are now just felt on the tongue; it will be found to be reduced, showing that the break-shock which was alone felt before has been reduced down to or even below the strength of the make-shock.

CHAPTER II.

THE GRAPHIC METHOD. MAXIMAL AND MINIMAL STIMULI. UNIPOLAR EXCITATION.

THE graphic method is applied to muscle in order to obtain a permanent magnified record of the change in form of a muscle during contraction, and further, to investigate the time-relations of the contraction. For this purpose it is necessary to describe the method of preparing the muscle and then three special pieces of apparatus: (1) a magnifying lever, the muscle lever, or myograph, which can write on (2) a surface either stationary or moving at a uniform rate, the drum, and (3) an instrument for recording time on the drum, the chronograph, which will be described in Chapter III.

The Muscle- and Nerve-Preparation.—The quickest way to kill a frog is to "pith" it. The articulation between the skull and the vertebral column can be felt with the tip of the finger; it is severed by a transverse cut with a pair of scissors, and then a probe or blanket-pin is inserted into the skull to destroy the brain. The spinal cord is destroyed in a similar way, and this final stimulation of the nerve-cells

causes a discharge of motor impulses to the muscles of the body, which give a series of convulsive twitches or contractions. These twitches quickly cease, the body and limbs are in a toneless, relaxed condition, and all reflexes have been abolished.

The frog is then placed belly downwards on a frog-board, and the skin at the ankle is divided by a circular incision; the tendo-Achillis is exposed and a thread passed under the tendon and tied just above the sesamoid bone. In this way a ligature is attached to the muscle with-

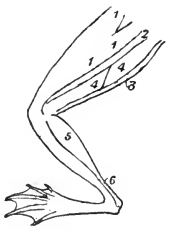


FIG. 20.

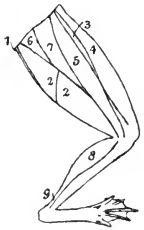


FIG. 21.

Muscles of the frog's leg. (After Ecker.)

FIG. 20.—Dorsal aspect.

1. Trinceps femoris.
2. Biceps femoris.
3. Rectus internus.
4. Semi-membranosus.
5. Gastrocnemius.
6. Tendo Achillis.

FIG. 21.—Ventral aspect.

1. Rectus internus.
2. Gracilis.
3. Adductor longus.
4. Vastus internus.
5. Sartorius.
6. Adductor brevis.
7. Adductor magnus.
8. Gastrocnemius.
9. Tendo Achillis.

out damage to or irritation of its fibres. The tendon is divided below the sesamoid bone, and a pull upwards towards the knee frees the gastrocnemius muscle and the skin from the remaining structures of the leg, which are cut away just below the knee. The gastrocnemius muscle is protected from drying and from contact with foreign substances by drawing down the "trouser" of skin. The sciatic nerve is now dissected in the following way. The skin over the posterior surface of the thigh is divided by a longitudinal incision in the middle line, the biceps and semi-membranosus muscles are separated, and the sciatic nerve is exposed. The nerve must not be pinched with forceps, for it is easily damaged. The muscles on each side of the urostyle and then the urostyle itself are cut away; the three constituent ends of the sciatic nerve are now exposed. The spinal column is divided transversely between the 6th and 7th vertebrae and the 9th, 8th, and 7th vertebrae are bisected. The piece of bone, from which the nerve to be prepared issues, can be grasped with the forceps without damage

to the nerve, and the sciatic nerve is freed from the surrounding tissues as far as the knee. The thigh is then severed from the body by a

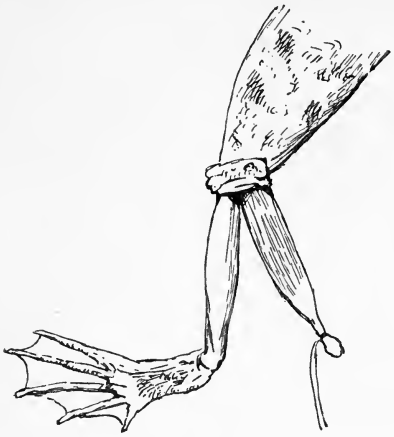


FIG. 22.

FIG. 23.

Diagrams of a muscle- and nerve-preparation. (Pembrey and Phillips.)

FIG. 22.—The first stage of dissection.

FIG. 23.—The second stage of dissection. The sciatic nerve exposed and the gastrocnemius muscle covered by skin.

transverse cut close to the articulation of the head of the femur (Figs. 22 and 23).

In order that the best results may be obtained the muscle- and nerve-preparation should be as fresh and irritable as possible, and in order to obtain this the following precautions should be observed. (*a*) All apparatus for the experiment should be in working order before the dissection is commenced. (*b*) The muscle must be prevented from drying by keeping the "trouser" of skin pulled down over it, and since the nerve is even more easily killed by drying, it should, when not required for immediate stimulation, be allowed to lie among the muscles of the thigh, the lymph of which will keep it moist and irritable. The nerve must not be placed upon the frog's

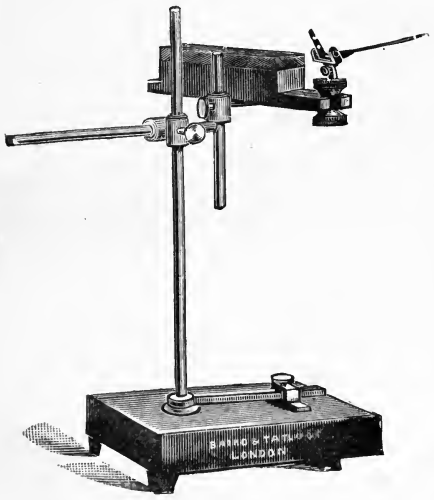


FIG. 24.—The crank-lever, muscle-board and stand.

skin, the secretions of which quickly injure it. (*c*) When the nerve is on the electrodes it must be kept moist by normal tap-water saline solution (·70 per cent. sodium chloride in tap-water) upon a piece of filter-paper, but care must be taken that the current from the electrodes is not short-circuited thereby. (*d*) The nerve itself should not be picked up by forceps, but should be lifted by the pieces of the vertebral column. Consequently the whole length of the nerve should always be dissected out; as a rule it should not be cut across in the thigh nor simply exposed in the thigh and two electrodes pushed under it.

The **Muscle-lever** takes one of two chief forms:

(*a*) The crank-lever (Fig. 24) consists of an L-shaped piece of metal, the horizontal arm of which is long and carries the writing

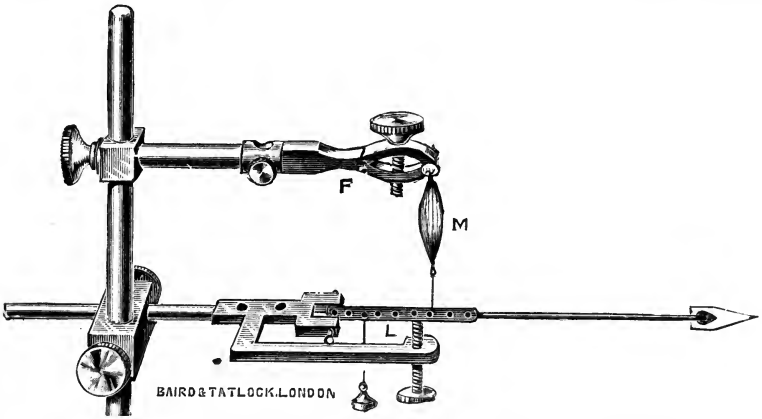


FIG. 25.—The simple lever with after-loading screw. F, clamp; L, lever; M, muscle.

point, whilst the vertical arm is short and to this the thread round the tendo-Achillis is firmly tied. The muscle rests, in the same straight line as the lever, on the muscle-board, a horizontal piece of wood covered with cork. The whole is carried on a vertical stand (Fig. 24), the arm of which is movable on the base, so that the writing point of the myograph can be swung towards and away from the drum without altering the position of the base of the stand. When the thread has been tied to the lever, a pin is pushed through the lower end of the femur into the cork; this gives the muscle a fixed point from which to pull. It is necessary to see that, when the muscle is at rest, the thread attached to the lever is taut, and that there is no "slack" to be taken in when contraction begins; further, the writing arm should be horizontal.

In this form of lever the movement of the writing point is at right

angles to the movement recorded. The magnification of the movement of the muscle recorded by the lever is calculated by dividing the distance of the writing point from the axis by the distance from the axis of the point of attachment of the thread from the tendon. The nearer to the axis the muscle is attached the greater will be the magnification. It is quite sufficient to magnify the movement of the muscle 5 times.

(*b*) The simple lever (Fig. 25) consists of two parts: a rigid femur-clamp to hold the piece of femur, and a horizontal writing lever below it to which the thread on the tendo-Achillis is tied. Care must be taken that the femur-clamp and lever lie in the same plane, and that the muscle is tied to a point on the lever vertically below the clamp. In this case the movement of the writing point is in the same plane as that of the movement recorded. The magnification, as before, is calculated by dividing the distance of the writing point from the axis by the distance of the point of attachment of the muscle from the axis.

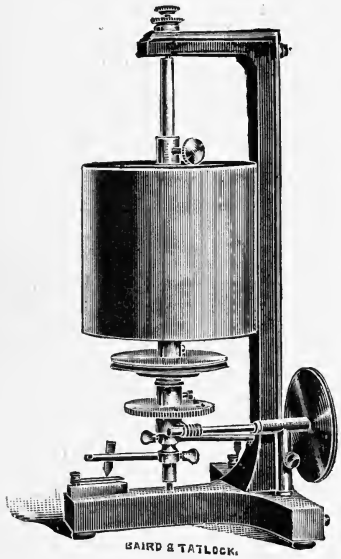


Fig. 26.—Kymograph.

The writing lever must be as light as possible (see page 27, Chap. III.), but it must be sufficiently rigid to prevent vibrations being set up in it. For this purpose writing levers are generally made of light metal, glass, Japanese cane or straw.

The actual writing point is made of thin metal foil or moderately stiff paper bent at its free end slightly over towards the drum. The writing point must lie as nearly as possible parallel to the recording surface, or, in other words, at right angles to a radius of the drum. Further, the bend near its end is necessary; it acts as a weak spring and keeps the writing point up against the recording surface in different positions of the lever. For the end of the lever describes a curved line, and the more it leaves the horizontal position the greater will be the distance of the end of the straw from the recording surface.

The **Kymograph** or recording drum (Fig. 26) consists essentially of a stout brass cylinder which is made to revolve round a vertical axis by either clockwork or string belting from a motor. It is necessary to have some arrangement by which the speed of revolution can be altered within wide limits; this is obtained by various mechanical devices in different patterns of drum, one of which is shown in Fig. 26.

The drum is covered with white glazed paper, the surface of which is then blackened by a thin layer of soot, obtained by revolving the drum through either the luminous part of a broad gas flame or the smoke of burning turpentine or camphor. The writing point of the lever, as the drum revolves, rubs off the layer of soot and leaves a white magnified image of the movement of the muscle or heart or whatever change is being recorded.

The white paper is of the same width and longer than the surface of the drum, and the under-surface of the overlap is gummed. The paper must be laid evenly and without wrinkles round the drum, the gum is then moistened and the paper fastened. The layer of soot obtained from the gas flame should be dark brown in colour, and care must be taken to revolve the drum sufficiently rapidly through the flame to prevent scorching or burning of the paper. The film of soot from camphor is less firmly attached to the paper, and must not be made too thick, otherwise the writing point does not, without undue friction, rub off enough of it to leave a distinct tracing. In recording it must be so arranged that the tracing does not come at the overlap, for the joint in the paper is liable to make the point of the lever jump. Further, it is very important that the drum should be made to revolve away from and not towards the writing point, in other words, the tracing as it is taken should pass from the writing point, not towards but away from the lever. When the tracing is finished, the paper is cut through at the overlap and the details of the experiment written on it. The tracing is preserved by drawing it once through a varnishing solution [1] and pinning it up to dry.

This graphic method, as we shall see, introduces several errors, but such accuracy as it has must depend upon the drum remaining a true cylinder; it is therefore very important that a drum should never be dropped or in any way dented.

Minimal and Maximal Stimuli.—If the strength of the stimulus applied to a muscle be varied within certain limits, it is found that the muscular response also varies, so that the greater the excitation the greater is the shortening of the muscle.

In order to demonstrate this, connect up a Daniell cell to an induction coil so as to give single induction shocks, placing a simple key in the primary circuit and a Du Bois key in the secondary circuit; cover and smoke a drum. Dissect out a gastrocnemius preparation and attach it to the myograph lever, arrange the electrodes to stimulate the muscle directly; one needle-electrode is used which passes through

[1] A rapidly drying varnish is made by dissolving 250 c.c. of the best white hard varnish in a litre of methylated spirits and then adding 10 c.c. of castor oil.

and fixes the lower end of the femur; the other wire from the Du Bois key is joined to a piece of capillary copper wire which has been threaded by means of a needle through the tendo-Achillis. In this way the current can be passed through the length of the muscle, and the very fine wire will not cause any obstruction to the free movement of the muscle when it contracts. Bring the writing point on to the surface of the stationary drum.

With the secondary coil at 20 cm. and the Du Bois key open, make and break the primary circuit, no contraction will take place. Gradually move up the secondary coil towards the primary, opening and closing the key in the primary circuit at each new position. With the secondary coil at about 16 cm. the muscle will contract at break but not at make, showing that the break induction shock is stronger than the make-shock. The contraction is recorded on the drum by a nearly vertical line, and shows a minimal contraction in response to a minimal stimulus; the make-induction shock is still a sub-minimal stimulus and no contraction results. Rotate the drum on a short distance by hand, move the secondary coil up 1 cm. and stimulate again. Repeat this process, moving the drum on after each contraction and increasing the strength of the stimulus after each make and break of the primary circuit (Fig. 27). As the strength of the stimulus is increased the contraction at break increases in height rapidly at first and then more

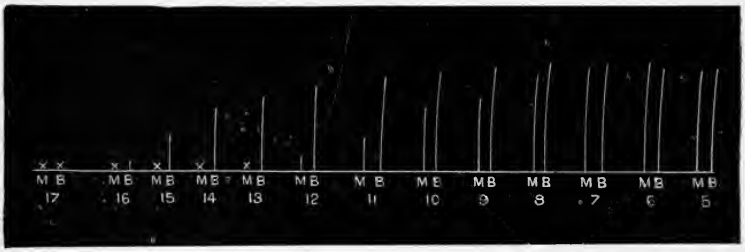


Fig. 27.—Heights of contraction of a muscle with different strengths of stimuli. M marks the make and B the break of the primary circuit. The numbers refer to the distances in cms. of the secondary from the primary coil. (A.P.B.)

slowly until, with the secondary coil at about 7 cm., a point is reached beyond which the height does not increase. At 7 cm., therefore, the break-shock and the contraction which it causes are maximal. All stimuli intermediate in strength between minimal and maximal are called sub-maximal. At a certain point the make-shock will be found to become an effective stimulus and cause a minimal contraction. As the make-shock is increased in strength, the contraction rapidly increases in height until, with the secondary coil at about 7 cm.,

it becomes maximal and of about the same height as the break contraction.

The higher the contractions become the more obvious is it that the writing point describes on the stationary drum, not a straight line, but an arc of a circle. The shortening of the muscle, after allowing for the magnification by the lever, is measured not by the length of this arc but by a perpendicular line dropped from its highest point on to the base line.

It is necessary to point out here that, when the primary circuit is made—and the same is true if it be broken—a momentary induced current is both made and broken through the nerve, and yet there is only one contraction of the muscle. It has been found that in a current of such short duration the break stimulus is ineffective because it falls within the refractory period of the make stimulus (see Chap. VII., p. 40). In both cases, whether the primary circuit is made or broken, the effective stimulus to the nerve is only the make stimulus of the induced current.

Unipolar Excitation.—Connect a battery to a coil so as to give tetanising shocks; connect a wire to one pole of the secondary coil and place its free end on the tongue. If the secondary coil be moved completely over the primary, faint shocks will be felt. The explanation of this phenomenon is that the making and breaking of the primary circuit causes free electricity to collect at the end of the wire connected with the secondary coil; when the E.M.F. of this charge is sufficient to overcome the resistance of the tissues of the body, the circuit is completed through the body, the floor and desk, and so back to the other pole of the secondary coil. With the wire still on the tongue, touch the other pole of the secondary coil with a moistened finger; much more powerful shocks are felt because a more direct circuit from one pole to the other of the secondary coil has been provided.

Repeat the experiment on a sciatic-gastrocnemius preparation in the following way, with either tetanising or single-induction shocks. Lay the preparation on a perfectly clean and dry glass-plate and place a wire connected with one pole of the secondary coil under the nerve; no contraction of the muscle takes place because the dry plate insulates the preparation and the secondary circuit cannot be completed. Now touch the muscle with a wire, the other end of which rests on a gas or water pipe; the muscle contracts because the circuit is completed through the earth. It is not even necessary that the conductor should touch the preparation, for, if a moistened finger is brought as near the muscle as possible without touching it, the muscle contracts, especially

if a moistened finger of the other hand touches the other pole of the secondary coil. In this case the human body acts like a condenser charged with electricity, which by its approach can stimulate muscle or nerve. Further, if the nerve be ligatured between the electrode and the muscle, or cut across and the two cut ends laid over each other, which will prevent the passage of a nervous impulse along it, contraction of the muscle is still produced, because the discharge takes place along the whole length of nerve and muscle between the electrode and the point by which the muscle is connected to the earth, so that any irritable tissue in the course taken by the charge is stimulated.

If, however, the muscle and nerve preparation is laid on an ordinary moistened muscle-board, the insulation is so slight that one electrode, connecting the nerve and the secondary coil, will by itself cause the muscle to contract.

It is in order to guard against accidental stimulation of muscle and nerve by unipolar action that a Du Bois key must always be placed in the secondary circuit, and must always be kept closed except when the tissue is being intentionally stimulated. The brass bridge of the key, which has many thousands of times less resistance than the tissue between the electrodes, affords a perfect closure of the secondary circuit and prevents static electrification of the electrodes.

Errors from unipolar action are liable to take place, especially in the study of the electromotive phenomena of muscle and nerve by the electrometer and galvanometer (see Chap. IX., Part III.).

CHAPTER III.

A SINGLE CONTRACTION OF A GASTROCNEMIUS MUSCLE.

In order to study the contraction given by a muscle in response to a single stimulus, it is not sufficient to inspect the curved line traced by the myograph-lever on a revolving drum. It is also necessary to study the length of time occupied by the whole twitch and the time-relations of different parts of it. For this purpose a time-tracing must be simultaneously recorded by a special apparatus, which generally takes one of two forms.

(1) The **Tuning Fork**; to one prong of this a writing point, similar to that on the myograph-lever, is attached. With the writing point lightly touching the blackened surface of the drum, a sharp tap is given to the fork, and the drum set in motion; care must be taken that the drum does not make more than one revolution, otherwise the time-

tracing will run over itself. The number of complete vibrations per second and the time value of each will depend upon the note of the fork. The most useful fork is one that gives 100 complete vibrations per sec. When more rapid vibrations are required the above method is not suitable, because the vibrations of a fork of a higher note cease so soon after a single tap.

In order to obtain a time-tracing in $\frac{1}{200}$ths or less of a second, it is necessary to use—

(2) A **Chronograph** or time-marker, which records on a drum the number of times per second a current through it is made and broken by

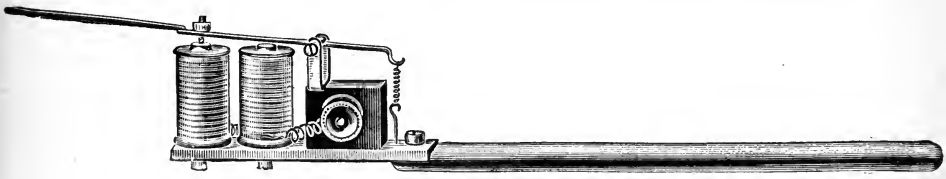


Fig. 28.—A time-marker.

another special piece of apparatus. The chronograph (Fig. 28) consists essentially of an electro-magnet, which, when the current through it is made, attracts and pulls down a metal lever carrying a writing point. When the current through the electro-magnet is broken, a spring at the other end of the lever raises the writing point.

The apparatus used to make and break a current through the chronograph at any definite known rate is a tuning-fork of the corresponding note. To one prong of the fork is attached a platinum wire which,

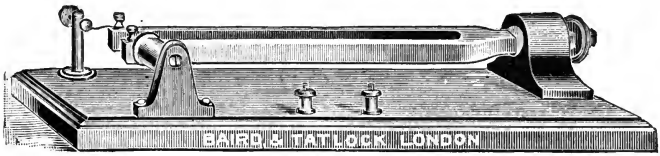


Fig. 29.—A tuning-fork with electro-magnet.

with each complete vibration of the fork, makes and breaks the chronograph circuit by touching and receding from a brass contact or mercury cup (Fig. 29). The tuning-fork, when once started vibrating by a tap, is kept vibrating automatically by an electro-magnet in the same circuit (Fig. 30). Thus, when the platinum wire touches the mercury cup the battery current is made through the chronograph and the writing point is pulled down; at the same time the current is made through the other electro-magnet, which attracts the tuning-fork and pulls the platinum point away from the mercury. Both electro-magnets now cease to act, the writing point of the chronograph is pulled up by the

spring, and the platinum wire of the tuning-fork again touches the mercury, thereby making the circuit again.

To record the contraction of a muscle in response to a single maximal induction-shock, the apparatus is set up in the following way (Fig. 31).

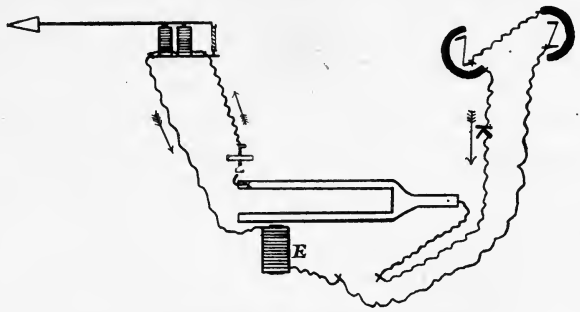


FIG. 30.—Diagram of the chronograph circuit.

Connect one pole of a Daniell cell to one top binding-screw of the primary coil, and the other binding-screw of the coil to a binding-screw on the base of the stand of the drum. The current passes through the metal work of the stand to a metal striker carried beneath the drum on its axle. As the drum revolves this striker touches a strip of naked wire attached to, but insulated from, the rest of the stand. The binding-screw in connection with this naked wire is connected to the other pole of the battery. It is only when the striker and naked wire are in contact that the primary circuit is completed.

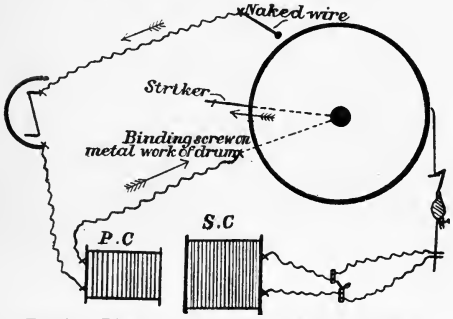


FIG. 31.—Diagram of the apparatus for recording a single muscular contraction.

A sciatic and gastrocnemius preparation is made and attached to the myograph-lever, which is weighted near its axis with 10 or 20 grams, and which should then be horizontal. The nerve is laid across the electrodes coming from the Du Bois key, and the secondary coil is arranged to give maximal induction-shocks. A tuning-fork giving 100 complete vibrations per second is arranged to write just beneath the myograph lever. Before the two writing points are brought into contact with the smoked surface, the drum should be made to revolve in order to see that it will rotate away from the writing points and at a suffi-

ciently rapid rate; the rate of rotation should not be less than 20 cm. per sec. Adjust the writing points to touch the smoked paper lightly, and with the Du Bois key open, and the fork vibrating, let the drum make one revolution and no more. The curve of the muscular contraction and the time below it in $\frac{1}{100}$ths of sec. will be recorded (Fig. 32). Close the Du Bois key, remove the tuning-fork, but do not alter the position of the base of the stand carrying the myograph. With the writing point of the lever accurately on the abscissa line of the muscle curve let the drum revolve so as to complete a base line beneath the actual curve corresponding to the muscular contraction. With the writing point still on the base line, rotate the drum by hand until the striker just touches the naked wire. At this position of the

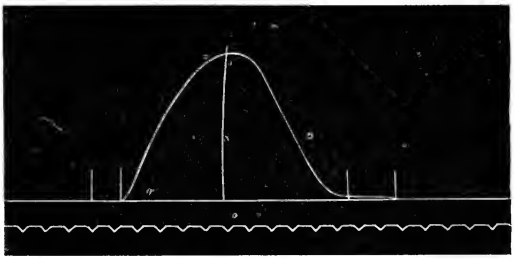


FIG. 32.—Single contraction of gastrocnemius in response to a maximal make shock. Muscle loaded with lever and 30 grms. at axis of lever; actual load on muscle, 6 grms. Magnification, 5. Temp., 15° C. Time marker, 100 per sec. (A.P.B.)

drum a maximal make induction-shock was sent into the nerve; with the finger on the lever make the writing point describe a vertical arc, which cuts the time-tracing below and the abscissa line above. In the same way, by rotating the drum by hand, vertical arcs are drawn through the muscle-curve and time-tracing at the three following points: (1) the point at which the curve leaves the base line, (2) the highest point of the curve, and (3) the point at which the curve regains the base line.

It will be noted that, during the single revolution of the drum, the primary circuit has not only been made but also been broken again by the striker leaving the naked wire. The nerve has consequently received a maximal make and then a maximal break shock, but has only responded by a contraction to the first; for, owing to the rapid rotation of the drum, the second stimulus has reached the muscle too soon after the first for the muscle to be able to respond (see Refractory period of muscle, p. 42). If, however, the drum is revolving but slowly, the second stimulus may follow the first after a sufficient interval of time for the muscle to partly respond to it. This leads

to a deformation of the curve (Fig. 32), in which the hump near the top of the up stroke of the lever is caused by the muscle responding to the second stimulus (see Effect of two successive stimuli, Chap. VII., p. 40). If with a slowly revolving drum it is desired to send into the nerve a single stimulus, it is only necessary to place the secondary coil at such a distance from the primary that the break but not the make shock is effective.

The curve (Fig. 32) occupies about $\frac{12}{100}$ths sec. and can be divided into three parts.

(1) The first part extends from the point at which the stimulus reached the nerve to that at which the contracting muscle began to raise the lever. This is the latent period, and is seen to last about $\frac{1}{100}$th of a sec. During this period several distinct processes take place; (*a*) a nervous impulse has to pass down the strip of nerve between the point stimulated and the muscle, this will occupy about $\frac{2}{1000}$ths of a second (see Velocity of nervous impulse). Of the remaining $\frac{8}{1000}$ths (*b*) the passage of the nervous impulse along the fine motor nerve-endings occupies about $\frac{3}{1000}$ths sec., and (*c*) the latent period of the muscle itself about $\frac{5}{1000}$ths of a sec. This in turn is due to several factors, of which two must be mentioned. When muscle fibres begin to contract a certain time must elapse before the muscle is able to exert a sufficient pull to move the recording lever; in other words, there is instrumental inertia to be overcome. Again, when muscle, which is highly extensible, begins to contract, every part of every fibre does not simultaneously begin to shorten; but the contracted part of a fibre stretches at first the uncontracted part, and is therefore not united to the lever by a rigid connection. It is only when the tension in the stretched part has sufficiently increased, or the fibre as a whole has passed into a state of contraction, that the lever begins to be pulled upon.

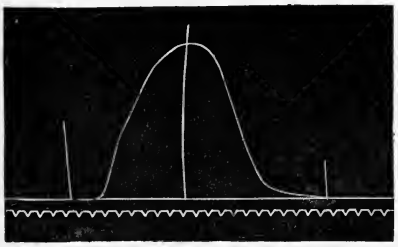


FIG. 33.—Contraction of the same preparation as in Fig. 32, recorded on a drum revolving at a slower rate. The hump near the top of the upstroke of the lever represents a second contraction in response to the *break* shock. Time marker, 100 per sec. (A.P.B.)

(2) The second period extends from the point at which the lever begins to rise to the point highest above the base-line. This is the period of active contraction or shortening of the muscle, and occupies about $\frac{5}{100}$ths of a sec.

(3) The third portion extends from the highest point of the curve to the point at which the curve rejoins the base-line. This is the period

of relaxation, and lasts about $\frac{6}{100}$ths of a sec. Relaxation is a passive process brought about by the falling lever and weight doing the same amount of work on the muscle as the muscle during its period of shortening has done in raising the lever and weight to a certain height.

The muscle-curve, although roughly a magnified record of the change in length of the muscle, is deformed by certain errors of instrumental origin, which it is necessary to mention in order to avoid, so far as they are preventible. The most important are the mass and length of the lever and the disposition of the weight along it. They affect all parts of the curve. The weight of the lever tends to prevent the muscle from beginning to raise it (inertia of position) and so lengthens the latent period; therefore the lever should be as light as possible. During the stage of shortening the lever, when once in motion, tends to be carried on by its own momentum after the muscle has ceased to

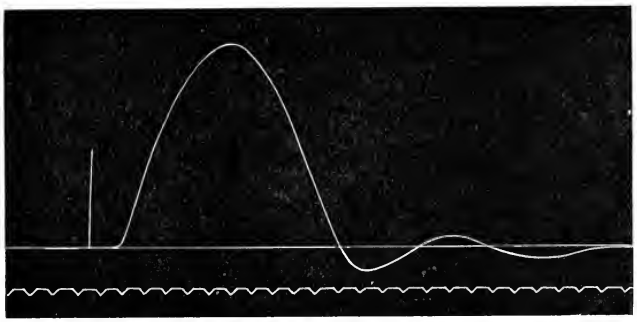


FIG. 34.—Single contraction of gastrocnemius. Muscle loaded only with a rather heavy lever. Magnification, 5. Temp., 15° C. Time marker, 100 per sec. (A.P.B.)

pull on it (inertia of motion), and so makes the muscle appear to have shortened more than it really has. For the same reason, during the period of shortening, the tension on the muscle is not uniform, but becomes less as the lever undergoes acceleration; during the relaxation exactly the opposite happens, a heavy lever as it falls again undergoes acceleration and increases the tension on the muscle throughout the relaxation and may even stretch it beyond its original resting length (Fig. 34). In order to reduce these errors the lever again should be as light as possible.

On the other hand, to attach to the muscle no other weight than that of a very light lever would introduce fallacies. For, unless the muscle is sufficiently weighted to keep it taut, there may be, when the muscle begins to contract, a certain amount of 'slack' to be taken in which would cause an apparent lengthening of the latent period.

Again, when the muscle does begin to pull on the lever, it will do so with a sudden jerk, which may cause a light lever to fly up out of control of the contracting muscle; this, again, makes the muscle appear to have undergone greater shortening than it really has (see, however, Chapter II., Part III.). Further, the relaxation of a muscle being purely passive, the period of relaxation of an insufficiently weighted muscle is much prolonged, and the writing may fail to reach the base-line again.[1]

In order to get over these instrumental difficulties, the muscle-lever must be as light as is consistent with rigidity, and the muscle must be suitably loaded, the weight being attached near the axis of the lever for the following reasons: the nearer it is to the axis, the less movement will it undergo, and therefore the less will be its inertia of movement and the more uniform the tension on the muscle throughout the curve. This disposition of the weight also helps to reduce the after-vibrations or 'shatter'-curves which are frequently seen following the relaxation (Fig. 34). Compare with this Fig. 32 taken from the same muscle; by hanging a weight of 30 grams near the axis of the lever the shatter curves have been nearly eliminated, and are represented by the slight oscillation between the two vertical lines at the end of the curve.

It may be pointed out that in the living body the muscles are always weighted when they contract, and even when relaxed they are under considerable tension; for they are really shorter than the distance between their points of origin and insertion, and their antagonists are always exerting a certain pull on them, and some muscles, such as the deltoid, are considerably stretched by the weight of a limb.

The length of the lever is of some importance; for, besides the fact that length reduces the rigidity of a light lever, a further deformation of the curve is introduced by increasing the magnification. As the writing point is raised, it tends to leave the drum, and in the course of a much magnified curve is only kept on the drum by the lengthening out of the spring formed by the writing point. Therefore the more the writing point is raised above the horizontal, the more the magnification is constantly increasing. For this reason the muscular movement should not be magnified more than is sufficient to make the record of it clear.

Although muscle curves, as accurate records of the muscular move-

[1] Muscles during the cold of winter, even when properly weighted, frequently show this 'contraction-remainder.' If cold be the cause, turn back the 'trouser' of skin and pour over the muscle some normal tap-water saline heated in a test tube to 25° C. Cf. footnote on p. 33.

ment, have fallacies inseparable from the method of recording them, it is possible to make two rough deductions from them:

(1) The amount of actual shortening a muscle undergoes during contraction can be calculated by measuring the vertical height of the top of the curve above the base line and dividing it by the magnification; in Fig. 32 the height is 20 mm., and the magnification 5, therefore the muscle became shorter by 4 mm. The length of the resting muscle when loaded by lever and weight was 25 mm., consequently the muscle during contraction became shorter by $4 \times \frac{1}{25}$, *i.e.* nearly a sixth of its original length.

(2) The amount of work done by the muscle during its contraction is the product of the load and the height to which it was raised, $W = L \times H$. In Fig. 32 the actual load which the muscle raised was not the whole of the 30 grams, hung near the axis of the lever, but a proportion of it, calculated by multiplying by the distance from the axis of the point of the suspension of the weight, and dividing by the distance from the axis of the point of attachment of the muscle; this fraction was $\frac{1}{5}$, and the actual load lifted 6 grams. The height to which it was raised was 4 mm.; consequently the work performed was 24 gramme millimetres.

CHAPTER IV.

THE CONDITIONS WHICH AFFECT SINGLE MUSCULAR CONTRACTIONS.

(*a*) **Different Muscles.** (*b*) **Veratrine.**—The curve produced by the contraction of a muscle may be altered not only by such influences as temperature, load, fatigue, and drugs, but also by the differences in structure of various muscles. The muscular fibres of the frog are found to present two varieties, clear and granular, which differ both in structure and in physiological properties. The gastrocnemius may be taken as an example of a muscle whose fibres consist largely of the clear variety, and the hyoglossus of the granular variety, *i.e.* a muscle in which the majority of muscle-fibres contain more nuclei and are relatively richer in undifferentiated living material, the sarcoplasm. The chief physiological difference between granular and clear muscles are, that granular muscles have a slower and more prolonged contraction, are less excitable, more easily tetanised, and less readily fatigued.

In mammals the same differences between red and white muscles can be shown to exist. Red muscles, such as the masseter or soleus of the rabbit, differ structurally in having more sarcoplasm and nuclei in their fibres, and are redder in colour owing to a much richer capillary net-

work between their fibres and to the presence of myohaematin in the fibres themselves; physiologically they are far less readily fatigued and show a contraction four or more times as long as that of the white gastrocnemius (Fig. 35).

For comparison with the single twitch of the gastrocnemius, that given by the hyoglossus may now be studied. This muscle, arising from the anterior edge of the body of the hyoid cartilage, runs forwards into the substance of the tongue.

A **Hyoglossus Preparation** is made by cutting off the whole of the lower jaw, including the tongue and hyoid cartilage. Place it on the myograph board, mucous surface upwards, turn the tongue

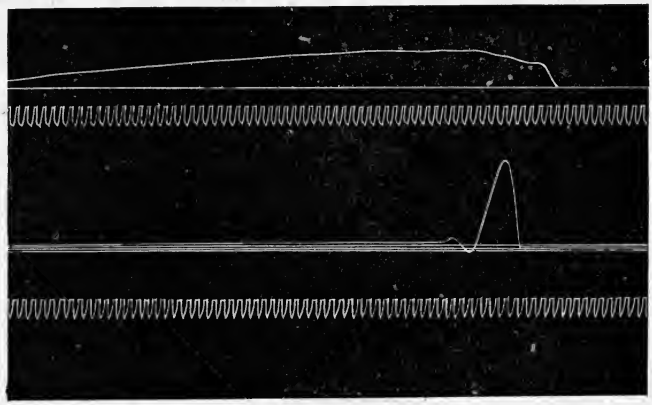


FIG. 35.—Comparison of contractions of red and white muscle of rabbit, stimulated indirectly. Upper curve is response of the red soleus and lower curve that of the white gastrocnemius. Time marker, 50 per sec. The tracing to be read from right to left. (M.S.P.)

forwards, and connect its tip to the lever by a thread. Firmly fix the hyoid cartilage by running a pin through it into the cork. Two needle electrodes transfix the base of the muscle just in front of the hyoid.

All the other connections are the same as when studying the single contraction of the gastrocnemius; a weight of 5 or 10 grams is placed near the axis of the lever.

Compared with the single twitch of the gastrocnemius, that given by the hyoglossus (Fig. 36) shows the following differences: the whole contraction lasts more than twice as long, the latent period is slightly longer, but it is the period of shortening and still more that of relaxation which is more gradual and prolonged.

Action of Veratrine.—A brainless frog is poisoned by injecting into the dorsal lymph sac 5 minims of a saturated (1 in 1000) solution of veratrine in normal tap-water saline. In order that the drug may

be rapidly absorbed it is important not to 'pith' the frog, but to destroy its cerebrum with a pair of Spencer-Wells pressure forceps. In about ten minutes it will be observed that the hind legs are very slowly and imperfectly flexed after a jump, and a few minutes later the frog will be seized by a spasm when it jumps. As soon as these

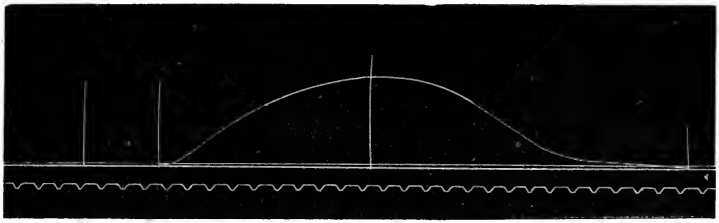


FIG. 36.—Contraction of the hyoglossus muscle. Time marker, 100 per second. (A.P.B.)

symptoms appear the remaining portions of the central nervous system are destroyed, and a sciatic and gastrocnemius preparation made.

In the meantime the action of veratrine may be studied on the hyoglossus preparation used in the previous experiment. Five minims of the veratrine solution are injected into the lymph sac in which the muscle lies. The drum is arranged to revolve at a slow rate of about 2 cm. in 10 secs., and a simple key instead of the "striker" of the drum is placed in the primary circuit. After waiting a few minutes the muscle is stimulated by a single maximal induction-

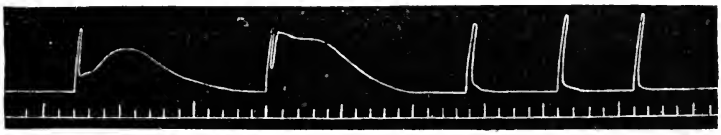


FIG. 37.—Contraction of the gastrocnemius muscle of a frog. The effect of veratrine. The first two contractions show the characteristic effect of the drug; further stimulation produced twitches without the prolonged contraction. The curve has been reduced to one-half the actual size. The time is marked in seconds. (Pembrey and Phillips.)

shock, and its contraction recorded. The curve shows that the response is a single slow contraction with an enormously prolonged relaxation. (Fig. 176.)

Replace the hyoglossus by the gastrocnemius and sciatic preparation and stimulate it in the same way. As soon as the first contraction is over, the muscle is stimulated again, and so on for half a dozen contractions. It will be seen that the first contraction (Fig. 36) consists of a smart initial twitch followed by a much longer contraction, and an even more prolonged relaxation. The second contraction shows the same characters to a less extent, and the subsequent contractions become of shorter and shorter duration until they reach the

normal. If the muscle be allowed to rest, the veratrine effect returns again. The absence, in the case of the hyoglossus, of the sharp initial twitch seen in the gastrocnemius contraction, is probably due to more complete poisoning of all the muscle-fibres. The gastrocnemius is more bulky, some of its fibres remain unpoisoned and respond with a normally rapid contraction, followed by the slower and more prolonged contraction of the poisoned fibres.

CHAPTER V.

THE CONDITIONS WHICH AFFECT SINGLE MUSCULAR CONTRACTIONS—CONTINUED.

(*c*) **Temperature**.—Since the shortening of muscle during its contraction is but the outward and visible sign of chemical changes taking place in the muscle, it is not surprising that changes in temperature should greatly affect the single muscle-twitch.

In warm-blooded animals whose bodily temperature does not undergo a greater variation than about 2° C., the effect of different temperatures on muscular activity is unimportant. But it is quite otherwise in cold-blooded animals whose range of bodily temperature is that of their external medium. In them, the muscular activity of which they are capable at any moment is determined largely by the temperature of their muscles. Again, the subject becomes important for warm-blooded animals when, from any cause, their bodily temperature is materially altered, as it may be by disease. These abnormal variations in their temperature may be sufficiently great to affect the muscular activity of which the animal is capable. More frequently, however, they are important because of the effect which an abnormally high bodily temperature, especially when long continued, may have upon the actual chemical constituents of muscle, and especially upon its proteids.

In order to study these effects, the apparatus is arranged to stimulate a muscle with single maximal induction shocks, using the "striker" of the drum, in the primary circuit. Either a hyoglossus or gastrocnemius preparation may be used; if the latter, it must be prepared without a covering of skin, in order that its temperature may be more readily altered. Also, the muscle must be stimulated directly and not through its nerve, since changes of temperature affect nerve (see p. 316).

It is important to use maximal stimuli, for cold increases the excitability of muscle, and a stimulus which is minimal at 5° C. will be sub-minimal at 25°. The lever should be weighted near its axis and the drum should revolve at a rate of about 20 cm. per sec.

Cold tap-water saline solution, which has been cooled by ice to nearly 0° C., is slowly poured upon the muscle; the temperature of the solution is noted, the muscle is stimulated, its contraction recorded and the point along the tracing at which the stimulus was sent into the muscle is marked. Swing the writing point off the drum, but do not move the base of the stand carrying the myograph. Take a series of superimposed curves at temperatures of about 3°, 13°, 23°, and 33° C. (Fig. 38). Sufficient time must be given and fluid used to allow the bulk of the thick gastrocnemius to attain approximately the temperature of the saline solution. In order to get exact results, it would be necessary to

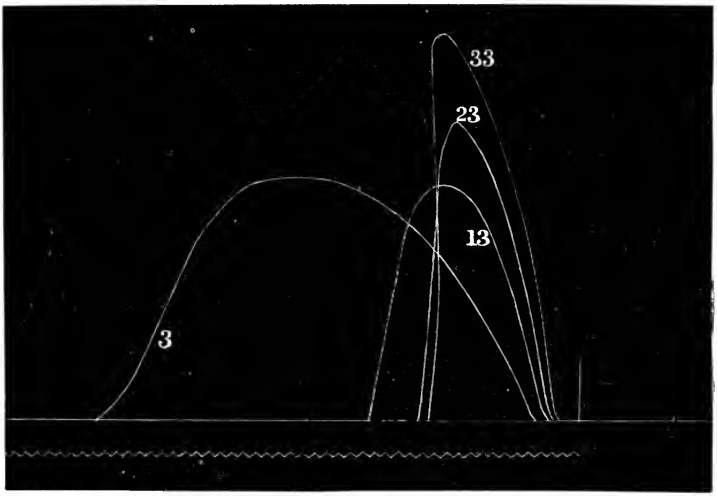


FIG. 38.—The effect of temperature upon the contraction of the gastrocnemius muscle. The time is marked in $\frac{1}{100}$ second. The tracing should be read from right to left. Figures on curve are the temperatures of the salt solution. (Pembrey and Phillips.)

suspend the muscle in the solution at a given temperature until its substance had attained that temperature.

It will be seen that cold lengthens the whole curve, especially the latent period and the phase of active contraction; the period of relaxation is relatively less affected, but a tendency to incomplete relaxation is often seen.[1] As the muscle is warmed, the liberation of energy becomes more and more rapid, consequently the time occupied by the whole twitch decreases progressively, and especially the latent period and period of shortening; the passive stage of relaxation is

[1] Cooled excised muscles, even when weighted, are liable to show a 'contraction-remainder,' or incomplete return to their former length after contraction. It is also seen after strong direct stimulation, in poisoning with veratrine, and as fatigue or death come on.

relatively less shortened, although muscle does become more extensible as its temperature rises from 0° to 30° C. (Fig. 38).

The relation between temperature and the height of the contraction is not quite so simple. Between 0° and about 15° C. the actual height of the contraction may fall slightly, and for two reasons: as the temperature increases, the irritability of the muscle decreases; further, other things being equal, the more slowly a muscle contracts, the more time it has to shorten up as much as it will in response to a given stimulus. From 15° to 25° the height of the curve rapidly increases; this is largely, if not entirely, instrumental in origin, and is

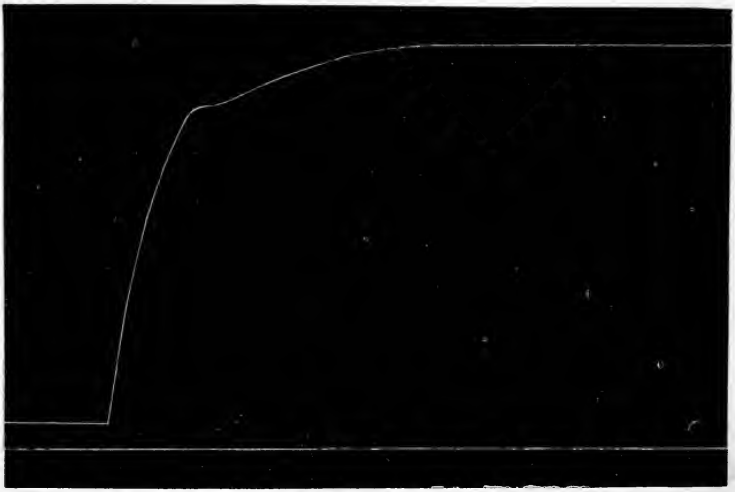


FIG. 39.—Curve of the shortening of the gastrocnemius muscle during heat-rigor. (Pembrey and Phillips.)

due to the fact that, as the liberation of energy becomes more rapid, the lever receives a considerable jerk from the rapidly contracting muscle. In other words, the increased height of the contraction is due, not to a greater liberation of energy, but to the greater rate at which the same quantity of energy is liberated. From 25° to 35° C. the irritability of muscle and its height of contraction rapidly fall.

Now pour on some solution warmed to 50° C. When the muscle-fibres reach a temperature of about 40° C. they undergo a rapid shortening (Fig. 39), which, as the temperature of the muscle rises, passes into the permanent shortening of 'heat-rigor.' This condition is due to coagulation of some of the muscle proteids, and in consequence the muscle becomes hard, opaque, inelastic, and has permanently lost its irritability.

CHAPTER VI.

THE CONDITIONS WHICH AFFECT SINGLE MUSCULAR CONTRACTIONS—Continued.

(*d*) **Load.**—In order to study the effect of variations in load upon a single muscular contraction, the apparatus is arranged for stimulating the muscle by a single maximal induction-shock; the drum being placed as a key in the primary circuit and arranged to rotate at a fast rate. Make a gastrocnemius-sciatic or hyoglossus preparation.

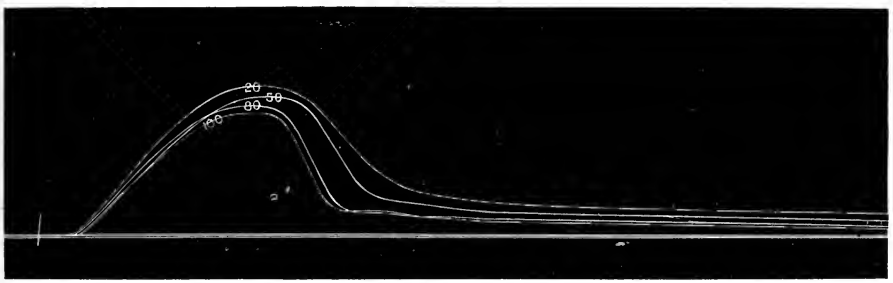


Fig. 40.—The effect of load upon the contraction of the gastrocnemius muscle (A.P.B.)

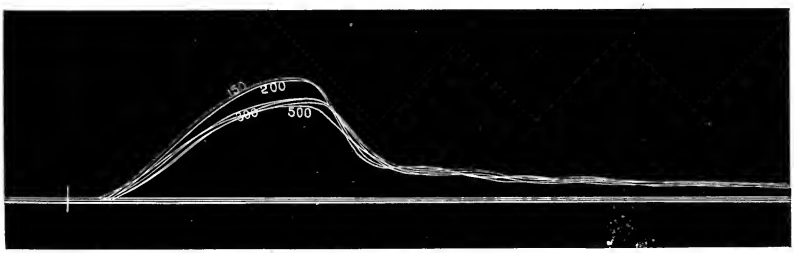


Fig. 41 is the continuation of the experiment in Fig. 40. Single contractions of the gastrocnemius with different loads. The figures on the curves represent the weights in grms. hung at the axis of the lever; actual load on muscle was in each case one-fifth. Magnification, 5. Temp., 12° C. (A.P.B.)

Record a single contraction of the muscle weighted only by the lever, mark the latent period and draw a base-line. Then hang on to the lever near its axis weights increasing by 20 grams at a time, and for each addition of weight record a contraction. The base of the stand carrying the myograph should not be moved during the experiments, but the curves should be superimposed as in Figs. 40 and 41. Each increase of weight stretches the muscle, consequently it is

necessary to bring back the writing point accurately on to the base-line before each contraction is recorded.

The general effects to be noticed are—that, as the load is increased, the latent period becomes slightly longer, the height of the contraction generally becomes less, the rise of the lever during the period of active contraction becomes more gradual, and the period of relaxation, which may be at first much decreased, gradually lengthens out again.

If the muscle be fresh and in really good condition, the early effect of increasing the load may be to increase the height of the first few contractions (Fig. 43). This stimulatory effect of initial tension on the power of a muscle to liberate energy during a subsequent contraction, is seen, within certain limits, in all kinds of muscular tissue (see p. 293); and it is of importance. For, in the body, as has been already pointed out, the skeletal muscles are, even when relaxed, under a certain tension produced by the pull of their antagonists and their being really shorter than the distance between their points of origin and insertion.

But when we study the work done by the muscle during a series of contractions with increasing loads, and not merely the height of the individual contractions, the stimulating effect of increased load is much more obvious. After the tracing has been varnished and dried, measure off the vertical heights of the curves corresponding to the different loads, and calculate the work done during each contraction (see p. 29). In the following table are given the details of the work done during the contractions recorded in Figs. 40, 41.

Number on Contraction.	Actual Load in grms.	Actual Height of Contraction in mm.	Work done in grm. mm.
20	4	4	16
50	10	3·8	38
80	16	3·6	57·6
100	20	3·4	68
150	30	3·3	99
200	40	3·2	128
300	60	2·8	168
500	100	2·6	260

It will be seen that, although the height of the contraction decreases as the load increases, the work performed increases throughout. This process of course has limits, which will be dealt with on p. 288. The important deduction to be made from these results is that muscle as

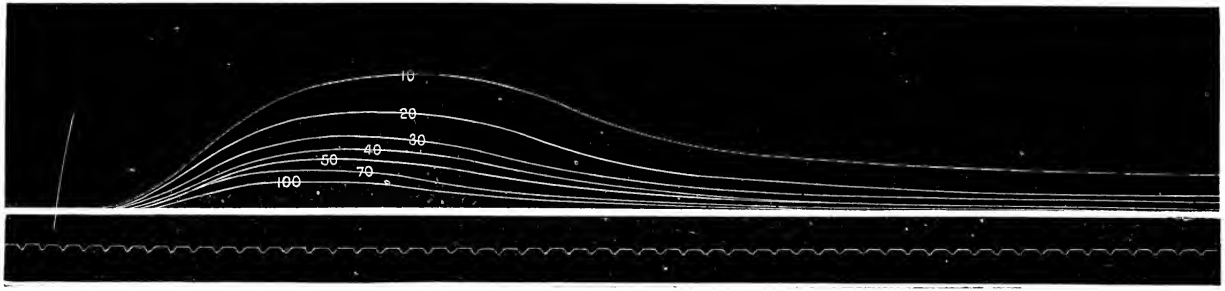


FIG. 42.—Single contractions of the hyoglossus with different loads. The figures on the curves represent the weights in grms. hung at the axis of the lever; actual load on muscle in each case, one-fifth. Magnification, 5. Temp., 12° C. Time marker 100 per sec. (A.P.B.)

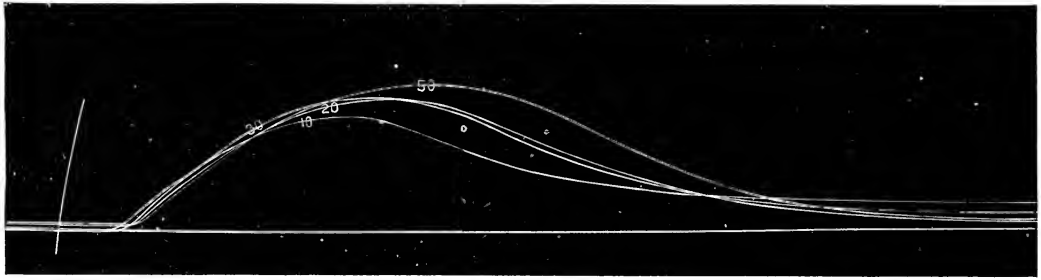


FIG. 43.—Single contractions of gastrocnemius, showing increased height of contraction as the load is increased. (A.P.B.)

a machine for doing work is found to have its output of energy regulated, not merely by the strength of the stimulus reaching it, but also to a large extent by the amount of work it is called upon to do (see p. 288).

Effect of Fatigue.—When discussing the fatigue of muscle it is necessary to draw a distinction between the fatigue of a movement produced by the voluntary contractions of the muscle concerned in it (see p. 304 . and the fatigue of a muscle caused by the artificial stimulation of the muscle itself or of the nerve supplying it (see p. 306). Further, in the second case there is a marked difference in the effect of continued stimulations on a muscle whose circulation is still intact (see p. 308), and on one which has been excised from the body. Here we shall deal only with the simplest case of a muscle excised from the body and stimulated directly and not through its nerve, in order to exclude any possibility of fatigue of nerve or of nerve endings.

Prepare either a hyoglossus preparation to be stimulated by two needle-electrodes, or a gastrocnemius-sciatic preparation to be stimulated by one needle-electrode and by fine capillary copper wire threaded through the tendo-Achillis, as the other electrode. The drum is placed in the primary circuit, so that each time it revolves the muscle receives a maximal make induction-shock; it should revolve at such a speed that the muscle will be stimulated once or twice a second. Weight the muscle near the axis of the lever, using 20 grms. for a hyoglossus and 50 grms. for a gastrocnemius preparation. With the Du Bois key closed, describe a base line and mark on it the point at which the stimulus will enter the muscle. Now open the Du Bois key, allow the drum to revolve, and record the first contraction and every tenth or twentieth subsequent contraction. For this purpose, directly the first contraction is over, the writing point is swung away from the drum, which goes on revolving and causing the muscle to contract. The base of the stand carrying the myograph must not be moved so that for each contraction the point of entrance of the stimulus will be the same. The writing point should be a fine one, otherwise the number of superimposed curves will to some extent obliterate each other.

When a series of curves taken in this way is examined (Fig. 43) it is seen that they show the following changes as fatigue progresses,—the latent period becomes slightly longer, the shortening of the muscle takes place more slowly and reaches its maximum more gradually, but the actual height of the curves does not begin to decrease much until the other features of fatigue are well marked; the lengthening out of the period of relaxation is the most marked feature, it is evident from the first, and, as it progresses, a 'contraction remainder' also appears.

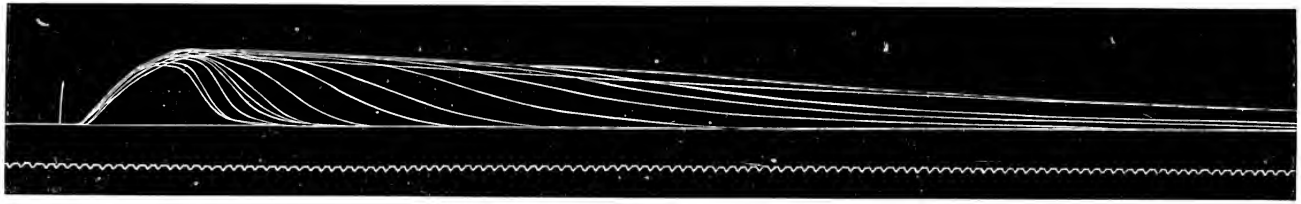


FIG. 44.—Series of contractions of gastrocnemius to show the change in contraction as the muscle becomes fatigued. Every twentieth contraction recorded. Actual load on muscle, 10 grms. Magnification, 5. Temp., 12° C. Time tracing, 100 per sec. (A.P.B.)

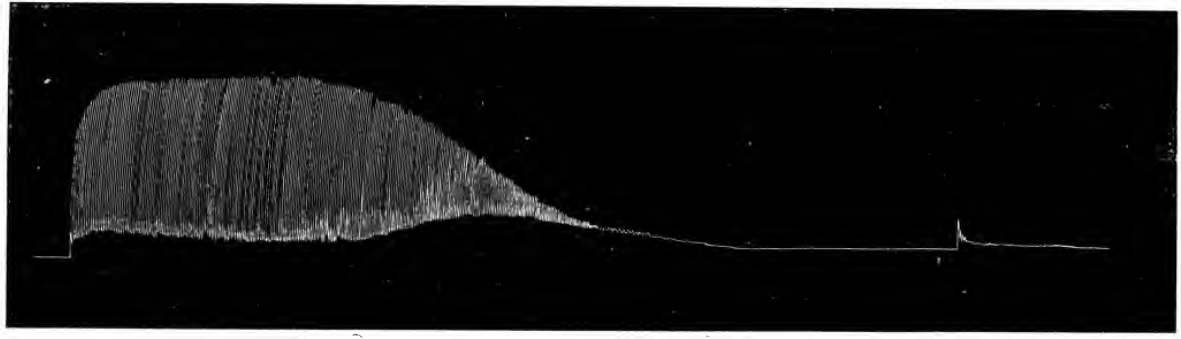


FIG. 45.—Exhaustion curve of excised and loaded gastrocnemius. Muscle was stimulated with a maximal shock every 5 secs. Exhaustion was complete at the end of 15 minutes. A slight recovery curve is shown at the end of 6 minutes' rest. (M.S.P.)

The rate at which fatigue comes on in a muscle under the above conditions is increased by raising the temperature and the load.

Another method of studying the effects of fatigue on a hyoglossus or gastrocnemius muscle is as follows. In this case the primary circuit is made and broken by hand, and the contractions are recorded as nearly straight lines on a drum revolving at the slowest possible speed. The secondary coil is moved up to the primary until both make and break shocks are maximal, and the muscle receives a stimulus once every 5 secs. In this way Fig. 45 was produced. It will be seen that the height of the contractions, after remaining fairly constant at the beginning, gradually decreases until, at the end of 15 minutes, the muscle was incapable of lifting the load. Further, it is seen that in the last two-thirds of the tracing the basal points of the twitches gradually fail to reach the base line, thus showing a 'contraction remainder.' If the muscle had been stimulated at shorter intervals, this appearance would have come on earlier; for, as soon as the period of relaxation began to increase, the next stimulus would have reached the muscle before there had been time for relaxation to be completed.

If the muscle be allowed to rest for a few minutes and then the stimulation is continued, it will be found that even excised muscle is capable of slight recovery from fatigue (Fig. 45).

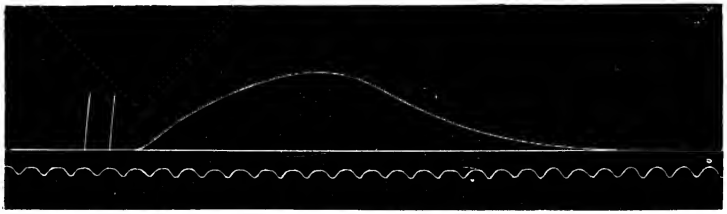
One other point shown by Fig. 45 must be referred to; the height of the first twenty twitches increases, showing a 'stair-case' effect. This short and temporary improvement in the condition of muscle, brought about by the repetition of a stimulus of constant strength, was at one time thought to be peculiar to cardiac muscle (see Heart); but although shown best perhaps by the heart, it is also shown by all forms of muscular tissue.

CHAPTER VII.

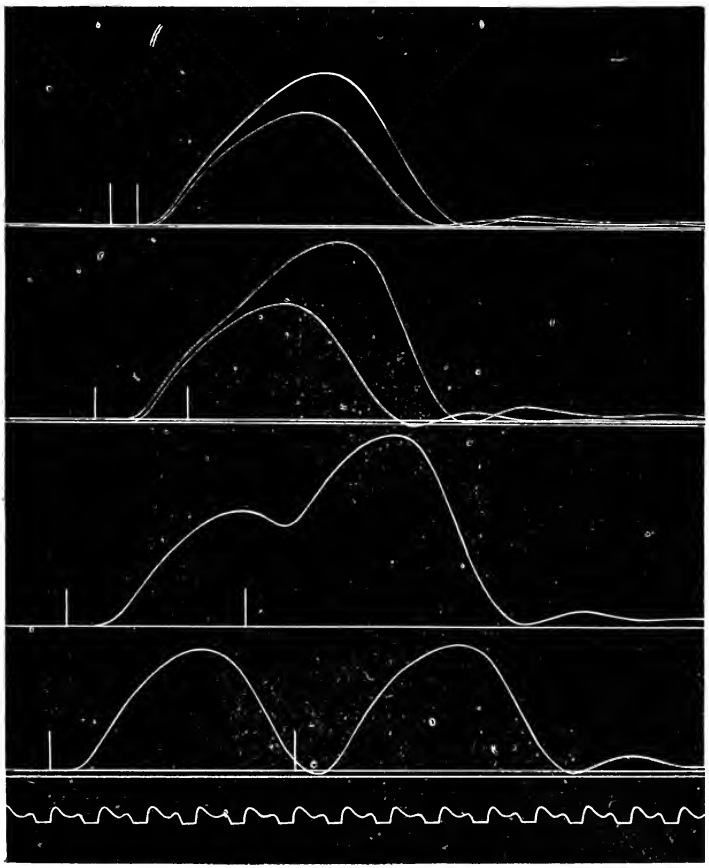
TWO SUCCESSIVE STIMULI. GENESIS OF TETANUS. TETANUS.

When a second stimulus reaches a muscle after the contraction caused by the first is over, the muscle responds with a second contraction similar to or perhaps slightly higher than the first (see Fig. 45). When, however, the second stimulus reaches the muscle before the contraction caused by the first is completed, the response given by the muscle to the second stimulus depends upon the exact phase of its twitch, in which it happens to be when the second stimulus reaches it.

In order to investigate this point, arrange the drum and circuits as



A



B

FIG. 46.—Effect of two successive maximal stimuli, with gradually diminishing intervals, upon the gastrocnemius. To be read from below upwards.

B. Time tracing, 50 per sec. In the two upper curves are shown both the contraction in response to the first stimulus alone and the combined contractions caused by the two successive stimuli. (M.S.P.)

A. Time tracing, 100 per sec. Recorded on a drum revolving at a much faster rate. The second stimulus was sent in well within the latent period of the first. (A.P.B.)

in experiments for recording a single maximal contraction on a rapidly revolving drum (p. 24); it is only necessary in addition to place a second 'striker' in the primary circuit through the drum. If the rate of revolution of the drum remains constant, then, by simply altering the angular distance between the two 'strikers,' a second stimulus can be sent in at varying intervals after the first. Make a gastrocnemius preparation and stimulate it either directly or through its nerve. Set the drum in motion and, with the Du Bois key open, approximate the 'strikers' until the muscle clearly to the eye just responds with a complete contraction to each stimulus. Close the Du Bois key, bring the writing point on to the bottom of the drum, describe a base line and mark on it the point at which each stimulus enters the preparation; then open the key, record both contractions, and close the key again. Now raise the myograph until the writing point will just clear the top of the curves, approximate the strikers a little, and again record the contractions, after marking a base line and the points of entrance of the two stimuli. This process is repeated until the 'strikers' are finally at such a distance apart that the second stimulus falls within the latent period of the first.

In this way Figs. 46 A and B were obtained. It shows that when a second maximal stimulus reaches a muscle during any part of its period of relaxation or of shortening, the rest of the contraction due to the first stimulus is omitted and the muscle starts off on a fresh contraction in response to the new stimulus. Since the second contraction may be as high as the first and starts with the writing point above the base line, it follows that the height of the second twitch above the abscissa is greater than and may be nearly double that of a single contraction; in other words, a summation of contraction has taken place. If, however, the second stimulus falls within the latent period of the first, then the muscle responds by a contraction only to the first stimulus (Fig. 46 A); that is, the muscle is refractory to the second stimulus so far as its being able to respond by a second contraction is concerned; therefore in skeletal muscle the 'refractory' period corresponds in time to the latent period (cf. the 'refractory' period of cardiac muscle, p. 64).

Genesis of Tetanus.—In order to study the response of a muscle to a series of stimuli, it is necessary to have an apparatus which will automatically make and break the primary circuit of an induction coil at any desired rate.

The **vibrating reed** is a convenient form and consists of a flat steel spring which can be clamped in various positions along its length; by altering the length of spring which is allowed to vibrate, the number of

vibrations per second can be changed. The spring has numbers stamped on its upper surface, corresponding to the position at which it must be clamped to give that number of complete vibrations per second. The free end of the spring carries a platinum point which makes and breaks contact with a mercury cup in connection with the primary circuit (Fig. 47). In order to maintain the vibrations of the spring it is usual to place above it, and in the same circuit, an electro-magnet, so that, when the spring makes contact with the mercury, it is attracted out of the cup again by the magnet. In performing a complete vibration, the spring will both make and break the primary circuit and, in order that the two stimuli may not cause contractions of unequal height, the secondary coil must be so placed that either the make shock is just

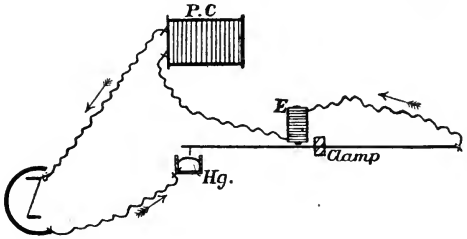


FIG. 47.—Diagram of the vibrating reed in circuit.

ineffective, in which case the number of effective stimuli per sec. will be the same as the number of complete vibrations of the spring, or the make and break shocks are made equal and maximal, in which case the number of contractions per sec. will be double that of the complete vibrations of the spring.

Place the vibrating reed in the primary circuit so as to give 10 effective stimuli per sec. Make a gastrocnemius and sciatic preparation, with the Du Bois key closed, set the spring vibrating and bring the writing point of the myograph on to the surface of the drum, rotating at a slow rate, about 3 to 4 cm. per sec.; open the Du Bois key and record the contractions for about 1 sec. Stop the drum, adjust the spring to give 20 effective stimuli per sec., and record the contractions as before. Repeat again with 30 stimuli per sec. Then remove the vibrating reed from the primary circuit, connect the battery with the coil so as to set the Wagner's hammer vibrating, and record the contraction of the muscle for a few seconds.

Since each twitch of a gastrocnemius at 20° C. lasts about $\frac{1}{10}$th sec., a muscle at that temperature could just respond without any summation to 10 stimuli per sec. If, however, the muscle is colder or fatigued, and each contraction therefore lasts longer, with 10 stimuli per sec., some slight summation may be seen, *i.e.* relaxation is not complete before the next

contraction begins, and the line joining the apices and basis of the successive contraction ascends slightly. With 20 stimuli per sec. the summation and fusion of each individual contraction is more complete; but the apex of each individual contraction will probably still be seen: the curve is therefore one of incomplete tetanus (Fig. 48). With 30 stimuli per sec. fusion may be complete from the first, *i.e.* complete tetanus, or if not complete at first, it gradually becomes so. This gradually increasing fusion (Fig. 48) is really due to fatigue: for the period of relaxation of the individual contraction tends to become longer and longer, and therefore the next stimulus reaches the muscle progressively earlier in each individual twitch, until a point is reached in which there is no time for the muscle to begin to relax between the stimuli, and fusion becomes complete. With the Wagner's hammer, which causes the muscle to receive 50 or more stimuli per sec., fusion is complete from the first. One other point is to be noted in nearly all these curves: at first the rise in the lever is very rapid, then it suddenly becomes more gradual, but, even when fusion has been complete from the first, the lever may still rise slowly for a short time until the muscle has reached the utmost shortening of which it is capable. If the stimulation is still continued, this height may be maintained for a short time, but sooner or later the lever will begin to drop, showing the onset of marked fatigue. In all cases when the stimulation ceases, the relaxation is at first extremely rapid, then becomes more gradual and a 'contraction-remainder' varying in extent according to the degree of fatigue is generally seen.

The same experiments may be performed with a hyoglossus preparation. This muscle, however, being of the 'granular' variety and having a contraction which lasts twice as long as that of the 'clear' gastrocnemius (see p. 30), is sent into complete tetanus with half the number of stimuli, *i.e.* about 15 per sec.

CHAPTER VIII.

THE PROPERTIES OF NERVE, MINIMAL AND MAXIMAL STIMULI.

A NERVE is not a unit; it is that branch of a nerve-cell which conducts an impulse to, or from, the periphery. A nerve-cell with its dendrites and axis-cylinder process or *axon* forms a unit, the *neuron*. It is convenient, however, to examine the characteristics of a nerve apart from its nerve-cell. The chief of these are *excitability* and *conductivity*. Excitability, or, as it is sometimes called, *irritability*, is the response to a

FIG. 48.—Incomplete tetanus of gastrocnemius. 20 stimuli per sec. Temp. 15° C. (M.S.P.)

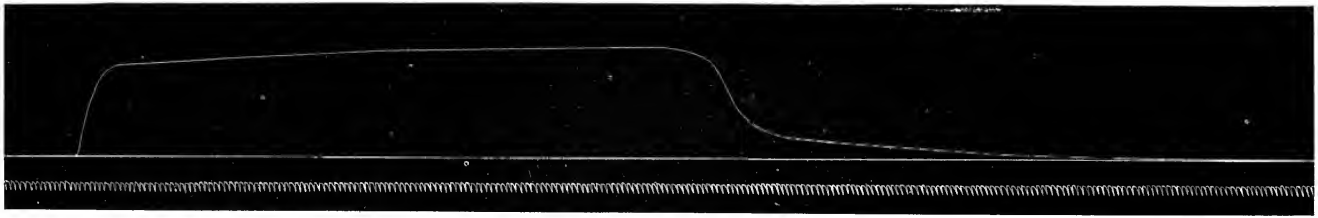


FIG. 49.—Same preparation as Fig. 48. 30 stimuli per sec. Time marker, 50 per sec. Temp., 15° C. (M.S.P.)

stimulus; a nervous impulse, the real nature of which is unknown, is started at the point stimulated, and is transmitted or conducted along the nerve.

Nerves can be stimulated by electrical, mechanical, chemical or thermal agents; of these the most important in experimental physiology is the electrical, for it can be finely graduated, is of extremely short duration, and can be applied repeatedly without damage to the nerve. The first experiments will therefore be the electrical stimulation of nerve.

The Electrical Stimulation of Nerve.—An induction-apparatus is arranged for single induction-shocks, and a simple pair of electrodes is connected with the secondary coil by means of a Du Bois key. A preparation of the sciatic nerve in its entire length and of the gastrocnemius muscle of a pithed frog is made, and near the origin of the nerve is applied the pair of electrodes.

On the passage of an induction-current through the electrodes the nerve is stimulated, and an impulse is sent down the nerve, reaches the muscle, and causes it to contract. This is *indirect* stimulation of the muscle, and is, if a weak current be used, not due to an escape of the electric current along the nerve towards the muscle. This is proved by the following experiment. A moistened thread is tightly tied round the nerve at a point between the electrodes and the muscle. The passage of a weak induction-current of the same strength as that previously used will stimulate the upper portion of the nerve, but the nervous impulse will not pass through the block produced by the thread. A breach in the physiological continuity has been produced, and the nervous impulse is not conducted through the ligatured nerve. The moistened thread would not prevent the passage of a purely electric current.

The response of the nerve to a stimulus bears within certain limits a relation to the strength of the stimulus. This can be shown by the following experiment.

Maximal and Minimal Stimuli.—The muscle of the preparation is attached to a myograph, the lever of which is arranged to write upon a drum covered with smoked paper. The electrodes are placed between the muscle and the ligatured portion of the nerve which was used in the previous experiment. The induction shock is made so weak that no response is obtained, and is then gradually increased until a contraction is observed with the break-shock Contraction does not follow each break-shock; the stimulus is *sub-minimal.* The contraction is recorded as a vertical line upon the stationary drum, and before each stimulation the drum

is turned by hand about half an inch. The strength of the current is slowly increased until a small contraction follows each break-shock; this is the *minimal* stimulus. The distance in centimetres of the secondary from the primary coil is noted upon the drum. The make-shock is weaker than the break, so that it is necessary to use only the one or the other in this experiment.

The intensity of the current is still further increased until the most powerful contraction of the muscle, as indicated by the height of the nearly vertical lines upon the drum, is obtained; the stimulus is now *maximal.* Any further increase in the strength of the stimulus is not accompanied by a bigger contraction; a *supra-maximal* stimulus only produces a maximal contraction, and is liable to damage the nerve.

It may be, as Gotch has recently suggested, that the difference between maximal and minimal stimulation depends upon the number of the constituent fibres of the nerve stimulated. A weak electric current may affect only a few fibres, and therefore the result will be only a slight contraction, due to the excitation of those muscle-fibres alone which are supplied by the nerve-fibres.

It will be found that the excitability of the nerve changes, so that with the same strength of stimulus there will not be the same minimal point. A loss of excitability readily occurs if the nerve be allowed to dry, but during this process there may be irregular fluctuations in the excitability of the nerve above and below the normal.

Mechanical Stimulation of the nerve can be shown by pinching the nerve with a pair of forceps; the muscle contracts, showing that a nervous impulse was produced. Such a method of stimulation injures the nerve, but by means of simple arrangements a nerve can be stimulated mechanically without damage. A light hammer worked by an electro-magnet may be used to tap the nerve, or small drops of mercury from a funnel may be allowed to fall upon the nerve. Such methods are useful in experiments in which an electrical stimulus might introduce a source of fallacy, but for ordinary experiments they are undesirable, since there is a difficulty in maintaining a constant strength of stimulus, and there is a danger of damage to the nerve.

Thermal Stimulation is next shown by the application of a hot wire to the nerve. The muscle contracts. The damaged portion of the nerve is cut away, and to the end of the living nerve is applied a crystal of common salt; the muscle soon shows irregular twitches due to the **chemical stimulation** of its nerve. Such forms of stimuli are obviously limited to special experiments, for the stimulus is not easily graduated and damages the nerve.

CHAPTER IX.

THE RELATION BETWEEN MUSCLE AND NERVE.

THE motor nerves by means of their end-plates are so intimately connected with the muscle-fibres that it is impossible to stimulate the muscle-substance alone by the direct application of a pair of electrodes to the intact muscle. The question, therefore, arises whether muscle possesses *independent excitability*, whether it can respond to a stimulus without the intervention of its nerve. The development of muscle from protoplasm, which is contractile and excitable although possessing no nerves, would suggest that muscle itself is excitable and can respond to a stimulus. This can be shown, for the fully developed muscle, after its nerve has been paralysed by the action of a drug.

Curare[1] is an alkaloid used as an arrow-poison by some natives of South America. The following experiments show that it paralyses the terminations of the motor nerves, but that the muscle still responds to direct stimulation :

(i) Two watch-glasses are almost filled with a 1 per cent. solution of curare in normal tap-water saline. Two muscle and nerve-preparations are made, care being taken to bisect the lower portion of the vertebral column and thus obtain the entire length of the sciatic nerve. The excitability of the nerve and of the muscle in the case of each preparation is tested by the determination of the minimal stimuli. Then the nerve of preparation A is placed in one watch-glass full of the poison, but its muscle is left outside upon a piece of filter-paper moistened with normal tap-water saline. The gastrocnemius muscle of the preparation B is placed in the solution of the drug and its nerve upon the damp filter-paper (Fig. 50). Stimulation of the nerve B will soon produce no contraction, even if the strongest induction-shocks be used; on the other hand, an examination of the nerve A will show that its excitability has practically undergone no decrease. Stimulation of the muscle B which has been exposed to the action of the drug readily produces a contraction. The poison, therefore, must act upon some portion of the terminations of the nerves, probably upon the end-plates, for both muscle-substance and nerve-trunk retain their excitability even after long exposure to the drug.

Muscle will contract on direct stimulation even after its nerves have degenerated. This experiment, however, is not suitable for a class, for it would be necessary to keep the animal alive for two or three weeks in order that the nerve-fibres might completely degenerate.

[1] It is prepared from various plants of the genus Strychnos.

A further experiment with curare can be made. (ii) The cerebral hemispheres of a frog are destroyed, and then the sciatic nerves are carefully exposed in each thigh; a strong ligature is passed under the sciatic nerve of one side, A, and is tied tightly around all the structures of the thigh except the nerve. The circulation of the blood is thus completely stopped in the structures below the ligature. Stimulation of either sciatic nerve produces a contraction of the muscles of the corresponding leg. Under the skin of the back of the frog are injected two or three drops of a 1 per cent. solution of curare. The poison is

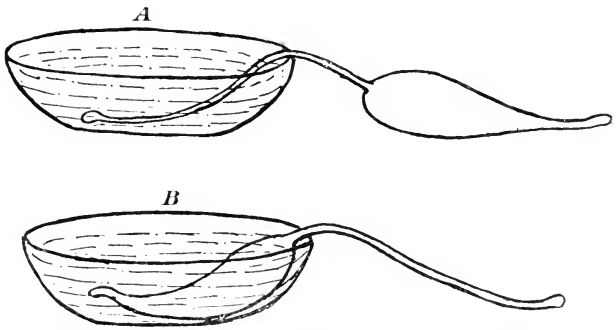


Fig. 50.—Diagram of the experiment on the action of curare.

absorbed by the blood-vessels and is circulated in all parts of the body except those below the ligature. Paralysis is produced, and the frog lies in a toneless condition and does not move if its toes be pinched. Stimulation of the sciatic nerve produces in the case of the ligatured leg, A, a contraction of the muscles, but in the case of the other leg, B, no contraction occurs. The muscles, however, of the leg, B, contract on direct stimulation.

Both nerves in their upper portions have been exposed to the poison, the muscles of both legs respond to direct excitation, but the ligatured leg alone to indirect stimulation. The ligature has prevented the poison from reaching the terminations of the nerves inside the muscles. It is upon these terminations that the curare acts.

The independent excitability of muscle can also be shown in the case of cardiac muscle. The apex of the ventricle of the frog's heart contains no ganglia, but it responds to a stimulus, and under appropriate conditions will even contract rhythmically.

Further experiments upon the independent excitability of muscle are given in the advanced part of the course (p. 315).

[1]This operation should be performed with a pair of Spencer-Wells pressure-forceps in order that no blood may be lost.

CHAPTER X.

THE EFFECT OF A CONSTANT CURRENT UPON MUSCLE AND NERVE.

MUSCLE and nerve consist of complex chemical substances, and contain about 70 per cent. of water in which various salts are dissolved. Moreover they are bathed in lymph.

The passage of a constant current through a liquid produces electrolysis; thus, in the case of water, oxygen is given off at one plate, hydrogen at the other. Animal tissues, containing, in addition to a large percentage of water, salts and proteids, are also the seat of electrolysis during the passage of a constant current; the ions are probably of a complex nature. These changes in nerve and muscle are shown by alterations in excitability and conductivity.

These it is necessary to consider in relation to the changes which occur at the anode and kathode during the make and break of the

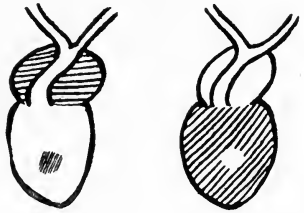


FIG. 51.—Diagram of the frog's heart to show the effects of the make and break of a constant current upon muscle. In A the ventricle is represented as pale and contracted, with a small shaded area to represent the flushed and uncontracted portion of the ventricle; that is, a local diastole during general systole. This condition can be produced by the make of the anode or the break of the kathode of a constant current. In B the ventricle is dilated and flushed, with a small pale area of contracted muscle; that is, a local systole during general diastole. This condition can be produced by the make of the kathode or the break of the anode.

constant current. The simplest experiment can be made upon the frog's heart.

The Effects of Anode and Kathode upon the Frog's Heart.—The brain and spinal cord of a frog are pithed and then the heart is exposed. Care should be taken to avoid the severance of large blood-vessels in order that the vascular system may be well filled with blood. The pericardium is opened and the heart is observed; the ventricle during systole is pale owing to the contraction of its muscle fibres forcing out the blood from its spongy walls; during diastole, when the muscle is relaxed the ventricle is flushed owing to its distension with blood. There are no blood-vessels in a frog's cardiac muscle.

The ends of two pieces of ordinary insulated wire are well cleaned and are connected with a Daniell battery; the clean free ends of the wires are bent back so that there will be smooth surfaces to apply to the heart. The wire connected with the copper of the battery is the *anode*, that with the zinc is the *kathode*.

In the frog's mouth is placed the kathode, for there good contact is obtained with a moist conductor; the anode is placed upon the ventricle. Now it will be found that during the systole of the ventricle that portion of the muscle which is around the anode will be flushed, uncontracted, and bulging outwards—*the anode at the make of the circuit produces a local diastole during general systole* (Fig. 51 A). The rhythmic power of the cardiac muscle around the anode is diminished, so that it remains uncontracted.

If now the wire be suddenly removed from the heart, the break of the anode causes an increased excitability of the muscle to which it had been applied, there is a local pallor; the cardiac muscle is here contracted during the general diastole of the heart. The *break of the anode produces a local systole during a general diastole.*

The kathode is now applied to the heart and the anode is placed in the frog's mouth. There is produced a *local systole during the general diastole of the heart.* The kathode increases the excitability of the cardiac muscle, and thus the fibres affected remain contracted. The end of the wire is kept in contact with the ventricle for about a minute and is then suddenly removed; a flushed and bulging spot will indicate the region to which the wire had been applied. The *break of the kathode produces a local diastole during general systole*, for the disappearance of the condition of katelectrotonus is accompanied by a fall in excitability.

This simple experiment shows that the make of the kathode and the break of the anode excite, that the make of the anode and the break of the kathode depress. This is also true in the case of nerve. (See Advanced Course, Part III., p. 328.)

CHAPTER XI.

THE ELECTROMOTIVE PROPERTIES OF MUSCLE AND NERVE.

In uninjured and resting muscle and nerve there is no electric current, but during activity a current, the '*current of action*,' is produced. Injury causes local activity around the damaged tissue, and is therefore accompanied by an electric current, the so-called '*demarcation* or

injury-current.' This electrical current produced by injury is, as Gotch pointed out, to be considered as a *current of action.* These facts can be demonstrated by the following experiments.

The Rheoscopic Frog. Galvani's Experiment, Contraction without Metals.—A long length of the sciatic nerve is dissected in a pithed frog and the muscles of the thigh are exposed and cut across. The trunk of the sciatic nerve is laid along the longitudinal surface of the muscles of the thigh, and then by raising the end of the nerve by a small glass rod the transverse section of the nerve is allowed to fall upon the cut surface of the muscles (Fig. 52). At this moment a twitch of the

FIG. 52.—Diagram of Galvani's experiment. Contraction without metals

muscles of the leg moves the foot or toes. The circuit of the electric current in the muscle has been completed through the nerve. The section of the muscle-fibres has produced a local contraction of the fibres, and this is accompanied by an electrical change which is sufficient to produce excitation when it is passed through an excitable nerve.

Secondary Contraction or Secondary Twitch.—Two muscle- and nerve-preparations are made; the nerve of A is so placed upon the muscle B that the cut surface of the nerve lies upon the tendon and its longitudinal surface upon the muscle-fibres (Fig. 53). The nerve of preparation B is stimulated by a weak induction-shock, and thus its muscle is excited and made to contract; the muscle A will also contract. The contraction of the muscle B is accompanied by an electrical current, the '*current of action,*' which passes through the nerve A and thus produces a contraction in the muscle A. This is not due to an escape of electrical current from the electrodes, for a secondary twitch can be obtained if mechanical or thermal stimuli be

used to excite the nerve of preparation B. Further, ligature of the nerve B with a moist thread will show that there is no escape with a weak induction-shock; the ligature destroys the physiological continuity and prevents the passage of the excitatory state but not that of an electrical current.

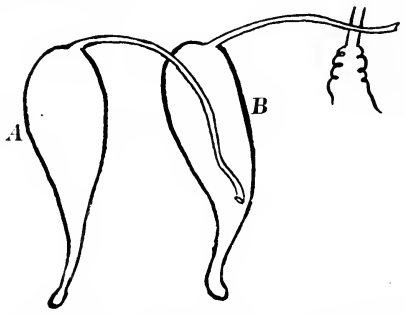


FIG. 53.—Diagram of the experiment on secondary twitch.

Secondary Tetanus.—If the nerve be stimulated with a rapid series of induction-shocks the muscle B goes into tetanus and its '*currents of action*' stimulate the nerve A, with the result that the tetanus is also observed in the muscle A. This 'secondary tetanus' can be produced by rapid mechanical stimuli.

Further experiments upon the electromotive properties of muscle and nerve are given in the Advanced Course (page 331).

CIRCULATION, RESPIRATION, AND ANIMAL HEAT.

CHAPTER XII.

THE ANATOMY OF THE FROG'S HEART AND ITS CONTRACTION.

Anatomy of the Frog's Heart.—At the mid-point of a horizontal line drawn through the posterior margin of the eyes a blanket pin is passed through the skull of a frog, and the cerebrum destroyed. To prevent bleeding the hole is plugged with a piece of lucifer match cut to a blunt point. The cerebrum can be destroyed equally well by the application of a strong pair of Spencer-Wells forceps to the skull. The medulla oblongata and spinal cord are left intact, so that the vasomotor control continues and the circulation is unimpaired. The frog is pinned on the corkplate belly uppermost. The skin over the abdomen is pinched up and slit up to the mouth. The abdominal wall is then divided slightly to one side of the mid-line to avoid cutting the anterior

abdominal vein. By a transverse cut the xiphisternum is divided and the junction of the anterior abdominal vein with the heart preserved. The pectoral girdle is next divided in the middle line. The inner blade of the scissors is kept hard against the sternum to avoid injuring the heart beneath. The divided halves of the pectoral girdle are pulled widely apart. The heart is now seen enclosed in a thin membrane—the pericardium. This is picked up with forceps and slit open. A slender band of connective tissue—the fraenum—connects the posterior surface of the heart with the pericardium. A thread is passed under the fraenum with fine pointed forceps and tied. The fraenum is then divided on the side of the thread remote from the heart.

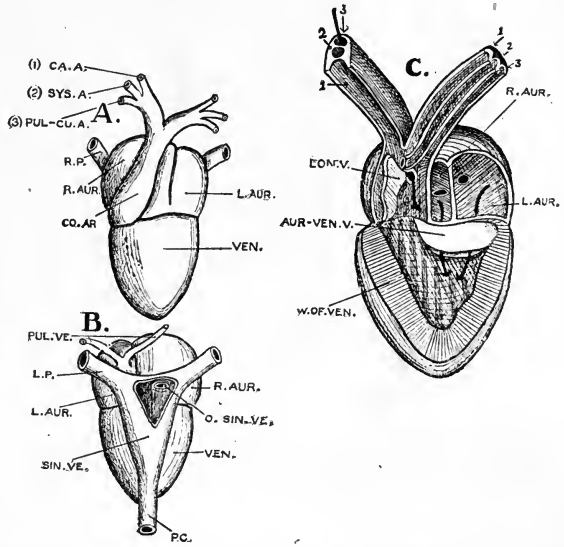


FIG. 54.—The frog's heart. A, Anterior view; B, Posterior view; C, Longitudinal section. (Mudge.)

By means of the thread the heart can be lifted up and turned over for examination. In the front aspect of the heart a single blunt pointed ventricle is seen with the bulbus arteriosus and the two auricles—the bulbus ascends over the right auricle from right to left. It separates into two aortae. Each aorta is divided by longitudinal septa into three channels which soon separate and become the carotid, the aortic, and the pulmono-cutaneous arches.[1] The auriculo-ventricular groove separates the auricles from the ventricle. On turning the heart over the sinus venosus is seen, and the white crescentic line which marks the

[1] The frog respires both by skin and lungs.

junction of the sinus with the right auricle. Entering the sinus from below is a large vein, the vena cava inferior, into which open the hepatic veins. Above there enter the two smaller superior venae cavae. These are seen on gently displacing the auricles. The small pulmonary veins enter the left auricle.

The Contraction of the Heart.—The venae cavae and sinus beat first, then the auricles, and lastly the ventricle and bulbus arteriosus. The blood is returned from all parts of the body to the sinus venosus, whence it passes to the right auricle. From the pulmonary veins the blood passes into the left auricle. The two auricles simultaneously contract and expel the blood into the ventricle. The two blood streams do not readily mix in the ventricle owing to the muscular meshwork within its cavity. When the ventricle contracts the venous blood on the right side is the first to enter the bulbus arteriosus. It is directed by a spiral valve within the bulbus into the pulmono-cutaneous arteries. The spiral valve is then driven over and closes the orifice of the pulmono-cutaneous arch, and the blood (partly arterial and partly venous) now passes into the systemic or carotid arch. The resistance is least in the systemic arch, so most of the blood at first takes this pathway. Finally, as the pressure increases in the systemic arch, the pure blood from the left side of the ventricle is expelled into the carotid artery. Between the auricles and ventricle there hangs the auriculo-ventricular valve. The bulbus arteriosus contains two sets of pocket-shaped valves in addition to the longitudinal spiral valve.

The ventricle becomes smaller, harder, and pale in colour during systole, as the blood is driven out of the muscular sponge-work of which it is composed. It reddens in diastole. Count the beats per minute.

The Tissue of the Heart possesses Automatic Rhythmic Power.—Excise the heart, cutting widely round the sinus venosus, and place it in a watch glass. Note the immediate effect and the after-effect on the rhythm. The beats may at first intermit and then become more frequent, but quickly settle down to about the same rate as before.

The Effect of Temperature on the Rhythm.—Pour on the heart some normal saline solution which has been cooled in ice. The frequency becomes greatly lessened. Replace the cold with warm saline (25° C.). The heart-beats become frequent as the temperature rises. If heated to 40°–43° C. the heart stops still in diastole, but may recover if quickly cooled. If not cooled the heart passes into the condition of *heat rigor*. Taking another heart, cut away the sinus at the sino-auricular junction. After a short period of inhibition both parts begin to beat, but with a

different rhythm. The sinus is the more injured, and beats at a slower rate. If the cut be made through the auricles, the sinus beat continues and is unaffected by the injury. Cut off the ventricle just above the auriculo-ventricular groove. After a period of inhibition both auricles and ventricle beat. The auricles recover first. Cut through the ventricle below the auriculo-ventricular groove. The **apex preparation** does not beat spontaneously. It responds to a prick by a beat, and may in some cases be taught to beat rhythmically by rhythmic stimulation. A crystal of common salt placed on the apex or the passage of the galvanic current through the apex preparation provokes its rhythmic contraction.

Rhythmic Contraction—the function of the Heart Muscle.—Cut out small pieces of the bulbus arteriosus, and place them under the microscope in a watch glass containing Ringer's fluid. The pieces will beat rhythmically. There are few if any nerve cells in the bulbus, and there are certainly none in some of these pieces, so the rhythm must be the function of the heart muscle.

A frog's heart painted with nicotine (1 per cent. solution) continues to beat. Nicotine paralyses nerve cells (Chapter XVII.).

Isolated portions of the mammalian heart will beat rhythmically for hours if fed through their nutrient arteries (with oxygenated serum). The serum is supplied from a reservoir which need be raised only slightly above the level of the heart. The preparation and the reservoir of serum are together enclosed in a strong glass chamber. The chamber is kept at body temperature, and into it oxygen is forced up to a pressure of two atmospheres. Under this pressure the oxygen dissolved in the serum reaches a tension which is sufficient to maintain the vitality of mammalian tissues (Porter).

Demonstration.—A hen's egg incubated 24-36 hours is broken, and the contents floated out into a dish. With the aid of scissors and forceps the investing albumin is removed. The beat of the heart may be observed in the embryo under the microscope. The embryonic heart beats before its muscular tissue become differentiated, and before any nerve-cells become included within its structure. The inherent power of rhythmic contraction is seen in the chick heart by the 24th hour of incubation, while the migration of ganglion cells into the heart does not take place till the 3rd day.

The structural elements of the heart are nucleated, branched, and cross-striated cells. The muscle-cells are joined together into networks and bands, so as to form one functional whole, and hence excitation of any one part leads to the contraction of the whole. The first part to begin to functionate in the embryo is the venous end. In

the mammalian heart it has been shown that muscle fibres of an embryonic type connect the auricles with the ventricles.

The above experiments prove that rhythmic contractility is the inherent function of the cardiac muscle. The muscle of the sinus and auriculo-ventricular junction is more embryonic in structure and possesses greater power of initiating rhythm. It is less excitable, and conducts a stimulus less rapidly than the muscle of the auricles and ventricle. The auricular and ventricular muscle is more differentiated in structure. The cross striæ are more marked. It does not so easily initiate rhythm. Owing to its greater excitability and conductivity it follows the lead of the sinus.

During the period of systole the heart is *refractory* to artificial excitation. The excitability returns with diastole, increasing as diastole proceeds. The energy of any cardiac contraction depends on the previous activity of the heart, on the pressure of the diastolic filling, on the resistance to systolic outflow, temperature, nutrition, etc. It is independent of the strength of the stimulus so long as the latter is efficient Owing to the refractory period, the slow rate of contraction, and the independence of the amplitude of contraction on the strength of stimulus, the heart cannot be tetanised.

By the study—with the aid of the capillary electrometer—of the electrical current of action which accompanies the systole, it has been shown that the contraction of the heart is a simple twitch, and not a tetanus. The current of action is triphasic in the mammal—(1) base negative, (2) apex negative, (3) base negative. The excitatory wave travels from base to apex and from apex to base, following the course of the muscle-bands, which start from the base, run to the apex, and, turning in there, ascend on the inner wall of the ventricle. The current of action travels at the same rate as the excitatory state. The power of slow, sustained contraction seems to depend on the richness of the heart-muscle in sarcoplasm. The heart-muscle possesses tone, and this varies with the temperature and nutrition. Muscarine, acids and chloroform weaken, while digitaline, caffeine, and alkalies increase the tone of the heart. The auricular muscle of the toad exhibits rhythmic alterations in tone.

Antiperistalsis is difficult to produce because the excitatory process in the ventricle is slow, and does not easily affect the more rapidly contracting auricle. The refractory period which persists during systole also prevents antiperistalsis. The excitatory wave is delayed in passing through the more embryonic type of muscle in the sino-auricular and auriculo-ventricular junctions, and therefore the auricle beats in sequence to the sinus and the ventricle in sequence to the auricle. By

cooling the sinus and warming the ventricle the sequence of the heart can be reversed, for the excitability of the ventricle is by these means raised, while that of the sinus is lowered.

By gently clamping a strip of tortoise auricle muscle between two little bits of cork an artificial block can be created, and the piece of auricle below the clamp then beats in sequence to the piece above the clamp. The natural delay in conductivity at the auriculo-ventricular junction is thus imitated (Gaskell). The conductivity is decreased by the clamp (see Advanced Course, page 346).

The nerve cells of the heart are placed in the least differentiated parts: in the sinus (Remak's ganglion), in the inter-auricular septum (v. Bezold's ganglion), and in the auriculo-ventricular groove (Bidder's ganglion). The nerve cells are the cell stations of the vagus nerve. The nervous system regulates, but does not initiate either the rhythm or sequence of the heart. The maintenance of the rhythm depends on the blood, and there is evidence to show that it especially depends on the oxygen, and on the mineral salts which are in solution in the blood.

The chief mineral salts, chlorides and phosphates of sodium, potassium, and calcium, are dissolved in the blood in minute traces, and are in a state of ionisation. The presence of these ions seems to be absolutely necessary for the production of the excitatory state. During diastole the production of synthetic compounds is, it is supposed, pushed beyond the limit of stability until there results in systole an explosive liberation of energy. As the mineral salts in the serum, with a due supply of oxygen and water, are sufficient to maintain the frog's heart in rhythmic activity for hours, it is clear that the heart muscle contains a large supply of explosive material in its sarcoplasm.

CHAPTER XIII.

METHODS OF RECORDING THE HEART.

The Suspension Method of Recording the Heart-beat.—The frog is placed on a cork plate which is fixed to the stand beneath the crank myograph lever. A fine pin is bent into the shape of a hook and passed through the tip of the apex of the ventricle. A thread is attached to this hook and to the lever. A sufficient weight is hung over the pulley on the axis of the lever so as to slightly stretch the heart. The tissues round the base of the heart are pinned down to the cork plate on which the frog rests.

The lever is provided with a long light straw. In place of the weight a fine rubber thread may be attached to the lever. One end of the

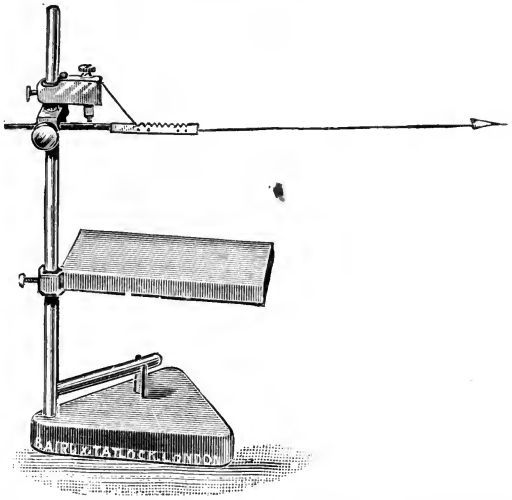


FIG. 55.—Suspension method of recording the contraction of the frog's heart, with use of rubber thread as a spring.

rubber thread is tied on to the short arm of the lever, and the other end is pinned to the cork of the myograph. By making the rubber thin

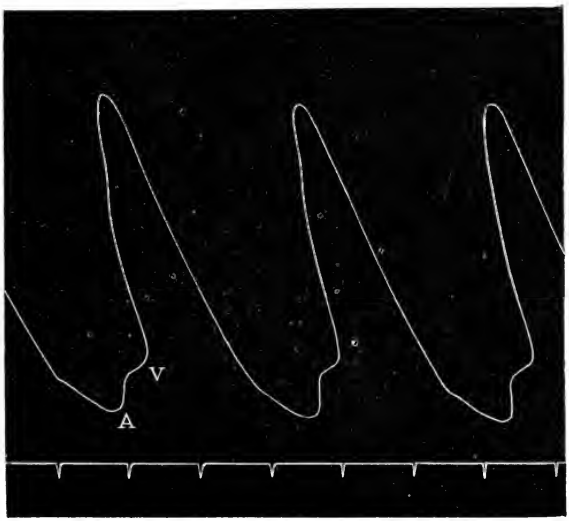


FIG. 56.—Contractions of the frog's heart. A=auricular, V=ventricular contraction. The time is marked in seconds. The curve should be read from left to right. (L.H.)

enough and adjusting its tension so that the lever is horizontal, a large excursion can be obtained.[1]

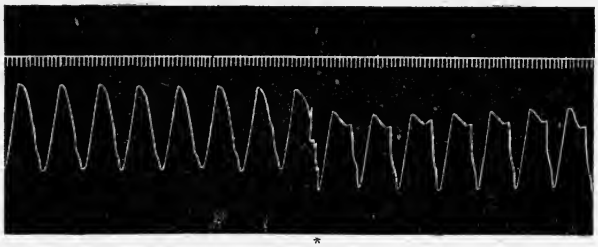


FIG. 57.—Contraction of the frog's heart. The curve should be read from right to left. The effect of rendering heart bloodless. Note the plateau on the top of the normal ventricular curve, and the pointed top after the blood has escaped at the point marked by the star. Time marked in fifths of seconds. (L. H.)

Record the heart-beats on a drum (slow rate). Note the auricular and ventricular curves, and the rounded top or plateau of the ventricular curve. Render the heart bloodless by opening an auricle. The apex of the ventricular curve becomes pointed. Internal tension excites the muscle of the heart to more prolonged and sustained contractions.

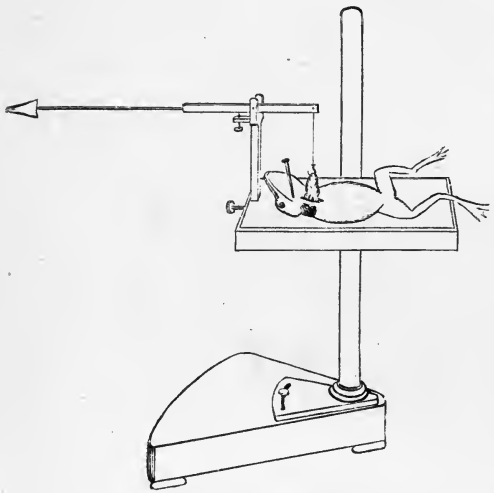


FIG. 58.—Lever, for recording the frog's heart. (Pembrey and Phillips.)

Another method of recording the heart is shown in Fig. 58. A long light straw lever is taken, and a needle is passed through it. The needle plays in holes in the brass upright. The thread from the heart is attached as shown.

The excised heart can be recorded by a similar lever represented in Fig. 59. A piece of lead is bent as shown and fixed to the cork plate

[1] With the form of heart-lever (Fig. 55) the contraction is represented by the down-stroke; with the lever (Fig. 58) the contraction is indicated by the up-stroke. The curves obtained with the former lever can be best compared with those made with the latter by turning the tracing upside down and reading from right to left.

by drawing pins. A needle passes through the straw lever and holes in the lead. A lump of modelling wax is placed on the long straw lever as a counterpoise, and another piece of modelling wax attached to the straw is arranged to rest on the heart.

Effect of Heat and Cold on the Excised Frog-heart.—Expose the heart of a pithed frog. Pass a small hook attached to a thread through

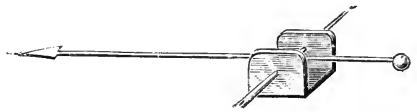


Fig. 59.—Method of recording the excised heart.

the tip of the ventricle. Excise the whole heart, cutting widely round it, and pin the tissues surrounding the base of the heart to a cork

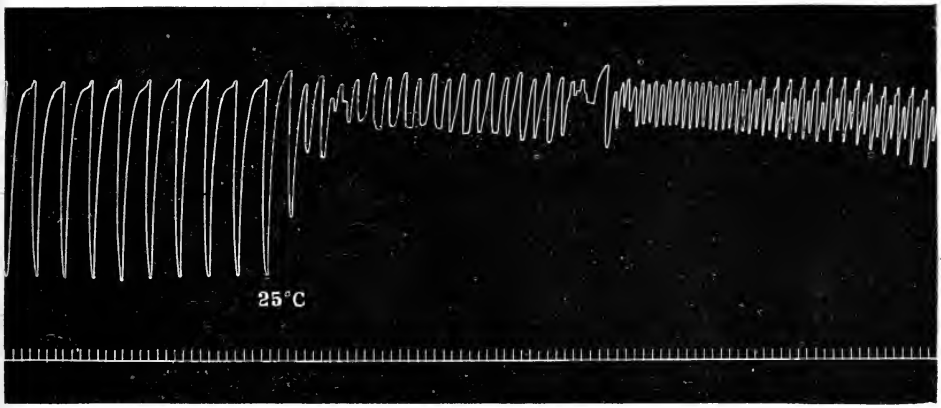


Fig. 60.—Contraction of the frog's heart recorded by the suspension method 15° C. and then immersed in saline at 25° C. The curve should be read from left to right. The time is marked in seconds. (L.H.)

which is attached to the bottom of the vertical limb of a T-piece. The T-piece is placed beneath the recording lever, and the thread which was attached to the ventricle is fastened to the lever. An elastic thread is used as a spring as in Fig. 55 and the record taken by the suspension method. Take a tracing of the heart when immersed in a beaker of Ringer's fluid at room temperature (12-15° C.). Ringer's solution is made by saturating 0·65 % NaCl with calcium phosphate and adding to each 100 c.c. of this solution 2 c.c. of 1 % KCl. Next fill the beaker with Ringer's fluid which has been kept in broken ice, and take another record. The cooled heart gives slow and forcible beats.

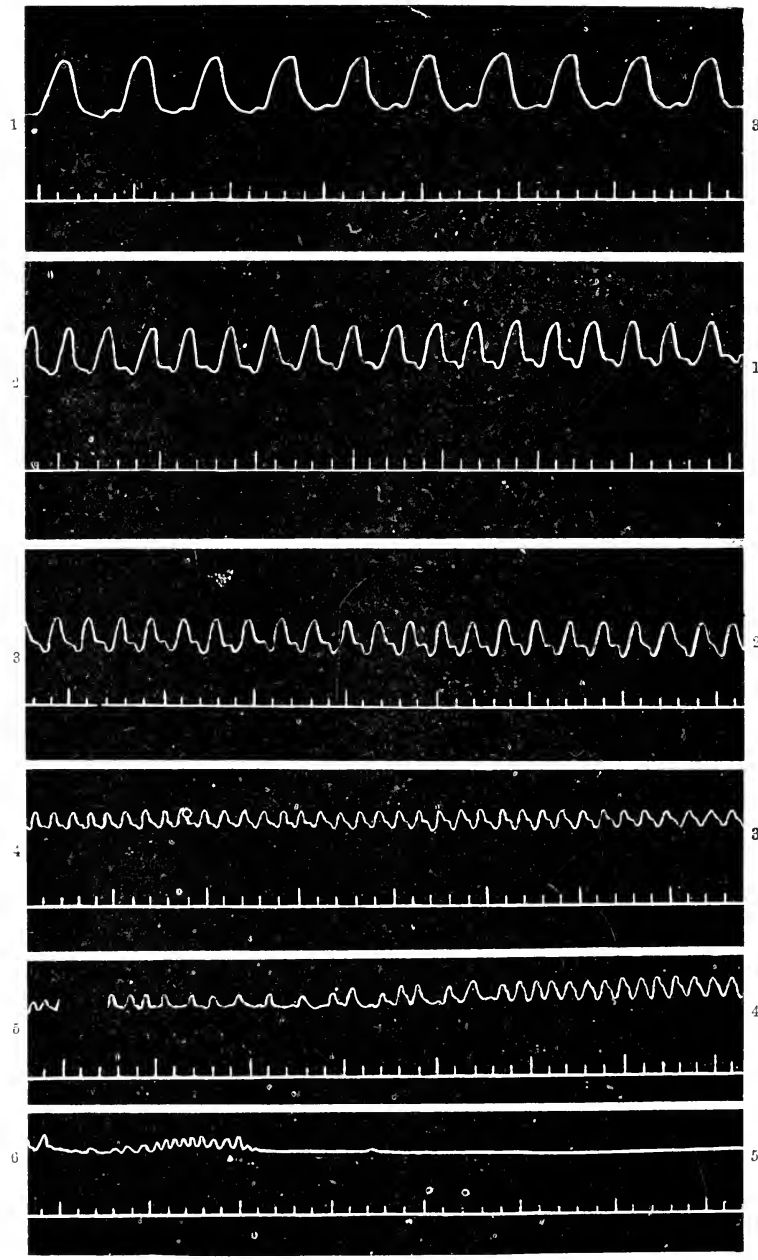


FIG. 61.—Contraction of the frog's heart recorded by the suspension method. Effect of pouring over the heart normal saline at the temperatures indicated. The water cools rapidly when this method is used, and the heart is not heated throughout its mass to the temperature indicated. (Pembrey and Phillips.)

The periods of contraction and relaxation are prolonged, the frequency greatly diminished. Now fill the beaker with Ringer's fluid at 25° C. The frequency becomes greatly increased, and the period of contraction and relaxation greatly shortened. A temperature of about 35° C. causes diminution of the tone of the heart. The ventricle ceases to follow the auricular rhythm, although it still responds to excitation. At 38° to 43° C. the whole heart ceases to beat, and gradually passes into the condition of heat rigor. The heat contraction, when once fully established, is not set aside by cooling.

CHAPTER XIV.

THE STANNIUS HEART.

The Stannius Heart.—The heart of a pithed frog is exposed and a thread is tied to the fraenum which is then cut away from the posterior surface of the pericardium. Pass a ligature under the two aortae and then by means of the thread attached to the fraenum gently pull the heart towards the mouth of the frog. The dorsal aspect of the heart is now readily seen. Draw the ligature round the white crescentic line which marks the sino-auricular junction and tie it exactly over this line. The sinus continues to beat, while the auricles and ventricle, after giving a few rapid beats, stand still. The sinus, with its more embryonic type of muscle, possesses the greatest power of initiating rhythmic contraction. The more specialised muscle of the auricles and ventricle is more excitable, and conducts an excitatory wave more rapidly, but is less capable of initiating rhythm. The excitatory wave which is started from the sinus is blocked by the ligature; thus the auricles and ventricle cease to beat. Prick the ventricle; it will respond by a single beat to each stimulus. The Stannius preparation is like a muscle preparation, and can be used to record the contraction of the heart and the latent period. Tie a second ligature just above the auriculo-ventricular groove. Both auricle and ventricle are excited by the ligature and start beating. The rhythm is no longer the same in the three chambers of the heart. The mere contact of the lever or electrodes resting on the Stanniused heart sometimes evokes rhythmic

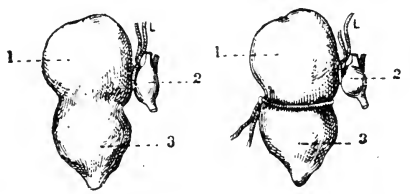


FIG. 62.—Stannius heart. The first and second ligatures (Hedon).
1, Auricles; 2, Sinus; 3, Ventricle.

contractions. The inhibitory effect of the first ligature has been attributed by some authors to excitation of the vagus nerve.

The Heart cannot be thrown into Complete Tetanus.—Set up a circuit for giving single induction shocks (see Fig. 16, p. 9). Apply

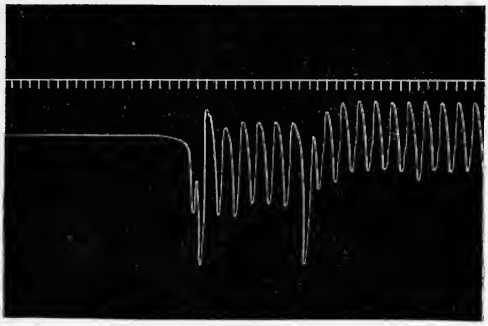


FIG. 63.—Contraction of the frog's heart recorded by the suspension method. The effect of tightening the first Stannius ligature at first gently and then firmly. The curve should be read from right to left. The time is marked in seconds. (L.H.)

the electrodes to the Stanniused heart and record the effect of rapidly repeated excitations. The heart gives an incomplete tetanus curve. Owing to the refractory period it cannot be completely tetanised.

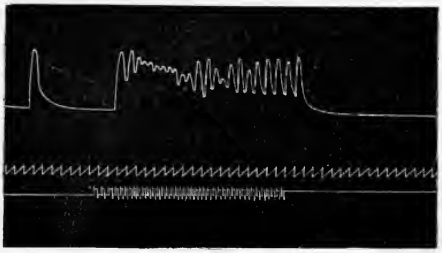


FIG. 64.—Effect of tetanising the Stanniused heart. The curve should be read from left to right. The time is marked in seconds. The third line shows the period of stimulation. (L.H.)

The Extra-systole and Compensatory Pause.—Excite with a single induction shock a rhythmically beating heart. The heart is recorded as in Fig. 55 or 58. An extra contraction excited during the diastolic period of the rhythmically beating heart is followed by a compensatory pause. Note that the heart does not respond when excited during systole—*the refractory period* (Fig. 217).

This period of inexcitability is seen in skeletal muscle (p. 42), but is of much shorter duration than the refractory period of the heart. The difference probably depends upon a slower metabolism in the cardiac muscle.

CHAPTER XV.

THE CARDIAC NERVES AND GANGLIA.

The Intra-cardiac Ganglia, and Nerves.—The vago-sympathetic nerves enter the sinus with the superior venae cavae, and form a plexus there which contains many ganglion cells (Remak's ganglion). The nerves pass on to enter the auricular septum, which also contains ganglion cells (v. Bezold's ganglion). Leaving the septum the nerves enter the auriculo-ventricular junction, where third groups of ganglion cells lie (Bidder's ganglion).

To see these structures (Fig. 65), forcibly inject the living heart with osmic acid (1% sol.), passing the needle of the hypodermic syringe into the auricle. The osmic acid almost instantaneously fixes the heart in distension. Cut out the heart and place it in a watch-glass of 1% osmic. After 5 minutes open the auricles under water, look for the septum and cut it out, including its attachments to the ventricle. Mount the septum in glycerine, and examine it microscopically. The nerve fibres and ganglion cells will be apparent in the septum.

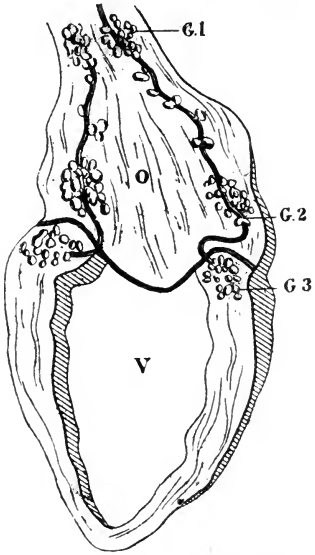


Fig. 65.—Inter-auricular septum and ventricle showing the vagus nerves and ganglia. G. 1 Remak's, G. 2 v. Bezold's, and G. 3 Bidder's. (Hedon.)

Dissection for Exposing the Vagus in the Frog.[1]—Lay the pithed frog on its back, and cut through the skin and sternum. Pin out the fore-limbs so as to pull the divided halves of the pectoral girdle widely apart. Open the pericardium and divide the fraenum. From the angle of the jaw on either side trace the thin band-like petro-hyoid muscles. These muscles arise from the skull, and circle round to the thyroid process of the hyoid. The petro-hyoids are crossed by two white nerves, which are clearly visible. One, the glosso-pharyngeal, curves round from the angle of the jaw, and disappears among the muscles of the floor of the mouth. The other, the hypo-glossal, takes the same direction, but lies nearer to the mid-line of the mouth.

The vagus, dividing into its cardiac and laryngeal branches, lies at

[1] See also another method of dissection, p. 69.

the lower border of the petro-hyoid muscle. It is a small nerve, and not easily seen. Having traced the nerve so far, cut away the lower jaw and as much of the larynx as can safely be removed. Next cut away the mucous membrane which covers the base of the skull and upper vertebrae. You will thus expose on either side a broad muscle, the levator scapulae inferior. This muscle arises from the skull round the jugular foramen, and is inserted in the scapula. Unpin the frog, and hold the skull in the left hand, so that, while the skull is horizontal, the body hangs vertically. Cut through the levator muscle, and under the upper part of this muscle observe the vagus ganglion and the vagus and glosso-pharyngeal nerves. Trace the sympathetic nerve, which is marked by black pigment, along the upper vertebrae to its junction with the vagus ganglion. The cardiac sympathetic fibres arise from the 3rd spinal nerve, and probably have their cell stations in the third sympathetic ganglion. Pass a fine thread (by means of a sewing needle) under the sympathetic at the level of the large brachial (2nd spinal) nerve. Tie it, and divide the nerve below the ligature. Pass a thread under the glosso-pharyngeal and vagus nerves, but do not tie it.

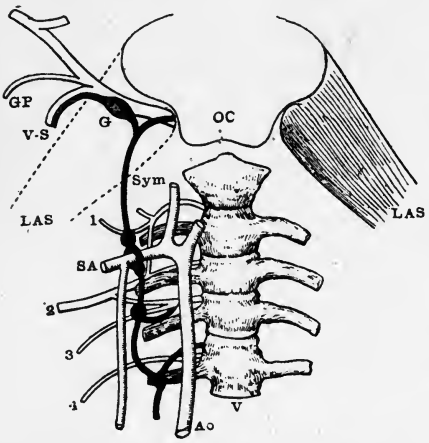


FIG. 66.—Diagram of the origin of the vago-sympathetic nerve (V.-S.). L.A.S. = levator anguli scapulae muscle. Ao. = aorta. 1, 2, 3, 4, = first to fourth spinal nerves. Sym. = sympathetic nerve. G.P. = Glosso-pharyngeal nerve. G. = vagus ganglion. (Gaskell.)

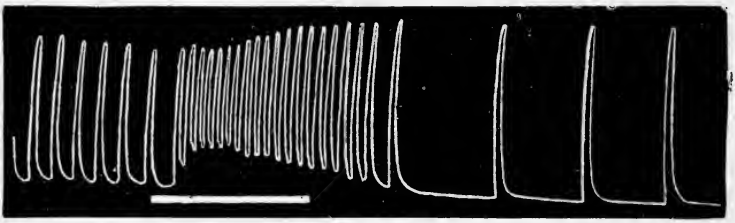


FIG. 67.—Contraction of the frog's heart. The effect of weak stimulation of the vago-sympathetic nerve. The white line marks the duration of excitation. Note the latent time, the acceleration and increased tone and the after-effect. The curve should be read from left to right. (Pembrey and Phillips.)

Place the frog again on the board, and record the heart by the suspension method (slow rate of drum). With the interrupted current

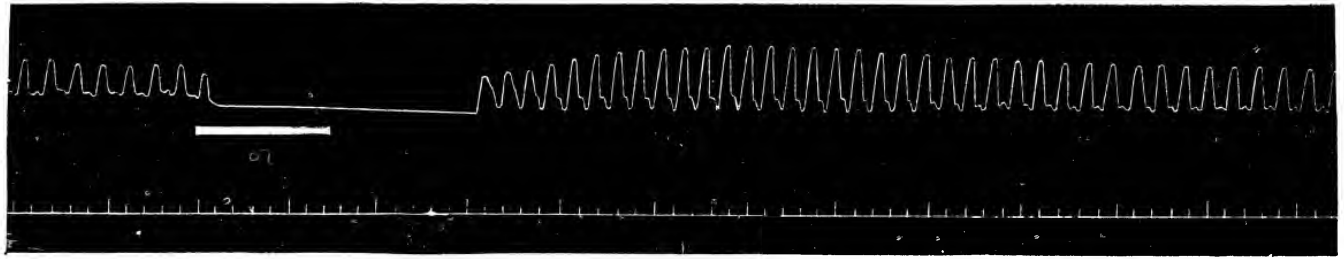


FIG. 68A.—Contraction of the frog's heart. The effect of strong stimulation of the vago-sympathetic nerve. The white line marks the duration of excitation; the time is marked in seconds. The curve should be read from left to right. (Pembrey and Phillips.)

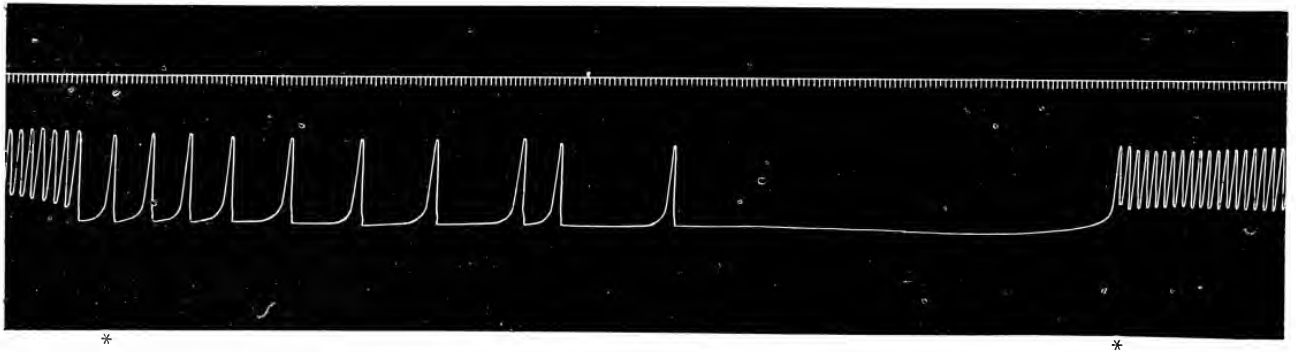


FIG. 68B.—Contraction of the frog's heart. Excitation of the vago-sympathetic nerve between the points starred. Note the escape from complete inhibition. The time is marked in seconds. The curve should be read from right to left. (L.H.)

stimulate the sympathetic. Use fine electrodes and a strength of current just comfortable to the tongue.[1]

The heart-beats are accelerated and augmented after a long latent period. This effect is prolonged for a considerable time after the excitation has ceased. The after effect is decreased frequency and amplitude.

Next pass the electrodes under the vago-sympathetic trunk. The heart-beats will either be arrested (inhibited) after a brief latent period or decreased in frequency and amplitude. There is a short after effect; the heart soon escapes, even if the excitation be continued. The after effect is usually increased frequency and amplitude. The returning beats frequently show the staircase effect. Sometimes the sympathetic effect overpowers the influence of the vagus. To stimulate

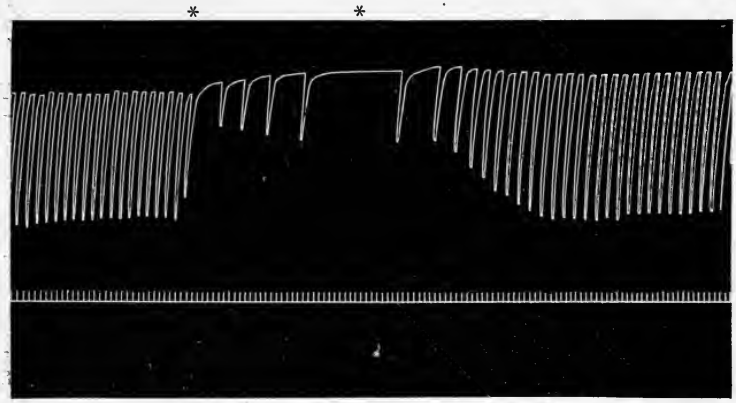


FIG. 69.—Excitation of vago-sympathetic. Note the after effect—a staircase augmentation of the heart-beat. The stars indicate the beginning and end of stimulation. The downstroke represents contraction. (See footnote, p. 60.) The time is marked in seconds. (L.H.)

the pure vagus fibres, the cerebrum is destroyed, the cervical cord divided, and the spinal bulb excited. During the state of complete inhibition the heart may not respond to mechanical excitation.

There is little evidence in support of the old view that the excitatory state is transmitted through the heart and the contraction regulated in sequence by the nerve ganglia of the heart. The ganglion cells, which wander into the embryonic heart some days after it has started beating, can be removed without disturbing the cardiac rhythm. Extra-cardiac

[1] The electrodes may be made of fine covered wires. The ends, for $\frac{1}{2}$ inch, are stuck together by melted paraffin. The paraffin is grooved with a knife, so as to lay bare the wires at a point $\frac{1}{8}$ inch from their end. The wires are passed through slits in a small piece of cork. The cork may then be pinned in any suitable position.

nerve-fibres from the vagus have their cell-stations in these ganglia. The sympathetic cardiac fibres have their cell-stations in the 3rd sympathetic ganglion in the frog, in the stellate or 1st thoracic ganglion in the mammal. Non-medullated nerve-fibres spin a fine network through all parts of the cardiac muscle. A great many of the cardiac nerve-fibres are centripetal or afferent, and convey impulses up the vagi to the spinal bulb, which reflexly control the tonus of the blood-vessels, and possibly the frequency of the heart and the respiration. The centrifugal cardiac nerves influence the frequency and force of the cardiac contraction and the conductivity of the cardiac muscle (chrono-, ino-, and dromo-tropic of Engelmann). The inhibitory fibres run in the vagus and arise from a centre in the spinal bulb which is in tonic action and curbs the heart. The function of the vagus is to decrease the frequency, force, and excitability The sympathetic fibres, which arise in the mammal from the anterior spinal roots in the upper thoracic region, antagonise the action of the vagus. The vagus, by reducing the heart-beat, causes anabolism, and the sympathetic katabolism of the cardiac muscle. The after-effect of vagus excitation is increased energy of contraction, while that of the sympathetic is exactly the opposite. The function of the cardiac nerves is to co-ordinate the beat of the heart to the needs of the body, and to co-ordinate the functions of the other organs of the body to the needs of the heart.

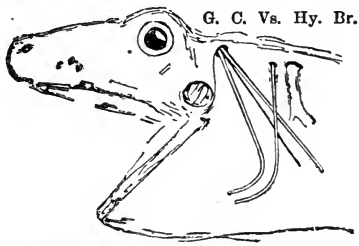


Fig. 70.—Diagram of nerves in the frog's neck. Dissection from behind. (Pembrey and Phillips.)

Dissection of Vago-Sympathetic Nerve from behind.—The skin in the mid-line of the back is divided and the scapula lifted up and cut away. The fore-limb is pulled outwards and then removed. A small plug of paper is placed in the frog's mouth to put the parts on the stretch. In front of the divided brachial plexus (Br., Fig. 70) there can be seen (Hy.) a much smaller nerve—the hypoglossal—which is the first spinal nerve in the frog and passes down to the floor of the mouth; (VS.) the vago-sympathetic, which can be traced from the skull, and runs by the side of the carotid artery (C.) and crosses underneath the hypoglossal nerve; (G.) the glosso-pharyngeal. This nerve issues with the vago-sympathetic nerve, but soon turns downwards and forwards to the floor of the mouth. The glossopharyngeal and hypoglossal nerves are then cut and a small piece of the bone containing the foramen from which the vago-sympathetic nerve issues is cut away from the skull. By means of this piece of bone the vago-sympathetic nerve can at any time be lifted up without damage and laid upon electrodes.

CHAPTER XVI.

THE SINO-AURICULAR JUNCTION. THE ACTION OF DRUGS.

Inhibition Produced by Excitation of the Sino-Auricular Junction.—The heart is recorded by the suspension method. Observe the white tendinous line which marks the sino-auricular junction. It is curved with its convexity upwards. This is known as the *crescent.* Pin the cork of the fine wire electrodes to the frog-plate so that the ends of the electrodes touch the crescent. The ends must not be more than 2 mm. apart. Start the drum (slow rate), record half-a-dozen beats, and then tetanise the crescent. The heart, owing to direct excitation of the muscle, at first

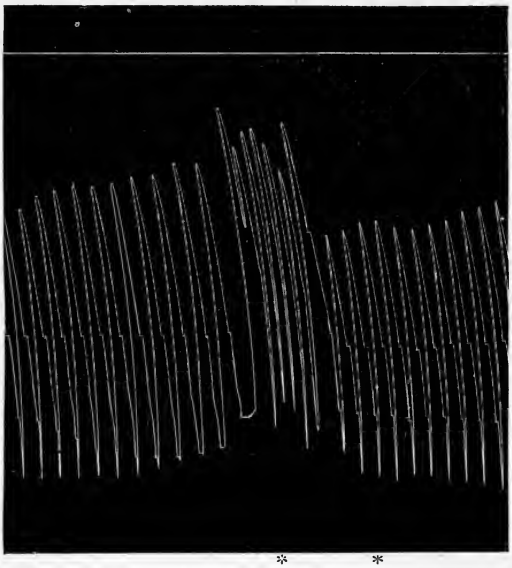


FIG. 71.—Contraction of the frog's heart. Excitation of the sino-auricular junction. Arrest of auricles and increased rate of ventricle (incomplete tetanus). A pause followed the cessation of the excitation. The curve should be read from right to left. The stars indicate the beginning and end of stimulation. (L.H.)

beats faster, and then is arrested in diastole. Sometimes the arrest does not take place till the excitation ceases. The heart soon escapes from arrest. The arrest is due to excitation of the intra-cardiac branches of the vagus. Mechanical stimulation of the ventricle during the arrest will cause a reversal of the natural sequence. The sinus continues to beat during the period of arrest. The excitatory wave is blocked in the auricular muscle.

Action of Muscarine and Atropine.—Dissect out the vago-sympathetic nerve and record the effect of excitation of (1) the vago-sympathetic,

(2) the crescent. Next with a glass pipette apply to the heart a few drops of nitrate of muscarine (10% solution). The tone, frequency, and amplitude of the heart will decrease until at last the heart becomes arrested in diastole. Mechanical excitation may still excite the heart to give a single contraction.

Now apply some drops of a 0·2—0·5% solution of atropine sulphate. The heart will begin to beat again, at first feebly, and then with

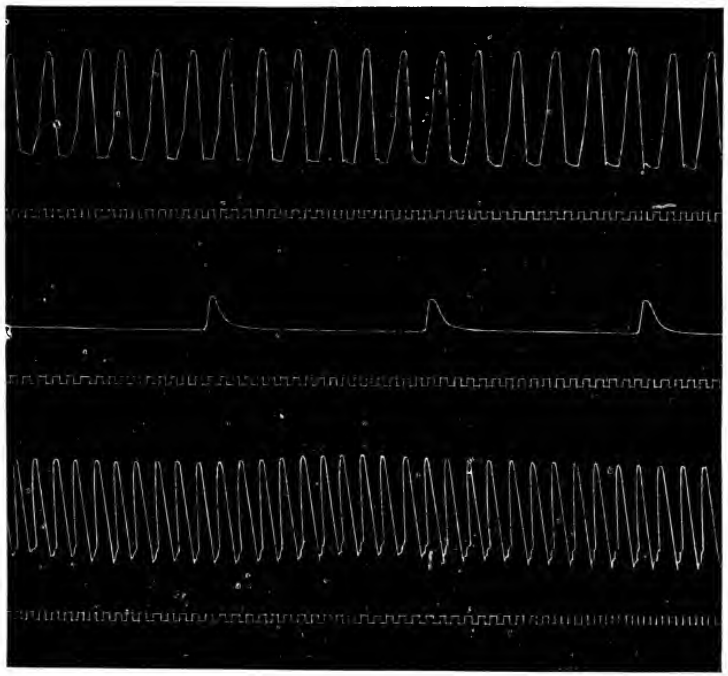


FIG. 72.—Frog's heart. 1, Normal; 2, three minutes after one drop of 10% solution of muscarine; 3, after the application of a weak solution of atropine sulphate. The time is marked in seconds. (Pembrey and Phillips.)

increasing amplitude. Muscarine abolishes the tone, rhythmic power, and conductivity of heart muscle, while atropine has in each respect the antagonistic action. This experiment succeeds on any ganglion-free strip of tortoise heart. After the application of atropine, excitation, either of the vagus or of the crescent, is ineffectual, for atropine paralyses the post-ganglionic fibres of this nerve. The effect of atropine cannot be antagonised by a further application of muscarine.

A 1% solution of pilocarpine acts in the same way as muscarine, and atropine acts as its antagonist.

Muscarine is an alkaloid obtained from the poisonous Fly Agaric—a

fungus. It is used as an intoxicant in Siberia. It is excreted, unchanged, in the urine, and it is stated that the urine is drunk when the

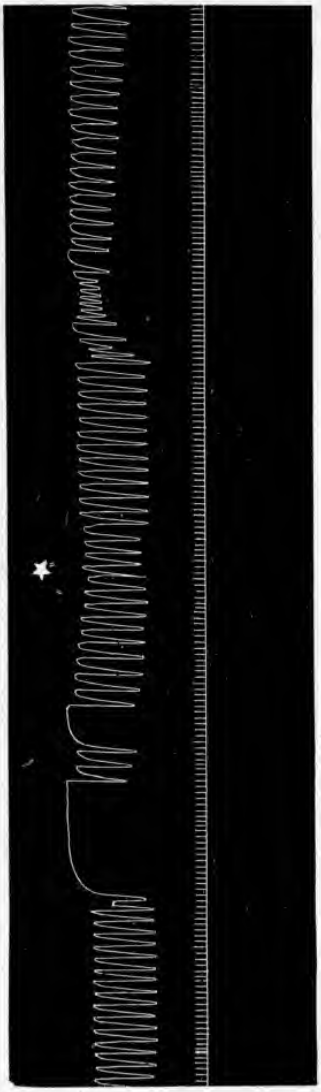


FIG. 73.—Contraction of the frog's heart. Effect of excitation of the sino-auricular junction before and after atropine. The atropine was applied at the point starred. The time is marked in seconds. The curve should be read from left to right. The downstroke represents contraction. See footnote, p. 60. (L.H.)

supply is short, and thus the intoxicant is handed on from one man to another.

Muscarine nitrate, $C_5H_{15}NO_3$, is prepared artificially from cholin, $C_5H_{15}NO_2$. Cholin is one of the decomposition products of lecithin.

CHAPTER XVII.

THE EFFECT OF NICOTINE, CHLOROFORM AND ETHER UPON THE HEART.

Action of Nicotine.—Dissect out the vago-sympathetic and record the beat of the heart by the suspension method. Record the effect of excitation of (1) the vago-sympathetic, (2) the crescent. Now apply to the heart a few drops of a 1 per cent. solution of nicotine. The frequency of the heart is at first lessened and then slightly increased, for the nicotine firstly excites and secondly paralyses the synapses of

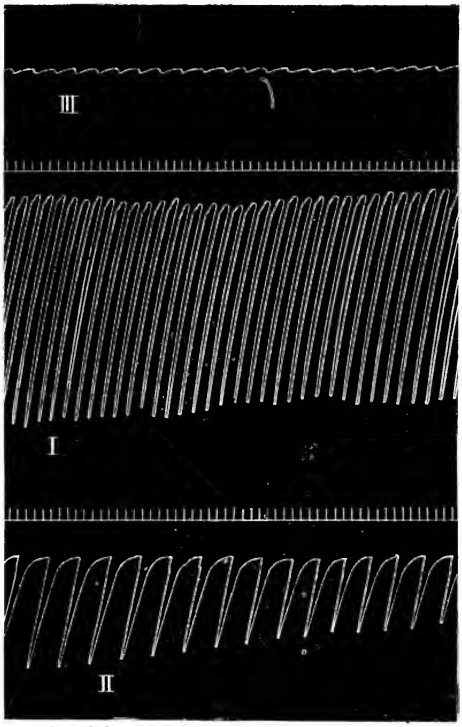


FIG. 74.—Contraction of the frog's heart. I. Normal heart-beat, II. and III. poisoned by nicotine. The downstroke represents contraction. The time is marked in seconds. See footnote, p. 60. (L.H.)

the vagus fibres with the cardiac ganglia. These ganglia contain the cell stations of the vagus fibres. Stimulation of the vago-sympathetic trunk no longer produces inhibition, but augmentation and acceleration. The cell stations of the sympathetic fibres are in the third sympathetic ganglion.

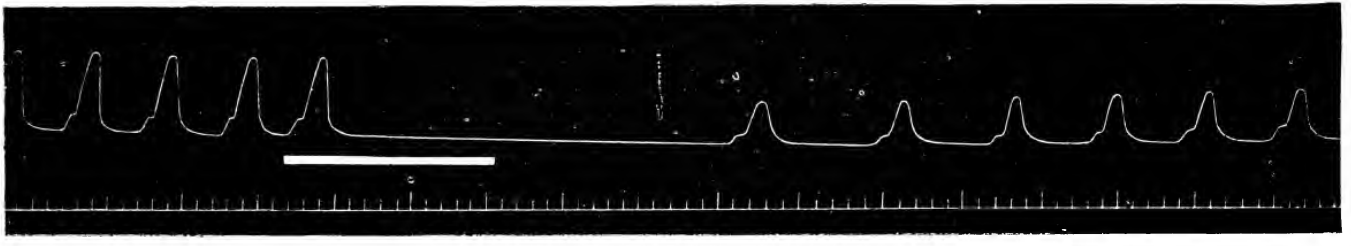


Fig. 75A.—Contraction of the frog's heart. Nicotine 1 in 1000 saline. Effect of exciting the sino-auricular junction during the period shown by the white line. Excitation of the vago-sympathetic on the contrary produced no effect. The time is marked in seconds. (Pembrey and Phillips.)

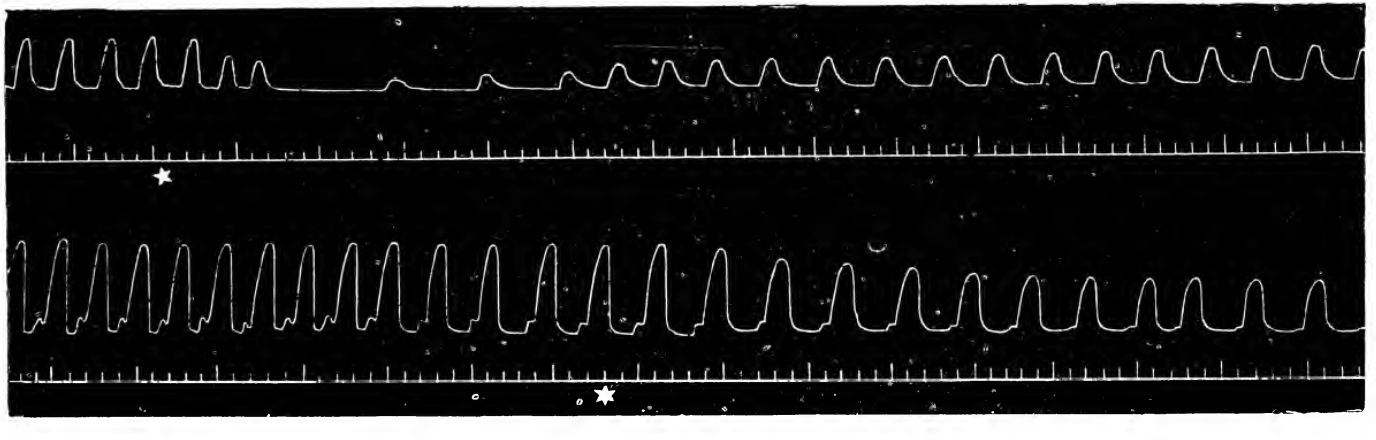


Fig. 75B.—Contraction of the frog's heart. Effect of applying a drop of chloroform to the frog-heart at the points starred. The time is marked in seconds. (Pembrey and Phillips.)

The vagus fibres are medullated as far as the cardiac ganglia, while the sympathetic fibres are non-medullated after leaving the third sympathetic ganglion (Fig. 75c). Stimulation of the crescent still produces inhibition, for weak doses of nicotine do not paralyse the post-ganglionic fibres. Nicotine is similarly employed to determine the cell stations of all the nerve fibres of the autonomic system (Langley). Too large a dose of nicotine paralyses the post-ganglionic fibres, renders the contraction of the muscle slow. At this stage stimulation of the sinus will cause a series of rapid beats due to the excitation of the cardiac muscle; this acceleration shows as an after-effect a prolonged period of diastole. Nicotine finally arrests the heart-beat by poisoning the muscle.

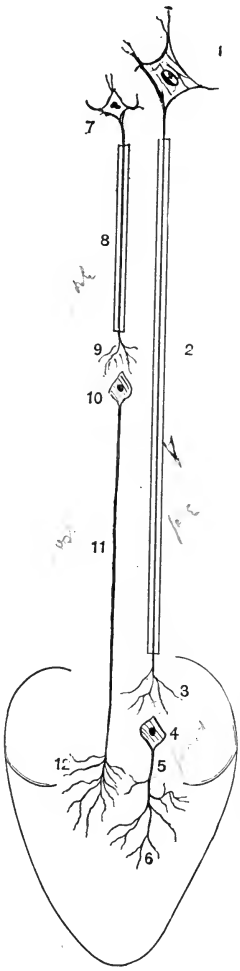


Fig. 75c.—Diagram of the nerves of the heart. 1. Nerve-cell of the vagus centre in the medulla oblongata. 2. Medullated inhibitory fibre of the vagus. 3. Its termination around a ganglion-cell (4) in the heart. 5. Post-ganglionic non-medullated fibre and its termination (6) in the muscular tissue of the heart. 7. Ganglion-cell giving rise to the medullated acceleration fibre (8) in the white ramus communicans. 9. The termination around the nerve-cell (10) of the ganglion stellatum. 11. The post-ganglionic non-medullated nerve fibre and its terminations (12) in the muscular tissue of the heart. The heart is roughly indicated in outline. (Pembrey and Phillips.)

Action of Chloroform and Ether.—Excise two frogs' hearts and place each in a watch glass containing 5 c.c. of Ringer's fluid. To one add one drop of pure chloroform and cover with another watch glass. The heart will become feeble, lose tone, and finally stop beating. It will take about ten times as much ether to produce the same effect on the other heart. Chloroform is ten times more potent a drug than ether. The causation of death from chloroform is cardiac failure. In the mammal the arterial pressure falls, and the vagus centre is rendered hyperexcitable by too concentrated a dose of chloroform. Failure of respiration and syncope may result from inhibition of the heart.

CHAPTER XVIII.

DISSECTION OF THE HEART. THE CARDIAC IMPULSE.

The Sheep's Heart.—The heart should, if possible, be obtained with the pericardium intact, and the lungs attached to it. Open the pericardium and test its strength. It is a strong, inelastic, fibrous bag, and prevents the over-distension of the right heart. The parietal layer of the pericardium is attached to the roots of the large vessels at the base of the heart, it thence runs over the surface of the heart, forming the visceral layer. The pericardium in man is attached below to the diaphragm, while above it is slung to the spine by the cervical fasciæ. The heart is thereby slung in position, and cannot twist over during changes of posture.

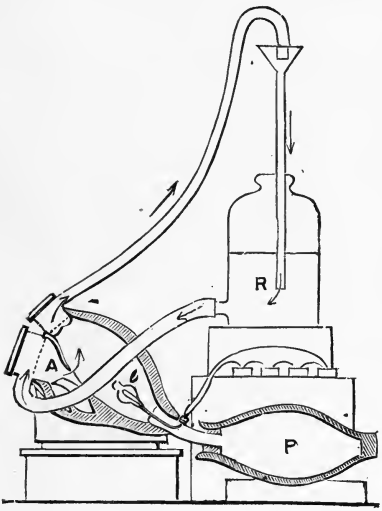


FIG. 76.—Gad's method of showing the action of the cardiac valves. (Fredericq.)

The front of the heart is recognised by a groove filled with fat, which runs obliquely down the ventricles from left to right. The groove starts from about the middle of the base of the ventricles to a point a little below the middle of the right margin of the heart. Running up the middle of the posterior and flatter surface of the heart is a similar shallow groove. The heart is divided by these grooves into a right and left side, and each of these is again divided by a groove containing much fat, which circles round the top of the ventricles. Above this groove lie the right and left auricles. Note the musculature of the left ventricle is thick and firm, that of the right ventricle thinner. Both the auricles are thin walled. The appendix of each auricle projects in front at the base of the heart, as a flat, crinkled, ear-shaped bag. The greater part of the auricles lies at the back and sides of the base of the heart, and is concealed by the aorta and pulmonary artery. The grooves on the surface of the heart mark the position of the septa, which divide the heart into four chambers. Trace the right and left coronary arteries, which issue from the right and left sinuses of Valsalva

in the root of the aorta, and run in the auriculo-ventricular groove. The left is the more important, and divides into the ramus circumplexus and the ramus descendens. Ligation of the left coronary artery causes fibrillar contraction, ending in arrest of the heart beat. Ligation of either of its chief branches may cause fibrillar contraction. Trace the coronary vein in the auriculo-ventricular groove. In the posterior wall of the right auricle find the opening of the vena cava inferior below, and that of the superior vena cava above.

In the posterior wall of the left auricle note the openings of the two pulmonary veins—in man there are *four*. The pulmonary artery arises in front of the base of the right ventricle, close to the anterior intra-ventricular groove. The aorta arises from the base of the left ventricle behind and a little to the right of the pulmonary artery. Tie a glass tube V.C. into the vena cava superior, and ligature the vena cava inferior and the left vena azygos. Tie a second glass tube P.A. into the pulmonary artery. The arterial tube should be two feet, and the vena cava tube one foot long. Fill the V.C. tube with water, the water runs through the right ventricle into P.A. Rhythmically compress the ventricles with your hand. The water sinks in V.C., and rises in P.A. Stop the compression. If the pulmonary valve is competent, the fluid in P.A. will not sink. Note that the root of the pulmonary artery is distended by the pressure of the column of fluid. The same experiment can be repeated, using one pulmonary vein and the aorta. Remove the tubes, and measure the diameter and compare the sectional areas of the two venae cavae, the pulmonary artery and the aorta. Extend the aorta and pulmonary artery. Both have extensile and elastic walls, and although empty, do not collapse. The pulmonary artery is very extensile. The venae cavae and pulmonary veins have thin inextensile walls which fall together.

Pass your finger down the superior vena cava through the right auricle into the right ventricle. Feel the size of the auriculo-ventricular orifice. Now cut through the pulmonary artery just above its origin, and look within. Note the three pulmonary semi-lunar valves. Put the pulmonary orifice under the tap. The pocket-shaped valves close and prevent the water entering the ventricle. Pass a finger through the pulmonary orifice, and another through the superior cava. The two fingers meet in the right ventricle.

Next cut open the right auricle, and observe that it surmounts the right auriculo-ventricular orifice like an inverted pocket.

Note the appendix with its fretwork of muscle—the inter-auricular septum with the fossa ovalis; the Eustachian valve, a membraneous fold in low relief, which lies immediately beneath the entrance of

the inferior vena cava. It is directed from the posterior wall towards the internal wall.

Note also the size and form of the auriculo-ventricular orifice. Cut away most of the auricle, and put the auriculo-ventricular orifice for a moment under the tap. The valve will float up. The flaps are brought into opposition by eddies the moment the ventricular pressure becomes greater than the auricular pressure. Note the shape of each flap, and the upward convexity of the valve flaps when closed, and the star-shaped figure formed by their opposition. Note also the papillary muscles and chordae tendineae. A band of muscle—the moderator band—crosses the right ventricle of the sheep's heart.

Next cut through the chordae tendineae, and then place the auriculo-ventricular orifice for a moment under the tap. The valve-flaps are now driven towards the auricle, and the flap is no longer competent. Introduce a pair of scissors between two of the valve-flaps, and cut down to the bottom of the ventricle. Then turn round the scissors and cut up close to the septum, towards, but not as far as, the pulmonary artery. Observe the columnae carneae and papillary muscles in the lower part of the ventricle. These pack together and obliterate the lower part of the ventricle during systole. Acting as elastic cushions, they rebound in diastole and produce a momentary negative pressure in the ventricle. Note the funnel-shaped, smooth-walled upper part of the ventricle—the conus arteriosus—which leads into the pulmonary artery. This part is not emptied during systole, and blood thus remains in contact with the auriculo-ventricular valve, and ensures its closure. Note the form of the flaps of this valve, and their attachment to the auriculo-ventricular ring. Some of the chordae tendineae are attached to the edges, and others to the under surface of the valves. Owing to the papillary muscles and chordae tendineae, the auriculo-ventricular valve presses on the blood during systole, equally with the rest of the ventricular wall.

Now lay open the pulmonary orifice and note the shape and attachment of the semi-lunar valves and the small nodule of tissue in the free edge of each flap. Observe also the sinuses of Valsalva. These favour the formation of eddies, which bring the valves in opposition the moment the intraventricular pressure becomes less than the pulmonary arterial pressure.

Cut open the left auricle in the same manner as the right, and observe the two flaps of the left auriculo-ventricular valve, the papillary muscles, etc., and thickness of the left ventricular wall. Cut across the aorta just above its origin and observe the three aortic semi-lunar valves. Insert the nozzle of the tap through this valve into the left ventricle and turn on the water. The auriculo-ventricular valve closes

and prevents the escape of the water. Lay open the left ventricle in the same manner as the right, carrying the first incision down the left side of the ventricle. Observe the entrance into the aorta and then lay this open. Note the orifices of the coronary arteries from the right and left sinuses of Valsalva.

Demonstration of Action of Valves in the ox or horse's heart. (Gad.) Two brass tubes with glass windows are tied one (7 cm. in diameter) into the left auricle, the other (5 cm.) into the aorta. The brass tubes are connected by side tubes to the bottom and top respectively of a reservoir containing water. A small hole is made in the apex of the heart, and a glow lamp is inserted into the left ventricle. The wires of the lamp are connected with two Grove cells. A tube connected with a rubber bag is tied into the apex. The bag is full of

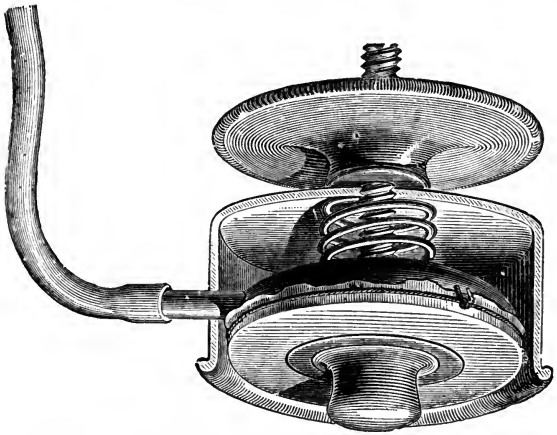


FIG. 77.—Marey's Cardiograph. The tube is connected with a recording tambour. Pressure is adjusted by the screw and spring.

water. On compressing the bag the auriculo-ventricular valves close, while the aortic valves open. On relaxation the aortic valves close, while the auriculo-ventricular valves open (Fig. 76).

The Cardiac Impulse.—Observe and feel the seat of the cardiac impulse when the subject is (1) standing erect, (2) lying horizontal on the left, and (3) on the right side. The impulse is felt in the fifth or fourth intercostal space about $1\frac{1}{2}$ inches below the nipple line, and $3\frac{1}{2}$ inches from the mid-sternal line. It shifts under the sternum when the subject lies on the right, and to the nipple line when he lies on the left side. Owing to the influence of gravity, a different part of the heart comes in contact with the chest wall in each posture. Apply the button of the cardiograph to the seat of the impulse, and fix it with tapes. One tape is fastened round the chest and one over the right

shoulder. Connect the cardiograph by means of a ⊥ tube with a recording tambour, and take records on a moderately fast drum. The ⊥ tube is used to regulate the pressure in the tambour. Set up a signal, spring key, and battery, in circuit. Listen to the heart sounds and mark the first and second sounds beneath the cardiogram (Fig. 78). The signal must write exactly under the writing style of the tambour. The reaction time of a trained observer for making signals in answer to sounds is 0·15 to 0·20 seconds. The curve is only typical when the button of the instrument is exactly applied to the seat of the impulse. Elsewhere the thorax is drawn in, as blood is expelled from the thorax during the period of systolic outflow (Fig. 79).

The impulse of the heart occurs where the ventricular wall touches the chest. It is produced by the sudden hardening of the ventricular muscle. During the first part of systole—the period of rising tension —the blood cannot escape from the ventricles.

For the demonstrations of the circulation see page 119.

CHAPTER XIX.

THE PULSE. HUMAN BLOOD PRESSURE.

Pulse.—Examine the radial pulse with the finger. Note (1) the size of the swelling, composed of the artery and its venae comites, which occupies the radial sulcus; (2) the tension of the artery, which is estimated by the pressure required to obliterate the artery and stop the pulse; (3) the condition of the arterial wall, which can be ascertained by rolling the vessel upon the bone; (4) the character of the pulse wave—its frequency, regularity, amplitude, and period of duration. Note also whether the chief secondary or dicrotic wave is perceptible.

Compress the brachial artery, and notice that the radial pulse ceases. Compress the upper arm, excluding the brachial artery. The swelling in the radial sulcus increases as the veins become congested. The pulse may be recorded by a sphygmograph. The principle of this instrument is a button resting on the artery and pressing against a steel spring. The spring in its turn is made to press either against a lever (Fig. 85) or a tambour. The lever is provided with a writing style, while if the tambour be used it is connected with a recording tambour. The Dudgeon sphygmograph is the most convenient. Apply it to the radial artery as in Fig. 81. The right position of the button may be found by marking the position of the pulse with ink. The pressure of the instrument can be varied both by the straps and by the dial which regulates the pressure of the spring. The instrument should be

applied with a pressure sufficient to flatten the artery, and then the pressure should be diminished until the maximal excursion is obtained. We have no means of accurately reading the pressure of the spring

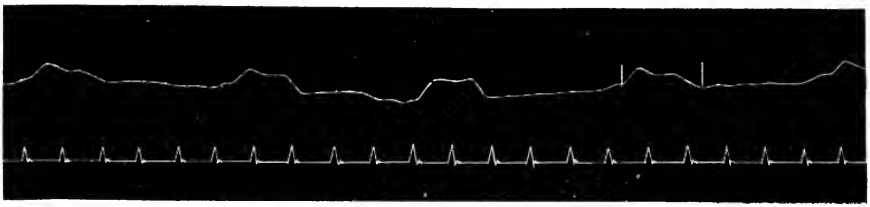


FIG. 78.—Impulse curve of boy aged 15. The moments when the heart-sounds were heard are marked. Time marked in fifths of a second. (L.H.)

or the changes of pressure indicated by the pulse curve. The instrument gives us the form of the pulse curve only. When the smoked paper is in position, and the writing style placed upon it, and the maximal excursion obtained, the clock is started and the record taken.

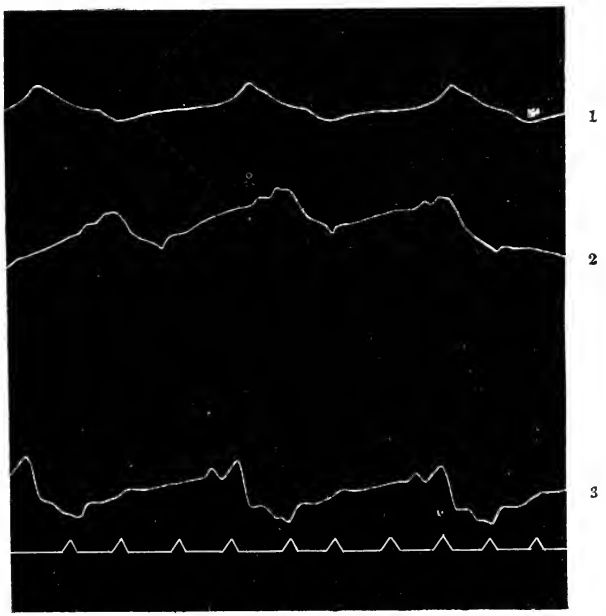


FIG 79.—Form of impulse curve changed by altering the position of cardiograph. In 3 the chest wall is sucked in during the systolic output. Time marked in fifths of a second. (L.H.)

The pulse curve consists of a primary and several secondary waves. The primary wave is the wave of expansion produced by the systolic output of the heart, and travels down the elastic arteries at a rate of about 5-8 metres a second. The secondary waves are produced by the

elastic vibrations of the wall of the large arteries which result from their sudden distension. The first, secondary, or predicrotic wave may perhaps be produced by reflection of the primary wave from the

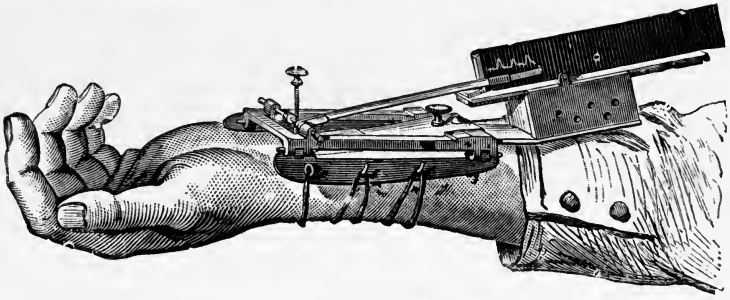


FIG. 80.—Marey's sphygmograph.

periphery. The second or dicrotic wave follows the dicrotic notch. The dicrotic notch is synchronous with the tension of the closed semilunar valves and the second sound of the heart.

The dicrotic wave, depending as it does on the elastic swing of the arterial wall, is most marked when the arteries are healthy, the arterial pressure low, and the heart-beats slow and powerful. The elastic wall of the aorta and large arteries, suddenly expanded by

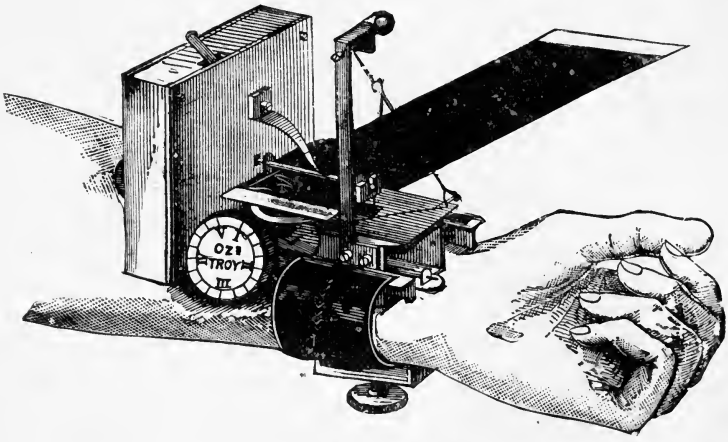


FIG. 81.—Dudgeon's sphygmograph.

the systolic output, then swing in and out like a stretched string when it is plucked.

Take another pulse tracing and forcibly inspire and expire during the record. The line of the tracing falls during inspiration and rises during expiration. This is due to the effect of respiration on the

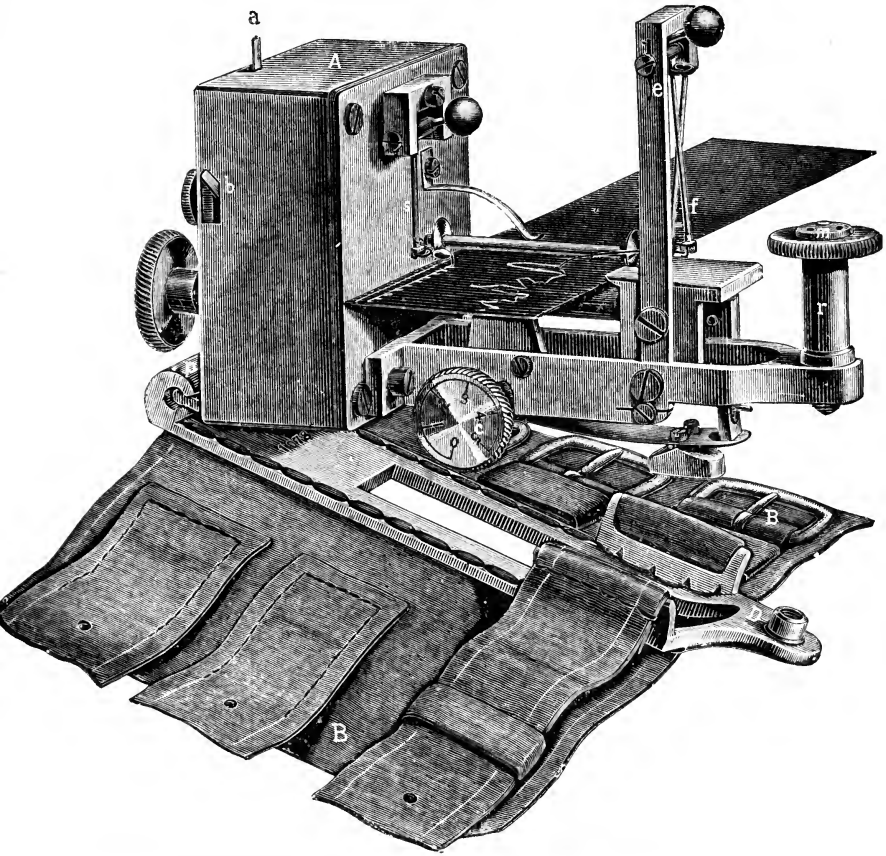


FIG. 82.—Sphygmograph provided with time writer (Jacquet).

FIG. 83.—Pulse tracing (sphygmogram) taken by Jacquet's sphygmograph. *a d*=the period of the pulse curve, *b*=the primary, *c*=the dicrotic wave. Time marked in fifths of a second.

venae comites which surround the radial artery. While taking a third tracing compress the upper arm of the subject with your hands, excluding the radial artery. The venous congestion thus produced will raise the line of the tracing and cut off the descent of the pulse curve (Fig. 84, 1). The swollen venae comites raise the instrument above the artery. It is important to remember that venous congestion may alter the character of the radial pulse.

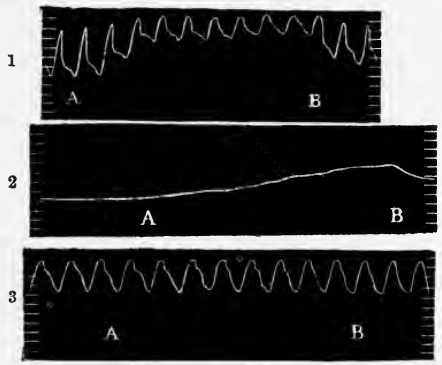


FIG. 84.—Effect of compressing femoral vein on sphygmogram. 1, Sphygmogram taken with instrument resting on femoral artery and vein of dog. 2, On femoral vein only. 3, On femoral artery only. (Hill, Barnard, and Sequeira.)

Blood Pressure in Man.—The pressure may be measured by the Hill-Barnard sphygmometer. This consists of a graduated glass tube, which expands into a capsule below and a small air chamber above. The capsule and the tube, almost to the zero mark, are filled with dilute glycerine acidulated with chromic acid; the capsule is covered by a rubber membrane. The air chamber is closed by a tap. In using the instrument the fluid is first set at zero. To effect this the tap is opened and the rubber membrane pressed until the fluid reaches the zero mark on the scale. The tap is then closed. The instrument is now pressed upon the artery until the position is found at which the fluid meniscus gives the maximal pulsation. The scale is read at this point, and the reading gives the mean arterial pressure in mm. Hg. While taking the reading the hand of the subject must be placed on the same level as the heart, so as to avoid the influence of gravity.

The air-chamber acts as a spring, and the instrument is a spring manometer. The zero is set by opening the tap before each reading, so as to avoid errors due to alterations of temperature and barometric pressure. The instrument is graduated empirically. The maximal pulsation is obtained when the mean pressure within and without the

artery are the same. If the pressure of the instrument be made greater than the mean, the artery will not expand to its fullest in systole. Similarly, if the pressure be made less than the mean the artery will not completely collapse in diastole.

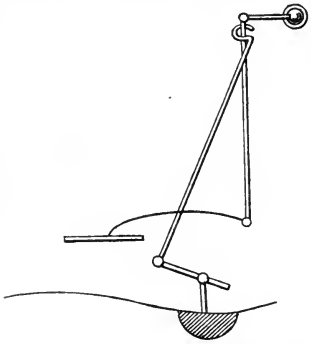


FIG. 85.—Arrangement of levers in Dudgeon's sphygmograph.

FIG. 86a.—Sphygmometer.

The mean arterial pressure is 100-110 mm. Hg in healthy young men. It may fall during sleep 10-20 mm. Hg, and rises to 130-140 mm. Hg during mental excitement or severe effort. It is normally higher in the erect than in the horizontal position. The effect of gravity is over-compensated. The reverse is the effect in states of

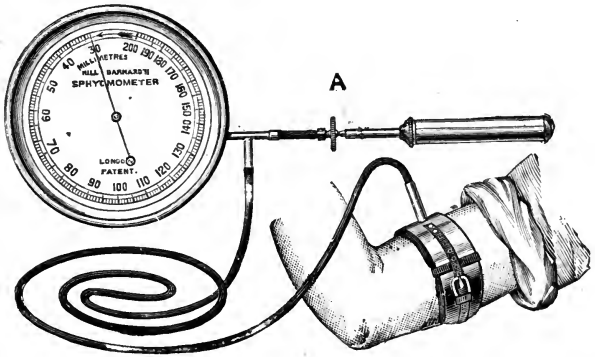


FIG. 86b.—Sphygmometer. The leather armlet encloses a rubber bag. The bicycle pump is used to raise the pressure. The spring manometer indicates the maximal pulsation and the pressure.

debility, and the pulse frequency is then greatly accelerated in the vertical posture. The arterial pressure is as constant as the body temperature from day to day. In the horizontal posture the arterial pressure will be found to be the same in all the big arteries. In the erect posture the pressure is higher in the femoral than in the carotid by the height of the column of blood which separates the two arteries.

The venous pressure may be obtained by pressing the instrument on to a vein on the back of the hand, emptying the blood out of the vein by digital pressure, and then diminishing the pressure until the vein suddenly fills. Note the pressure when this happens.

CHAPTER XX.

BLOOD. THE HAEMOGLOBINOMETER AND THE HAEMACYTOMETER.

Gowers-Haldane Haemoglobinometer.—The maximal error of this admirable instrument is not more than 0·8 per cent. The standard solution in tube D is a 1 per cent. solution of ox blood saturated with coal gas.[1] The oxygen capacity of the ox blood from which the standard was prepared was 18·5 per cent. This was determined by displacing the oxygen from laked ox blood with ferri-cyanide

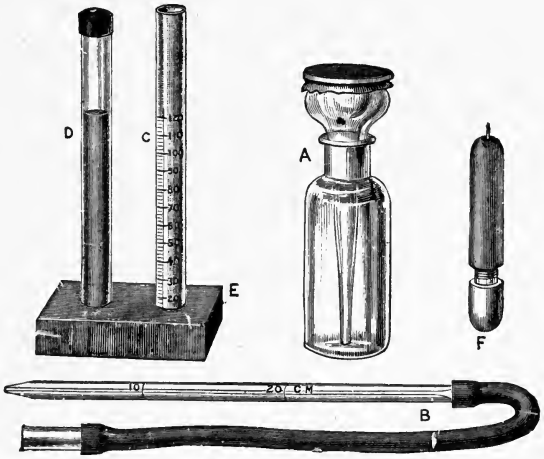


FIG. 87.—Gower's haemoglobinometer.

of potassium, and measuring the amount of gas. The percentage of haemoglobin corresponding to 18·5 per cent. is about 13·8 per cent. The normal human blood when saturated with CO and diluted with water to the mark 100 in tube C corresponds in tint to the standard, and has therefore an oxygen capacity of 18·5 per cent.

Add distilled water to tube C up to the mark 20. Take exactly 20 c.mm. of blood in the pipette, and blow it into C. Pass a narrow glass tube connected with a gas burner into the free part of tube C. Turn the gas on and push the glass tube down near to the blood. The gas

[1] Coal gas contains carbon monoxide as an impurity.

tube is then withdrawn, and tube C quickly closed with the finger to prevent the gas escaping. The tube is then inclined up and down about a dozen times, so that the haemoglobin becomes saturated with CO.

Distilled water is then added drop by drop from the dropping pipette A, until the tint appears equal to the standard. After half a minute read the percentage, and then add another drop or drops till the tints appear just unequal. Read the percentage again, and take the mean of the two readings as correct. In comparing the tints hold the tubes against the skylight, and frequently change the tubes from side to side. The average percentage of woman's blood is 11 per cent., and that of children's blood 13 per cent. below the average of adult men.

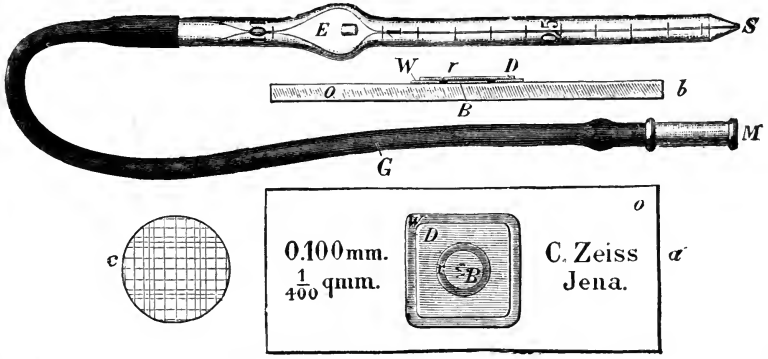


FIG. 88.—The Thoma-Zeiss haemacytometer.

Therefore, in calculating the percentage for women add about $\frac{1}{9}$, and for children about $\frac{1}{13}$ to the percentage found. Many other forms of haemoglobinometer have been contrived, but in comparison with this instrument none of them are worth notice.

The number of Corpuscles in the Blood.—The Thoma-Zeiss **Haemacytometer** consists of a counting chamber and an accurately calibrated pipette.

The finger behind the nail is cleaned with alcohol and ether, and a drop of blood is drawn by the stab of a lancet-shaped needle. The finger should not be constricted by a ligature during this operation. The point of the pipette is placed in the drop, and the blood is aspirated as far as the mark 1. The traces of blood on the point of the pipette are then removed, and the pipette is dipped into Hayem's fluid.[1]

[1] Sodium chloride, g. 2; sodium sulphate, g. 10; corrosive sublimate, g. 1; water, g. 400.

This fluid is sucked up until the diluted blood reaches the mark 101. The tip of the mouth-piece is then closed by the finger, and the pipette shaken. The glass bead in E mixes the blood and Hayem's fluid. The bulb contains 1 part blood and 99 Hayem's fluid.

Now blow gently into the mouth-piece, reject the first few drops, and then place a drop upon the centre of the counting chamber. The cover-slip is then placed in position, and the counting chamber is placed on the stage of the microscope, and left at rest for a few minutes. When the corpuscles have subsided, count the number in 10 squares, and take the average. Count those corpuscles which happen to lie on the lines on two sides of each square only. Each square covers an area of $\frac{1}{400}$ sq. mm., and has a volume of $\frac{1}{4000}$ c.mm., therefore 1 c.mm. contains 4000 times the average number found in a square. The dilution of the blood was 1·100. Thus the number in a square × 4000 × 100 = number of corpuscles in 1 c.mm. of blood.

In counting the white corpuscles it is best to dilute the blood with 1 per cent. acetic acid. This destroys the red corpuscles and brings the white clearly into view. By comparing the number of the red corpuscles in a square with the percentage of the haemoglobin, the worth of the corpuscle in haemoglobin is obtained.

$$\frac{\%\text{ of Hb}}{\text{No. in sq.}} = \text{'worth' of corpuscles.}$$

The average number of red corpuscles is 5,000,000 per 1 c.mm.; of white, 10,000 per 1 c.mm.[1]

Specific Gravity of the Blood.—A number of test-tubes are taken and filled with mixtures of glycerine and water, which vary in specific gravity from 1030 to 1075. A pipette is taken with the point bent at a right angle. The skin is pricked behind the finger nail, and a drop of blood is drawn into the pipette. The blood is blown in small droplets into the middle of the solution in several of the test tubes until the solution is found in which the blood neither sinks nor rises. The specific gravity of this solution is determined with the hydrometer. The behaviour of the droplet must be noted at the moment when it enters the solution. The blood quickly alters owing to osmotic change. The specific gravity of the blood is about 1060, of the plasma 1026-29. The specific gravity of fragments of muscle or other tissues may be determined in the same way. The method is thus employed to determine the amount of tissue-lymph in the organs.

[1] After using, clean the pipettes of these instruments. Suck water, alcohol, and ether up them in turn, and let the liquids run out. Never blow down the pipettes.

CHAPTER XXI.

INSPECTION AND AUSCULTATION OF THE THORAX.

Inspection.—The normal chest has the shape of a truncated cone. It is well and symmetrically expanded; the sternum and vertebral column are erect, while the anterior wall of the thorax is slightly arched forwards. The transverse section is oval, with the wider diameter from side to side. The sub-costal angle is wide. Measure the chest with a tape. The average measurement round the chest is 35 inches, or rather more than half the height of the man. With the calipers measure the side to side diameter; it averages 10-10½ inches at the level of the nipple. The antero-posterior diameter is 7½ inches on the average. Measure the difference in the circumference between the inspiratory and expiratory positions. It averages 2½-3½ inches. Take the shape of the chest with the cyrtometer. This consists of two pieces of lead piping hinged by a piece of rubber tubing. The cyrtometer is moulded round the circumference of the thorax, then opened and removed, to be again placed in position on a sheet of paper. The shape is then traced on the paper with a pencil.

Observe during respiration that the abdominal movements are most marked in men. In children and women the movement of the upper part of the chest is more noticeable.

Inspiration.—The distensile lungs follow at every point the inspiratory enlargement of the thorax, and air and blood enter the lungs in increased volume. The vertical diameter of the thoracic cavity is increased by the contraction of the diaphragm. This dome-shaped muscular sheet flattens until the acute angle between the thoracic wall and the diaphragm becomes an obtuse angle. The pull of the diaphragm on the lower ribs is antagonised by the pressure of the diaphragm on the abdominal contents. The quadratus lumborum fixes the twelfth rib, while the serratus posticus pulls the last four ribs backwards. The central tendon of the diaphragm is slung to the pericardium, and scarcely varies in position. The movements of the diaphragm may be observed in man by means of the Röntgen rays. The antero-posterior and transverse diameters of the thorax are enlarged by the external intercostal muscles, the intercartilaginous parts of the internal intercostals, and the levatores costarum. The scalene muscles fix the upper two ribs. By the elevation of the ribs the sternum is thrown forwards, the spine backwards, and the thorax is enlarged both from before backwards, and from side to side.

The movement of the upper ribs is chiefly forwards, and that of the lower ribs backwards. The elasticity of the costal cartilages and the sterno-clavicular articulations permit this movement. The bulk of the lungs below lies behind and above in front.

The thorax expands when the pleural cavity is opened in the corpse, for the elasticity of the lungs pulls the thoracic walls inwards. In quiet inspiration, therefore, the muscles have only to overcome the elasticity of the lungs, and help the thoracic cage to spring outwards.

Expiration is brought about by the elastic recoil of the lungs and abdominal wall, and by the weight of the thorax.

Extraordinary Respiration.—Let the subject run up and down stairs until dyspnoea results. Every muscle is brought into play which elevates the ribs, or fixes the origin of the muscles which elevate the ribs. In expiration the abdominal muscles depress the thorax and force up the dome of the diaphragm. Owing to the torsion of the thoracic cage an elastic recoil takes place both after inspiration and expiration, and thus no time is lost.

During severe muscular exercise the abdominal wall is tightened, and the diaphragm, its descent being resisted, raises the thorax.

Vocal fremitus.—Place the flat of the hand on the chest and tell the subject to say "ninety-nine." The vibration of the voice—vocal fremitus—is propagated through the bronchi to the wall of the chest.

Percussion.—Firmly place the index finger of the left hand on the chest and strike it with the middle and ring fingers of the other hand. The sound resonates over the lungs. It is dull when the thigh is percussed, for there the vibrations are damped. Note the feeling of resistance to percussion. Map out the areas of resonance. On the right side the resonance stretches from the apex of the lung in the supra-clavicular fossa to where the liver dulness begins at the 6th rib. On the left it extends to the cardiac dulness at the 4th rib. The cardiac dulness reaches from the mid-line to a line which is convex outwards, and runs from the sternal end of the 4th costal cartilage to the apex of the heart. The apex-beat of the heart is felt about $3\frac{1}{2}$ inches from the mid sternal line, and in the fourth or fifth intercostal space. It shifts to the right or left as the subject rolls over on to the right or left side.

In the axillary line the pulmonary resonance extends to the 8-9 rib, while behind it reaches to the 10-11 rib. Over the scapula the note is less resonant.

Observe the influence of inspiration and expiration upon the limits of pulmonary resonance.

Auscultation.—The respiratory and cardiac sounds can be heard by placing the ear against the chest, or by means of the wooden or binaural stethoscope. Listen over the trachea, or at the level of the 7th cervical spine. The harsh blowing inspiratory and expiratory sounds are separated by an interval. These 'bronchial sounds' are produced by the vibration of the orifices of the air-way and vocal cords.

Another breezy sound, the 'vesicular murmur,' is heard wherever the lung is in contact with the chest wall. It increases during inspiration and dies away during the first third of expiration. This sound is probably produced by the separation of the moist surfaces of the bronchioles and alveoli. It may be due to the conduction of the bronchial sounds to the alveoli.

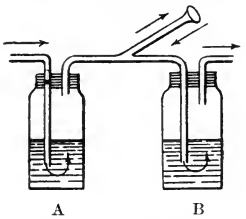


FIG. 89A.—Arrangement for demonstrating CO_2 in expired air by means of lime water.

Carbonic Acid in Expired Air.—Two flasks are arranged as in Fig. 89A and lime water is placed in both. Breathe through the mouthpiece. The inspired air passes through A, which remains clear, while the expired air passes through B, which becomes turbid. If the precipitate of calcium carbonate in B be collected, and a little hydrochloric acid added to it, effervescence will take place owing to the decomposition of the calcium carbonate.

Deficiency of Oxygen in Expired Air.—A large bottle is inverted over a basin of water. A bent tube passes under the water and into the bottle. Breathe in and out of the bottle through this tube. Then lift up the bottle and put a lighted taper into it. The taper immediately goes out. A flame will not burn in air that contains less than 17 per cent. O_2; a man can live in 10 per cent. O_2. A flame is therefore a safe test for deoxygenated air (choke damp) in wells and mines, etc. The deoxygenation of the air is due to the oxidation of iron pyrites, FeS_2, etc., in the soil. A well becomes filled with such air when the barometer falls. The foul air may be removed by raising and lowering an open umbrella.

An apparatus is made by Messrs. Siebe and Gorman for exploring mines, wells, etc., which contain foul air. It consists of a mask, an air-bag fitted with inspiratory and expiratory valves and containing potash to absorb CO_2, an oxygen bottle to supply oxygen to the bag.

For the demonstration of the respiratory movements, gases, and exchange, see pages 136 *et seq.*

CHAPTER XXII.

BODY HEAT AND SECRETION OF SWEAT.

The Normal Temperature.—The average temperature of man is 98°·4 F. (36°·89 C.). It is taken by means of a clinical thermometer which is either inserted in the rectum or mouth or the subject micturates over the bulb of the thermometer. Take the temperature of your mouth at each hour of the day. Chart out the results on a temperature chart and observe the daily variation. Take the temperature before and immediately after muscular exercise, such as a fifteen minutes' run. The temperature may rise to 100°—101° F. (37°·78—38°·33 C.) or even more on a hot day. A rise of temperature can be constantly observed if the thermometer be placed in the rectum or stream of urine; the buccal temperature may for the reasons given below show a *fall* in temperature during muscular work. It is important to remember that the daily range in the internal temperature of a healthy man may be from 97°·0 F. (36°·1 C.) to 99°·6 F. (37°·56 C.); and that observations taken in the mouth, even when it is firmly closed, are liable to be low, owing to the danger of cooling of the tissues of the mouth, externally by cold air, internally by the inspired air.

Heat Regulation.—Take a large frog, and insert a small thermometer in the cloaca or flex up the thigh, and insert the thermometer between it and the abdomen, and record its temperature. Place the frog in warm water at 30° C. After 10 minutes observe its temperature. It will have reached the same temperature as the water. Cool the frog again in cold water and take its temperature again. Then place it for 10 minutes in a thermostat heated to 35° C. In the dry warm air the frog's temperature will not rise to more than about 30°-33° C. This is owing to the evaporation of water from the frog's skin. Take the temperature of a small mammal in the rectum and then place it in the thermostat at 30° C. for 10 minutes. The temperature of the animal will scarcely vary. Note the quickened respiration of the animal. This increases the evaporation of water from the lungs. Note the way it sprawls out its limbs so as to increase the loss of heat by radiation, convection, and conduction. A man cannot bear for more than a few minutes immersion in a bath of water at a temperature of 44° C., but he can stay for twenty minutes in a dry atmosphere heated to 121° C. The body temperature is then regulated by sweating. It takes 5·55 Cal.[1] to evaporate 1 gram of

[1] A calorie is the heat required to raise 1 g. water 1° C. A large Calorie spelt with a capital C is the heat required to raise 1000 g. water 1° C.

water at room temperature (15° C.). Place two jars of water in the thermostat, and let the water of one jar be covered with a layer of oil. This jar, owing to the prevention of evaporation, will rise more quickly in temperature.

Loss of Heat.—Observe the diminution in temperature of a jar of boiling water when exposed for five minutes to room temperature. Contrast the diminution of temperature during similar periods of time when the jar is jacketed with (1) a cotton bag (2) a flannel bag. Clothes diminish the loss of body-heat by entangling layers of stationary air and surrounding the body with these layers. Conductors of heat and convection currents are stopped by the stationary air in the clothes. Wool garments entangle more air than cotton. Fur and feathers act in the same way. A rabbit covered with tar or varnish dies (unless clothed) owing to the loss of heat. A man is able to regulate his temperature under such conditions.

Effect of Anaesthesia on Body Temperature.—DEMONSTRATION. A small mammal is anaesthetised with chloral or urethane after its rectal temperature has been taken. The animal is laid on the table with its limbs spread out. In the course of an hour the rectal temperature may fall three or four degrees. This is chiefly due to the cessation of muscular movement. The same effect follows curarisation; section of the spinal cord in the lower cervical region; the administration of alcohol. Alcohol produces cutaneous vaso-dilatation. It is important to protect anaesthetised patients from exposure to cold. Drunkards who fall asleep on the roadside on a winter's night are easily frozen to death. Large doses of these drugs paralyse the central nervous system which regulates the temperature of the body.

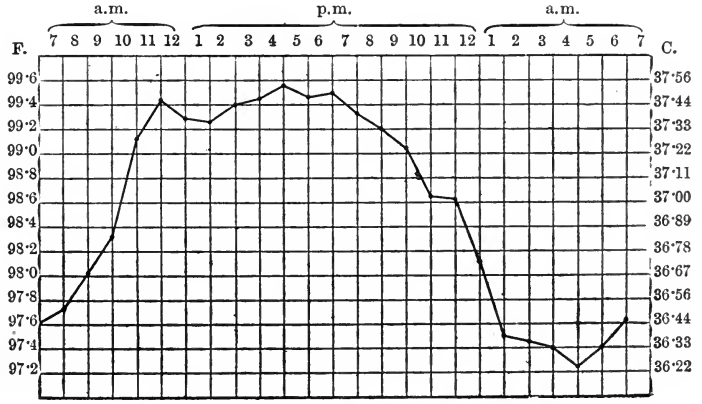


FIG. 89B.—Daily variation of Temperature of Man. (M.S.P.)

NERVOUS SYSTEM.

CHAPTER XXIII.

THE FUNCTIONS OF THE CENTRAL NERVOUS SYSTEM.

The Effects of Removal of both Cerebral Hemispheres.—In the frog the cerebral hemispheres contain only a single layer of nerve-cells and have reached only a very low stage of development. If the cerebral hemispheres be destroyed by rapidly compressing the anterior part of the skull between the blades of a pair of Spencer Wells' forceps

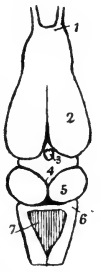


FIG. 90.—Diagram of the frog's brain. 1, Olfactory lobe; 2, cerebrum; 3, pineal gland; 4, thalamencephalon; 5, optic lobe; 6, cerebellum; 7, fourth ventricle and medulla oblongata.

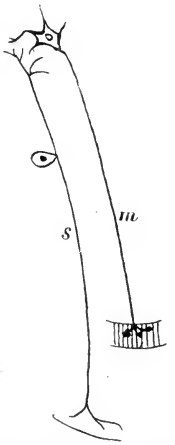


FIG. 91.—Diagram of a reflex arc, m = the motor nerve arising from a nerve-cell in the anterior horn of the spinal cord and ending by a motor end plate in a muscle; s = the sensory nerve arising from a nerve-cell in the posterior root ganglion and forming a series of dendrites around the motor nerve-cell above and possessing a sensory nerve ending in the skin below.

there will be no loss of blood and the optic thalami will escape injury. The first effect of the operation will be a general depression of the nervous system, a condition known as *shock*. This will quickly pass off and the brainless frog will show spontaneous movements, will swim if placed in water, will turn over if placed upon its back, and will behave generally as a normal frog.

If, however, the **corpora striata** and **optic thalami** be destroyed, the frog will show no spontaneous movements, will not feed, and will soon die unless the evaporation of water from its skin be prevented by placing it in a shallow plate filled with water and covered by a bell-jar. The destruction of these portions of the central nervous system produces marked shock, but, after this has passed off, the frog will still be able to jump, swim, maintain its equilibrium, and perform other complicated and co-ordinated movements when it is stimulated in the appropriate manner.

The **cerebellum** and **medulla oblongata** are now destroyed by passing a blanket-pin through the foramen magnum of the skull, and by lateral movements of the pin breaking up the nervous tissue. The frog now lies in a limp, toneless condition; shock is well marked, and does not pass off quickly. The respiratory movements of the nares and of the floor of the mouth cease. The circulation of the blood is disordered by the destruction of the vaso-motor centre (page 354).

The "**Spinal Animal.**"—The frog now possesses only its spinal cord, but it still shows co-ordinated movements. Its hind legs possess tone, and are drawn up against the flanks; if one leg be pulled away from the body, or be stimulated by pinching a toe, it will be withdrawn from the source of irritation. The movements are of a reflex nature, a response to a stimulus (Fig. 91).

When placed upon its back such a frog does not right itself, and when thrown into water it generally sinks to the bottom, and may or may not swim for one or two strokes.

If such a frog be suspended by the lower jaw, it does not move unless stimulated.

A small piece of filter-paper soaked in strong acetic acid will, if placed upon the skin of one flank, act as a stimulus, and the leg of the corresponding side will be raised to wipe off the offending body. If this experiment be repeated five minutes after the frog has been dipped in a beaker of water to remove the acid, and the leg be held down by the hand, then the leg of the opposite side will be raised in an apparent endeavour to wipe off the irritating piece of paper. The frog is again dipped in the beaker of water to remove the acid.

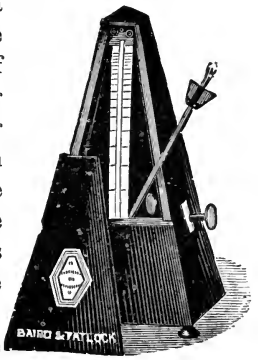


FIG. 92.—A metronome.

Türck's experiment upon the time of response of the spinal animal to a stimulus can now be performed. A small beaker is filled with dilute sulphuric acid (1 in

1000), and is gradually raised until the toes of one of the hind legs dip into the acid; this moment is noted, and then the interval between the application of the acid and the withdrawal of the toes is measured by a watch or a metronome (Fig. 92). After washing off the acid the experiment is repeated with acid of the strength 1 in 500. In each case the time of response is much longer than the true time of a reflex action.

CHAPTER XXIV.

THE ACTION OF STRYCHNINE AND OF CHLOROFORM.

THE cerebrum of a frog is destroyed by means of Spencer Wells' forceps, and then under the skin of the back are injected 10 minims of a saturated solution of strychnine (1 in 6700). In two or three minutes it will be noticed that the frog cannot readily recover its hind legs after a jump, and soon the reflex excitability of the spinal cord is so augmented that a slight touch or puff of wind upon the skin causes a general spasm of the muscles. Convulsions quickly follow, and the rigid body of the frog rests on the mouth and toes, a position known as *emprosthotonus*. This attitude is due to the different strength of the various muscles; all are thrown into contraction, but the stronger overcome the weaker. The muscles are somewhat relaxed after the spasms, but are again sent into tetanus by the slightest touch applied to the skin.

The *tonic* contractions are followed by prolonged twitches or *clonus*.

If during the stage of convulsions a probe be pushed down the vertebral canal, and thus the spinal cord be destroyed, the convulsions cease at once, showing that the strychnine acts upon the ganglion cells and their dendrites in the spinal cord. (See page 361.)

The action of strychnine should be contrasted with that of chloroform. Under the skin of the back of a frog, whose cerebrum has been destroyed by Spencer Wells' forceps, are injected 5 minims of chloroform. The first effect is one of stimulation, but this stage of excitement is quickly followed by marked inco-ordination and weakness. In about ten minutes there is marked anaesthesia, paralysis, and total absence of reflexes. If the frog be kept moist in a shallow plate full of water, and covered by a bell jar, it may recover from the effects of the chloroform in about eight or nine hours.

THE PHYSIOLOGY OF VISION.

CHAPTER XXV.

THE DISSECTION OF THE EYE.

THIS can be conveniently carried out on the fresh eye of an ox or sheep.

1. Notice in the front of the eye the transparent circular area, the **cornea**, continuous with the greyish opaque border, the **sclerotic**. This coat is continued over the sides and back of the eye, but will be found covered with fat. The external eye muscles may be traced in the fat, and their tendinous insertion seen in the front of the sclerotic. The optic nerve will be seen penetrating posteriorly. The greyish surface of the sclerotic in front is covered by a thin membrane, the **conjunctiva**, which is continued as a lining for the eyelids.

2. Having removed the fat from a portion of the upper surface of the eye so as to expose the sclerotic, make a pair of incisions passing along the surface from before backwards, and starting a few millimetres behind the corneo-sclerotic junction, let these incisons meet posteriorly. Then carefully peel up the sclerotic towards the cornea. Observe the dark underlining of the sclerotic, the **lamina fusca**. Note the **choroid** now exposed, and anteriorly observe that it is covered by a number of pale fibres passing forward to the corneo-sclerotic junction, forming the **ciliary muscle**.

3. Remove carefully the piece of the choroid lying exposed, and note a pale membrane lying beneath, the **retina**.

4. Place the eye in a glass basin of water, and make an incision right round the eye through all the coats, so as to separate the posterior from the anterior half. Examine the posterior half in the water. Note the thin retina floating away from the choroid, eccentrically in this the **optic disc** where the optic nerve enters the eye, and the blood-vessels radiating from this region. The **vitreous humour** of jelly-like consistency will remain attached to the anterior half of the eye. Looking through this, note the **crystalline lens**, at the side of this the radial folds of the choroid forming the **ciliary processes**. The thick portion

of the retina can be traced as far as these processes, where it terminates with a wavy edge, the **ora serrata.**

5. Remove carefully the vitreous humour, and note that it adheres to the ciliary processes by its outer coat, the **hyaloid membrane.** On removing the vitreous from the more central portion, note that it appears adherent to the posterior surface of the lens. The posterior layer of the lens capsule is continuous with the hyaloid membrane. If necessary, cut away the vitreous humour so as not to dislocate the lens.

6. Make a radial incision from the edge of the sclerotic down to the edge of the lens. Carefully separate the iris and ciliary region from the lens, and the **suspensory ligament** will be seen passing from the ciliary body mainly towards the front surface of the lens. Carefully separate the lens from this, and the suspensory ligament continuous with the capsule of the lens will float up away from the iris.

7. Cut round the upper half of cornea near its junction with the sclerotic. The **anterior chamber** will be exposed containing a clear fluid, the **aqueous humour.** Note the thickness of the cornea. At the back of the anterior chamber is seen the black curtain of the **iris,** with its central aperture the **pupil.**

8. Notice that the fresh vitreous humour and lens when placed in water are not easily seen; they have almost the same refractive index as water. After death the lens slowly becomes turbid.

9. Hold up the lens and look through it towards a lighted match; it will give an inverted image.

10. Notice the segmentation of the lens; it is peculiar, and may be roughly compared to a segmentation similar to that of an orange combined with the concentric lamination of an onion.

CHAPTER XXVI.

THE REFRACTING MEDIA OF THE EYE.

Kühne's Artificial Eye.—The nature of the refraction produced by the various media of the eye is conveniently illustrated by means of this instrument (Fig. 93). It consists of an oblong box, one of the long vertical sides being generally made of opaque material, the other of glass. The front end of the box is bounded by a curved glass surface, the hinder end is a plane sheet of glass. Various accessories are supplied with the instrument, such as a double convex lens which can be placed in the axis of the box behind the cornea, a frosted glass

screen which is used as a receiving surface for the refracted rays, and an opaque screen with a central hole.

The box is first filled with water, and in order to make rays of light the clearer, a few drops of some fluorescent solution (*e.g.* eosin) are added to the water. An external luminous object is then arranged. This may be conveniently done by placing a metal plate, in which a vertical arrow has been stencilled out, in front of a good source of light, such as the naked arc light of an electric projecting lantern, with the condenser and focussing lens removed. This stencilled plate is placed four or five feet from the front of the instrument.

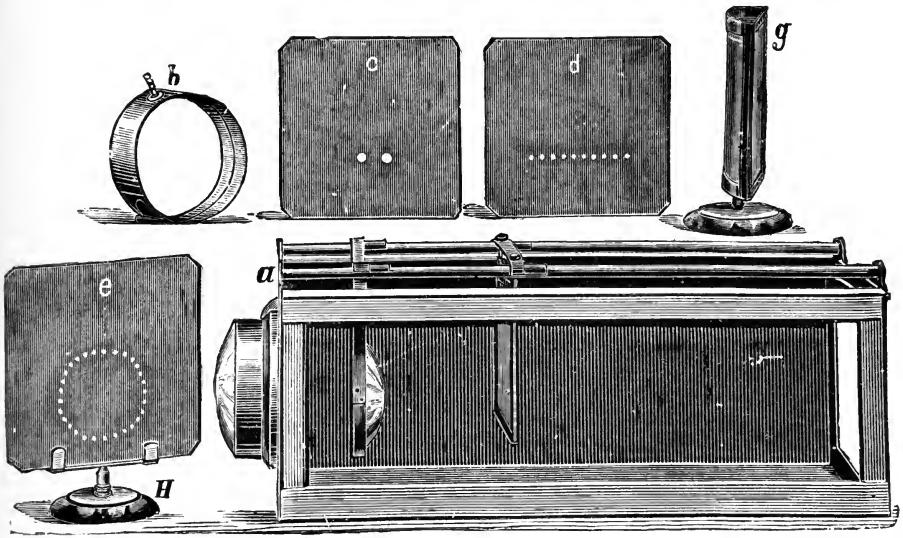


FIG. 93.—Kühne's artificial eye.

1. **The Action of the Cornea.**—If the illuminated arrow be placed approximately in the optic axis of the artificial eye, the rays of light will be seen passing through the box and converging somewhat in their progress. If the frosted glass screen be placed in the box, however far back it be arranged, no image of the arrow will be obtained. If, however, a screen be placed some distance behind the box an image will be formed. We have here illustrated the fact that without some specially strong refracting medium in the eye, external objects would be focussed behind the position of the retina and therefore not clearly visible. This is the case after the operation for cataract in which the crystalline lens is removed.

2. **The Action of the Crystalline Lens.**—Let the double convex lens supplied be now placed in the box at the front end. This at once

causes a much greater convergence of the rays, and it will be possible to obtain an image of the arrow upon the frosted glass screen, when this is placed about three inches from the hinder end of the box. This image may be easily seen on looking obliquely through the glass end, or may be projected by a convex lens on a lantern screen sufficiently clear for a number of observers to see.

3. **The Action of the Iris.** The iris improves the definition of the image by cutting out the more circumferential rays which in consequence of spherical aberration would not be focussed in the same plane as the more central. If the opaque screen having a central hole about an inch and a half in diameter be placed in front of the convex lens the total amount of light passing behind the lens is decreased, but the image is now much more sharply defined.

4. **The Position of the Image.**—It will be noticed that if the illuminated arrow point upwards the image on the artificial retina will point downwards. Images on the retina are therefore always inverted, the lower half of the retina corresponding to the upper half of the field of vision and conversely. By experience we always refer images on the retina to their proper position in the field of vision. This rectification corresponds to what is done by the second convex lens in projecting the retinal image upon the lantern screen. The effect of this second lens is to re-invert the image, so that on the lantern screen the image appears in the same position as in the original object.

5. **Accommodation.**—It is not possible with the artificial eye to mimic the changes that occur in the lens on accommodation. A clear image of objects at different distances can only be obtained by shifting the artificial retina backwards or forwards.

ACCOMMODATION.

1. The eye is able to see objects at varying distances from the eye. It has the power of adapting itself so as to form a clear image on the retina of different objects. Unless the eye had this power images of external objects at different distances would not always be formed at a constant distance behind the crystalline lens, where the retina is situated.

EXPERIMENT. Standing about 15 feet from a window and looking towards it, hold up a needle about two feet from the eye. If the needle be seen clearly the window sashes will be blurred, since the image of these will be in front of the retina. If the window sashes be looked at and seen clearly then the needle will be blurred, since the image of this is behind the retina.

2. **Range of Accommodation. Determination of Near and Far**

Points of Accommodation. Line of Accommodation.—At a certain distance close to the eye the power of accommodation is lost.

EXPERIMENT I. Hold a needle about 2 feet from the eye and gradually bring it nearer; it is for a certain time possible to obtain a clear image. At a certain distance, in spite of effort, the image begins to get blurred. The least distance at which one obtains a clear vision of the needle corresponds to the *near point* of accommodation. This is generally about 8 inches. In short-sighted persons a *far point* of accommodation may also be shown. If the distance between the two objects be not too great, although they are both in the line of sight, they may be seen clearly at one and the same time. That is to say that accommodation of a certain degree will enable the observer to see objects at varying distances from the eye. The maximum distance at which two objects in the line of sight may be separated will vary with the distance of the nearer of them to the eye. As the nearer object recedes from the eye **the line of accommodation** or the distance between the two objects increases.

EXPERIMENT II. Place two pins in the line of sight and note the distance apart at which they are both visible as single objects at the same time. Make observations with the nearer at 20 cm., 50 cm., 2 m. It will be found that the line of accommodation lengthens with a greater distance from the eye.

3. **Formation of Image in Excised Eye.**—The excised eye is accommodated for objects at a distance.

EXPERIMENT. Remove the sclerotic and choroid from a fresh sheep eye, and place it, cornea outwards, at the end of a cylinder of brown paper. Direct it towards the window, and on looking down the tube an inverted image of the window will be seen.

This experiment can be still more easily performed on the eye of a freshly-killed albino rabbit, which, for convenience of handling, should be fixed in a ring of modelling wax or clay. In this case the sclerotic and choroid are sufficiently thin to obviate the necessity for their removal.

4. **Action of Iris in Accommodation, and its Changes with Variations in Amount of Light.**—The iris cuts off the more peripheral rays impinging on the cornea, otherwise the clearness of the image on the retina would be diminished. This is especially the case when viewing near objects, as here the angle of incidence of the circumferential rays is greater.

EXPERIMENT I. In not too bright a light direct the subject's attention from a far to a near object. It would be noticed that the pupil becomes smaller.

Experiment II. Make the subject close one eye and shade the open eye from the direct light. Observe the size of the pupil when the eye is shaded. Then remove the shade; the pupil will be seen to diminish in size. From this experiment it may be inferred that the amount of light entering the eye is controlled by the iris.

Experiment III. Make a pinhole near the edge of a card, and hold the card about fifteen centimetres from the right eye, so that it does not interfere with the field of the light. Let a good source of light be placed about 60 centimetres from the eye, and allow a thin paper-screen to shield the light from the right eye. The left eye, when open, will look directly at the light, the right eye at the pinhole, and the illuminated paper through the hole. Close the left eye, and accommodate as nearly as possible for the distance of the pinhole. Note the size of the hole. Then alter the accommodation by attempting to look far away through the pinhole. The hole will immediately become distinctly larger, though less definite, on account of the blurring of the edges. Keep varying the accommodation, and the edge of the hole will similarly vary.

Whilst accommodated for far distance open the left eye. The sudden entry of light in the left eye will cause reflexly a diminution in size of both pupils. The pinhole will now become smaller. Close the left eye again and it enlarges. The size of the blurred image of the pinhole depends upon the size of the pupil, and hence variations in size of the pupil appear as variations in size of the pinhole.

5. **The changes in the Lens during Accommodation. Purkinje-Sanson Images.**—During accommodation for a near object, the ciliary muscle contracts, with the consequence that the suspensory ligament is slackened. The lens by its natural elasticity becomes more curved in its anterior aspect, and its thickness through the optical axis is increased. This change of curvature can be measured by means of the ophthalmometer. The existence of such a change may be inferred from the following experiments in which observations are made upon the images reflected from the anterior surface of the cornea, the anterior surface of the lens, and the posterior surface of the lens.

Experiment I. (Preliminary). In a dark room place on a table, rather to the right of the observer, a convex lens mounted on a stand. Hold a watch glass a few inches in front of the lens, with the convex surface of the glass forward. Still more to the right let a lighted candle be placed. The candle and the observer's eye should be symmetrically arranged on either side of the optic axis of the lens and watch glass. Observe the images reflected from the surface of (*a*) the watch glass; (*b*) the anterior surface of lens; (*c*) the posterior

surface of lens. The images at (*a*) and (*b*) are erect; at (*c*) is inverted; the image at (*b*) appears to be the most deeply situated of the three.

EXPERIMENT II. In a darkened room let the observer bring a lighted candle near the eye of the subject, rather to one side of his optic axis. The observer places himself so that his eye is symmetrical in position to the candle on the other side of the optic axis of the subject. When properly adjusted there should be observed reflected from the eye of the subject three images the first bright and erect, reflected from the cornea; a second near the centre of the pupil, but much feebler than the first, and apparently the most deeply situated of all the images, this being reflected from the anterior surface of the lens; a third image represented by a mere spot of light differs from the other two in being inverted. If now the accommodation of the subject be shifted from a far to a near point, the middle image will advance but grow smaller, and will approach the corneal image. The other images do not alter.

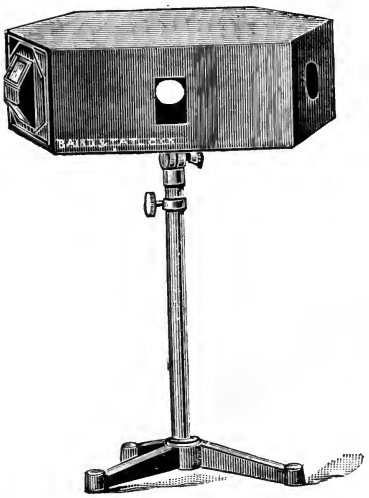


FIG. 94.—The phakoscope.

During varying accommodation it is found that this image is the only one to change, thus indicating that the change is in the anterior surface of the lens.

EXPERIMENT III.—**The Phakoscope.**—This instrument is specially adapted for viewing the reflected images of Experiment II. It is represented in Fig. 94. Fig. 95 represents diagrammatically the arrangement and course of the rays of light. It consists of a dark box, roughly triangular in shape, with the angles of the triangle bevelled off, and at *S* and *O* fitted with windows (Fig. 95).

The observer's eye is at *O*, the subject's at *S*. At *C* two prisms are arranged vertically so as to allow two illuminated squares to fall upon the eye at *S*. The eye at *S* can either be focussed for the vertical needle at *W*, or (since this lies in an opening) for distant objects beyond the opening. With the alteration of the lens corresponding to the change of accommodation, the images from the anterior surface of the lens will vary as in Experiment II.

6. **The Protrusion of the Lens during Accommodation for near objects.**—The actual bulging forward of the lens during accommodation for near objects may be seen in the following experiment.

EXPERIMENT. Using only one eye, let the subject of the experiment have arranged in the optic axis two objects, one about ten inches, the other distant. The observer takes a position so as to view the subject's eye in profile, but somewhat obliquely, so that he can just see the further side of the iris. When the subject's eye is accommodated for the near object, more of the pupil shows and the further side of the iris becomes narrower. This is obviously the result of bulging forward of the lens. This is independent of the variation of the diameter of the pupil for the different objects.

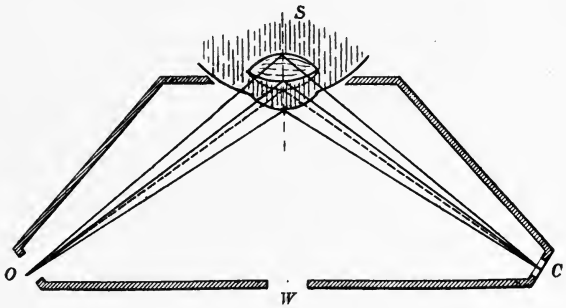


FIG. 95.—Diagram of the course of the rays of light in the phakoscope.

7. **Scheiner's Experiment.**—If the eye be accommodated for an object at any particular distance, the effect of preventing the retina receiving all the rays from the object (as by a screen with holes pricked in it and held close to the cornea), is simply to diminish the brightness of the image, on account of the lessening of the amount of light entering the eye. Any object at a distance for which the eye is not accommodated will form a blurred image on the retina, and if rays from the object by this partial screening of the retina have several paths by which to impinge on the retina, there will be formed upon the retina as many blurred images as there are openings in the screen. When, however, the eye is accommodated for this second object, these blurred images fade into one clear image.

EXPERIMENT I. To form a screen take a thin piece of cardboard and prick two holes in it, separated by less than the diameter of the pupil. About one-sixteenth of an inch will answer. Place in a strip of wood about a yard long two vertical needles, distant eight and twenty-four inches from the eye. Close one eye and with the other, holding the screen close to cornea, look at one of the needles. The other needle

will be also seen, but represented by a double blurred image. If the more distant needle be accommodated for, a double blurred image of the nearer will be obtained. Cover one of the holes in the screen with another card. If the right hole be covered the left blurred image will disappear, and conversely. Let the eye be now accommodated for the nearer image. A double blurred image of the more distant needle will be seen. If the right hole of the screen be now covered the right blurred image will disappear, and conversely.

EXPERIMENT II. A slight modification of this experiment and the material requisite is provided in the Milton Bradley Pseudoptics, Section I., exp. 4.

EXPERIMENT III. Experiment I. can be most instructively performed with Kühne's Artificial Eye. A special screen for the experiment is provided in which one hole is covered with red mica. Accommodation for the different distances is provided by shifting the retinal screen backwards or forwards, and the illuminated arrow can be used as an external object. It is found that if the screen be shifted forward so as to accommodate for objects beyond the arrow, that two blurred images of the arrow obtain. Covering either hole will block either image. But when the eye is accommodated for a more distant object it will be observed that covering the left hole removes the left *retinal* image. If the images be projected, as before, on the lantern screen, the opposite image will of course be removed. The apparent contradiction between Experiments I. and III. is obviously due to the fact that in I. the images are referred to the field of vision, in III. (without the use of further projection on the lantern screen) they are actually viewed as formed on the retina.

EXPERIMENT IV. The near point of accommodation can be conveniently ascertained by noting the least distance at which a single image of a needle can be seen, when using the perforated screen of Scheiner's experiment.

EXPERIMENT V. In Experiment II. note that the thread on which the needle hangs remains clear as a single thread for a certain distance on either side of the needle, but that beyond this distance it gradually bifurcates into a double thread. This singleness of the thread corresponds to the length of the line of accommodation. (See Experiment II., page 101.)

CHAPTER XXVII.

THE RETINA.

1. **The Blood-vessels of the Retina.**—The blood-vessels supplying the retina are distributed to the anterior portion of the retina, the main vessel entering the eyeball at the spot where the optic nerve passes in. These blood-vessels then lie between the vitreous and the sensitive part of the retina, and under certain circumstances they may throw shadows upon this portion of the retina.

EXPERIMENT I. **Purkinje's Figures.**—Make the subject of the experiment turn one eye inwards, and with a lens concentrate a good light upon the exposed sclerotic, focussing the light so as to make a small but strongly-illuminated area. Let the subject look towards a dark wall. Give the lens a gentle rocking or circular movement. The field will appear to the subject as reddish-yellow, and dark figures will be seen by the subject appearing in the field, which branch and have the character of the retinal blood-vessels, of which they are really the shadows. In the direct line of vision a small area will be seen free from these branching shadows. This is the yellow spot.

EXPERIMENT II. Through a pinhole in a card held close to the eye, look at a brightly and evenly-illuminated surface, as a white cloud or a sheet of thin white paper held in front of a lamp. Give the card an up-and-down movement, and a number of vessels will be seen running horizontally in general. Move the card from side to side, and vertically-running vessels will be apparent. Give the card a circular movement and the general distribution will be visible. Note that in the direct line of vision is a small area in which no vessels are seen, the macula lutea or yellow spot.

EXPERIMENT III. Remove the objective from a microscope, arrange the mirror for a good light, and move the microscope in the same way as the card was moved in Experiment II. The results will be as in that experiment.

In all these experiments the movement of the light or the illuminated field is essential. The retina appreciates these shifting shadows better than if they were continually applied to any fixed point of its surface. Further, a moving object will arouse attention more readily than one of constant position, which tends to be neglected.

2. **The Circulation in the Blood-vessels of the Retina.**—EXPERIMENT.—Look through a thick piece of blue glass at a white cloud. Many finely-illuminated points will be seen traversing the field. These

again are followed by slight shadows. Fix the gaze and note that these points move in constant directions. They probably represent small local collapsings of fine capillary blood-vessels, caused by temporary clogging of the red corpuscles. The re-filling of the vessel brings about the shadow following the bright point.

3. **The Blind Spot.**—A certain region of the retina, to the inner side and somewhat below the macula lutea, is insensitive to light, inasmuch as the optic nerve here enters the eyeball, and the layer of the retina which reacts to the stimulus of light is here absent. This insensitive region is spoken of as the optic disc or blind spot.

Experiments showing the nature of the blind spot may be conveniently carried out with the material in Section H. of the **Milton Bradley Pseudoptics** series.

EXPERIMENT I. Using cards H.2, or H.3, close the left eye and fix the gaze of the right eye on the cross. At a distance of about eighteen inches the tree in H.2 or the red disc in H.3 will disappear.

EXPERIMENT II. Arrange the cards H.4 and H.5 at such a distance that when the left eye is closed and the right eye gazes at the cross, the house in H.4 or the red spot in H.5 falls on the blind spot. It will be found that similarly, with the right eye closed and the left eye fixating, the cross, the church, and the yellow disc will be invisible. Having found the proper distance, open both eyes and place the card H.4x close to the nose and in the plane of the septum of the nose. It will be found that when the gaze is directed to the cross the surface of the cards appears uniformly white.

EXPERIMENT III. If a dot and a cross be drawn about four inches apart, the dot being about half-an-inch above the horizontal level of cross, and if then the left eye be closed and right eye gaze at the dot, at the distance of about a foot, the cross will be invisible, as its image falls on the blind spot.

When any image falls upon the blind spot it is invisible. By imagination *we fill in this region* of any image falling upon the retina by sensations similar to those in the neighbouring regions. This is well illustrated in the following experiments.

EXPERIMENT IV. Using the cards H.6, H.7, H.8, and H.9, and ascertaining the distance at which they should be placed, as in Experiment I., notice that when the coloured discs fall upon the blind spot, the place of the discs is taken by a combination of the background on which the discs lie. In H.9 in particular there seems no break in the chequered pattern forming the background to the red disc.

The blind spot may be *mapped out* with ease in the following manner.

Experiment V. Let the head rest in a fixed position, as by placing the chin in a tin mug, and place a sheet of white paper vertically in front of it at a distance of eighteen inches. Put a dot in the centre of the paper. Close one eye and with the other fixate the dot. Take a thin strip of white card-board and blacken about two millimetres of the end. Move the blackened end over the region of the field of vision corresponding to the blind spot, and note the points where the black area disappears, marking them on the white paper. A sufficient number of these points can be taken, and a curve drawn through them will indicate the margin of the field of the blind spot.

4. **The Yellow Spot.**—The experiments performed to exhibit the retinal circulation have shown that there is a certain region in the direct line of vision where the retinal blood-vessels are not visible. This region is coloured by a pigment which absorbs the blue and green of the spectrum, and therefore appears of a reddish-yellow colour and is called the yellow spot.

Experiment. Take a flat-sided bottle containing a fairly strong solution of chrome alum, or use a sheet of purple or violet gelatine. Look with one eye closed through the coloured medium at a bright white surface. A rose-coloured oval spot will appear in the centre of the field. The pigment of the yellow spot absorbs the blue and green, and transmits the rest, and hence the predominant red tinge imparted to the area corresponding to the macula lutea.

5. **Acuteness of Vision in different Regions of the Retina.**—In order to differentiate similar objects grouped closely together it is necessary that these should subtend an angle of a certain magnitude with respect to the eye. To be more precise, the angle subtended is at the nodal point of the schematic eye, and this angle again is equal to that subtended at the nodal point by the image of the differentiated objects on the retina. In order that objects be differentiated it is apparently necessary that their contiguous margins and the space between should form an image on the retina, which is of certain length. Helmholtz found that a subtended angle of 63·75″, equivalent to a retinal distance of ·00463 mm., was necessary for discrimination. As far as this method of investigation is concerned it appears to connect visual acuity with the distribution of the cones.

Experiment I. Set up in a good light the parallel line diagram used in the experiment on chromatic aberration (Experiment III.). Or arrange a series of five black wires, separated by their own diameter, against the sky. Walk backward from either of these objects till they can just be no longer discriminated. Calculate the size of the retinal image.

The visual acuity diminishes rapidly on the retina as we recede from the fovea. The diminution is more marked in the vertical than in the horizontal meridian.

EXPERIMENT II. Place on a card two dots, each 2 mm. in diameter and separated by a distance of 2 mm. Let the gaze be fixed on a mark on a vertical white sheet of paper, and let the card be moved in a horizontal meridian gradually nearer the mark till the two dots can be discriminated. Compare the vertical and horizontal meridia in this respect.

The acuteness of vision at the fovea is ordinarily tested by noting the distance at which letters, which at a given distance subtend an angle of 5′ at the eye, can be read. This method may be applied either to ascertain what error of refraction may exist in the eye, or if this be absent or corrected, what the acuteness of vision in the particular eye is.

EXPERIMENT III. Using Snellen's or Jaeger's test types, ascertain whether the letters can be correctly named at the normal distance in a good light. If this distance can be exceeded or if it cannot be reached an expression for the condition of the acuteness of vision may be written as follows:

$$V = \frac{d}{D}$$

where d = distance of person from the types and D = number of smallest type which a person can read at that distance.

6. **Mechanical Stimulation of the Retina. — Phosphenes.** — The retina can be stimulated by pressure on the sclerotic. An image will be produced which is referred to the opposite portion of the field of vision.

EXPERIMENT I. Close one eye and turn it as far as possible towards the nose. Press with a pencil point on the sclerotic, through the eyelid, at the edge of the orbit on the outer side. Note the circle of light which appears on the nasal side. The retina is stimulated just beneath the pressure and the image is referred to the nasal side of the field of vision.

EXPERIMENT II. Standing before a light close the eyes and move them quickly from side to side. When they reach the extreme position there will be seen in front a gradually disappearing blue spot with a yellow halo. This is due to a mechanical stimulation of the retina at edge of the blind spot, resulting from the effect of the movements on the optic nerve. Note in which eye the image is most distinct in either of the lateral movements.

7. **The apparent Inversion of Shadows thrown upon the Retina.**—If a beam of light falling upon the retina be intercepted by some object

close to the cornea, an erect shadow of the said object will be thrown upon the retina. This, however, will be projected into the field of vision as an inverted image.

EXPERIMENT. The Experiment No. 6, Section I. in Milton Bradley Pseudoptics, illustrates the nature of retinal shadows well.

8. **The Perception of Colour in the Peripheral Portion of the Retina.**—The sensibility of the retina for colour varies in different zones of the retina, and for different colours. Blue and yellow can be recognised at a greater distance from the fovea than red and green. Still more peripherally all colours appear as black, grey, or white.

EXPERIMENT I. Milton Bradley Pseudoptics, Section H, Experiment No. 1, conveniently illustrates the variation in the sensibility of the retina for colour.

EXPERIMENT II. If a perimeter or campimeter be used the boundaries of the field for the different colours can be defined. (See use of perimeter.)

9. **The Perception of Light in different Regions of the Retina.**—A faint light is often more easily seen when its image does not fall on the fovea, but a few degrees away from this. The recognition of a light at sea on a dark night is often facilitated by directing the gaze some ten degrees to the right or left of the supposed luminous object. Faint stars again may be seen more readily if not directly gazed at.

10. **Idio-Retinal Light.**—Let the eyes be carefully covered, but not pressed on; let the observer be placed in a perfectly dark room. After a time the effect of recent external light will pass off, but still fine clouds of light may be seen passing over the retina. This is supposed to be due to the action of the blood on the peripheral portion of the visual apparatus.

11. **After-images.**—After-images may be of two kinds, those which reproduce the original body in all its brightness, those that are the reverse in brightness to the original body. The first are called **positive** after-images, the second are **negative** after-images. Positive after-images may be either of similar colour to the original body or complementary in colour, negative after-images are always complementary. They are due to certain changes taking place in the retina and are best observed in the early morning after waking.

EXPERIMENT I. Close the eyes for two minutes to rest them and then for the briefest possible interval look at some bright source of light as the lamp or the window, closing the eyes again. A bright positive after-image of the source of light will be seen.

EXPERIMENT II. Look at the incandescent filament through a piece of red glass, as in Experiment I. The positive after-image will appear

red. Again look at the filament but for a prolonged period of about half a minute. On closing the eyes the after-image will appear bright but greenish in colour.

By an alteration of light and dark backgrounds the after-image may be changed from negative to positive.

EXPERIMENT III. Look at an incandescent lamp for half a minute and so get a well marked after-image. If the eyes be directed to a white surface the after-image will be negative, if to a dark surface it will appear positive.

EXPERIMENT IV. Note the colour of the after-images in Experiment III., and the gradual change in colour which they show. If the after-images tend to fade blink the eyes several times rapidly and they will become more marked. Notice especially the effect of blinking on the negative after-image seen on the white surface. It will become during the shutting of the eyes converted into a positive after-image.

FIG. 96.—Disc for the experiment on after-images of motion.

EXPERIMENT V. Look at an incandescent lamp with the right eye, the left eye being closed. After the lapse of half a minute, shut the right eye and look with the left at a dot on a white sheet of paper, as far as possible without blinking. After a time the field will gradually darken and a positive after-image will be seen. This is really the after-image seen with the right eye, which is not visible till a certain amount of retinal insensibility has occurred in the left eye.

EXPERIMENT VI. After-images of motion may be shown by gazing at the disc in Fig. 96 slowly rotated and then shifting the gaze to some uniformly mottled surface.

CHAPTER XXVIII.

THE OPTICAL DEFECTS OF THE EYE.

1. **Spherical Aberration.**—This is probably of little consequence in the eye, as the action of the iris eliminates it largely.

2. **Chromatic Aberration.**—Rays of coloured light are refracted differently according to their position in the spectrum. Those of shorter wave length, as the violet and blue, come to a shorter focus than do those of longer wave length, as the red.

EXPERIMENT I. Look through the upper part of a window towards the sky. Pass a card before the eye with the edge parallel to the upper side of the window frame. If the card be passed from below upwards, when it has covered about half the pupil the frame will be seen to have a border of blue. If the card be passed from above downwards, when it covers half the pupil the edge of the frame will be seen to have a reddish-yellow fringe. In the first case the less refracted red constituents of the margin of the white light are cut off by the card, in the second case the more refracted blue rays.

EXPERIMENT II. Look at the incandescent filament of an electric lamp. Pass a card across the pupil with the edge parallel to the filament. When the edge of the card is almost covering the filament, the filament is seen to have a red fringe on the side nearer the card, and a blue fringe on that more remote.

EXPERIMENT III. Von Bezold's experiment. Draw a series of vertical parallel bars one millimetre wide, separated from one another by a width of one millimetre. Look at these lines with imperfect accommodation. If the figure be placed about 60 cms. away, and the eye accommodate for an object (*e.g.* a pin) about 20 cms. away from the eye, the white intervals may become a rather dark red, and the black bars appear a bright greenish yellow.

3. **Astigmatism.**—It is frequently the case that the curvature of the cornea, or lens, in the vertical meridian is greater than that in the horizontal meridian. Therefore, accommodation for a horizontal bar at a certain distance means under-accommodation for a vertical bar at the same distance. Persons who have such a spoon-shaped cornea are said to suffer from **regular astigmatism.**

The cornea, or lens, may have irregular curvatures in various meridia, resulting in **irregular astigmatism.**

EXPERIMENT I. Adopting the method of ascertaining the near point of accommodation in Scheiner's experiment (Experiment IV.,

page 105), observe the near points of accommodation for a pin held vertically and one held horizontally. Note if the distances are identical.

EXPERIMENT II. Draw a rayed figure as follows: First, draw two lines intersecting in the centre at right angles, and each about 5 cm. long. Bisect each right angle by two other lines intersecting at the same point, and each of these smaller angles bisect further by four other lines. Bring this rayed figure to the near point of accommodation. Observe which of the lines can be distinctly seen, and which are blurred. It will generally be found that the horizontal and those adjacent will be clearly seen, when no effort will bring about definition of the vertical.

EXPERIMENT III. Using Kühne's artificial eye, place in front of the cornea the special glass trough (filled with water) designed for exhibiting the nature of astigmatism. This has a plane surface posteriorly. The anterior surface, however, is that of a cylinder, curved in horizontal meridia but not in vertical meridia. Substitute for the arrow used in earlier experiments as a source of light a stencilled cross, each bar being about 5 cm. long and $\frac{1}{2}$ cm. wide. Before introducing the astigmatic lens, ascertain the position of the retinal screen necessary for definition of the luminous object. Then place the lens in position. The image will become changed. It will be found that the upper and lower edges of the horizontal bar and the ends of the vertical bar are still distinct, but otherwise definition of the vertical bar is absent. In order to obtain definition of the vertical bar it will be necessary to move the screen much closer, when a reversed effect will we seen—viz., definition of the vertical bar, its end, however, blurred; the end of the horizontal bar clear, but its edges altogether undefined. At no intermediate position between the two foci can a clear image of the cross be obtained, and it will be necessary in order to compensate for the presence of this lens, convex in horizontal meridia, to introduce a second lens, plane on one surface, and concave in horizontal meridia. This indicates the method of correction of the defect in the human eye.

4. **Errors of Refraction.**[1]—In this division of the optical defects are included the conditions of **Myopia** or **short-sight**, **Hypermetropia** or **long-sight**, **Presbyopia** or **the sight of old people.**

The normal eye in which the far point of accommodation is practically infinity and the near point 20 cm. (8 ins.), is spoken of as **emmetropic.**

Presbyopia.—As a result of advancing age the power of accommodation for near objects may become diminished. Parallel rays are still focussed on the retina, but the ciliary muscle is unable to bring about

[1] Properly speaking, *astigmatism* should be included in this section. We have thought it best, however, to consider it in a separate section.

sufficiently increased curvature of the crystalline lens to accommodate for objects as near as eight inches. It may here be mentioned that in the normal eye continuous exercise of the full power of accommodation rapidly produces fatigue. It is impossible without fatigue to use more than a half to two-thirds of the full accommodation for any protracted period. The normal-sighted person instinctively avoids placing near objects, *e.g.* a book, closer to the eye than about sixteen inches. Similarly a presbyopic person whose near point is, say, ten inches, will hold a book at about twenty inches distance. Unless the illumination be extremely good the small size of the retinal image causes some difficulty to occur in reading. This, however, may easily be corrected by assisting the crystalline lens through using convex glasses, the degree of convexity corresponding to the extent of failure of accommodating power. These are, of course, only necessary when looking at objects close to the eye. It may be mentioned here that the distance of the near point gradually increases from infancy to old age. According to Landolt it is about 3 inches at 10 years of age, 4 inches at 20 years, $5\frac{1}{2}$ inches at 30 years, almost 9 inches at 40 years, 16 inches at 50 years, 40 inches at 60 years, at 70 years about 13 feet, and at 75 there is practically no near point, in other words the power of accommodation is generally lost.

Ametropia.—This is a term applied to all conditions of the eye in which the retina does not lie at the focus for parallel rays. The retina may lie in front of this focus when we have the condition of *hypermetropia*, or behind when *myopia* is the result, or the focus may be a linear one for any distant luminous point when we have the condition of *astigmatism*.

Hypermetropia.—In this condition the antero-posterior axis of the eye is generally too short. By some effort of accommodation, distant objects may form a clear image on the retina, but the individual suffering from this optical defect does not possess sufficient power of accommodation to focus clearly near objects. Though the emmetropic condition with much facility of accommodation is acquired at about the age of eight years, before this stage is reached the eye is naturally hypermetropic. A young child with marked hypermetropia and deficient power of accommodation will in viewing near objects (*e.g.* reading), make every effort with both eyes to accommodate for such objects. Included in this effort would be an exaggerated action of the muscles producing convergence of the optic axes of the eyes, leading to squint, but such squint will frequently be removed on correcting the optical defect.

EXPERIMENT. Using Kühne's artificial eye, place the retinal screen in the position necessary to obtain a clear image of the external luminous arrow. In this position of the retina the condition of the eye

may be regarded as emmetropic. Now move the screen about an inch nearer the corneal surface. The image at once becomes blurred. This represents the condition of the hypermetropic eye. Now place in front of the cornea a very weak convex lens. The image will become much clearer, and with little difficulty a lens of sufficient converging power may be chosen which will exactly correct the defect.

Myopia.—This defect is usually congenital, the result of the antero-posterior diameter of the eye being too long. As a result parallel rays are brought to a focus in front of the retina, and the eye cannot form a clear image of an object beyond a certain distance (far point of accommodation). The most common cause of acquired myopia in children is the reading of books with insufficient light. The child brings the book close to the eye to get a sufficiently large image of the words and this finally leads to a myopic state.

EXPERIMENT. Using again Kühne's artificial eye, which, as in the last experiment, is first adjusted as the emmetropic eye, shift the retinal screen about an inch away from the cornea. The arrow now becomes blurred and the eye resembles the myopic eye. Place in front of the cornea a concave lens. The image will become much clearer if the degree of concavity corresponds to that of the defect. It is necessary in this case to use a lens of dispensive power in order that the image may be thrown back on to the retina.

CHAPTER XXIX.

THE INSTRUMENTS USED IN THE CLINICAL INVESTIGATION OF THE EYE.

The Measurement of the Field of Vision.– If the eye be fixedly directed to some particular point it is possible to see objects at some distance from this point. The area in which objects can be seen with the eye thus fixated is spoken of as the *field of vision.* With the head fixed and the eye allowed to move as far as possible in any direction a much larger area can be viewed. This area is spoken of as the *field of regard.*

Though fairly satisfactory results can be obtained by using a comparatively simple form of apparatus called a campimeter, it is customary to employ an instrument called a **perimeter** to obtain accurate details of the extent of the field of vision.

The perimeter (see Fig. 97), consists of a quadrant upon which a white spot can be moved, and this quadrant can be revolved about a line continuous with the optic axis. At *K* is the chin rest, double, so as to enable either eye to be adjusted against *O.* The subject having taken

his position covers one eye and fixes the eye that is to be examined on the mark at *f*. The quadrant is then placed, say in the vertical meridian, and at the back of the wheel which revolves with the quadrant is inserted in the frame a special chart adapted for recording

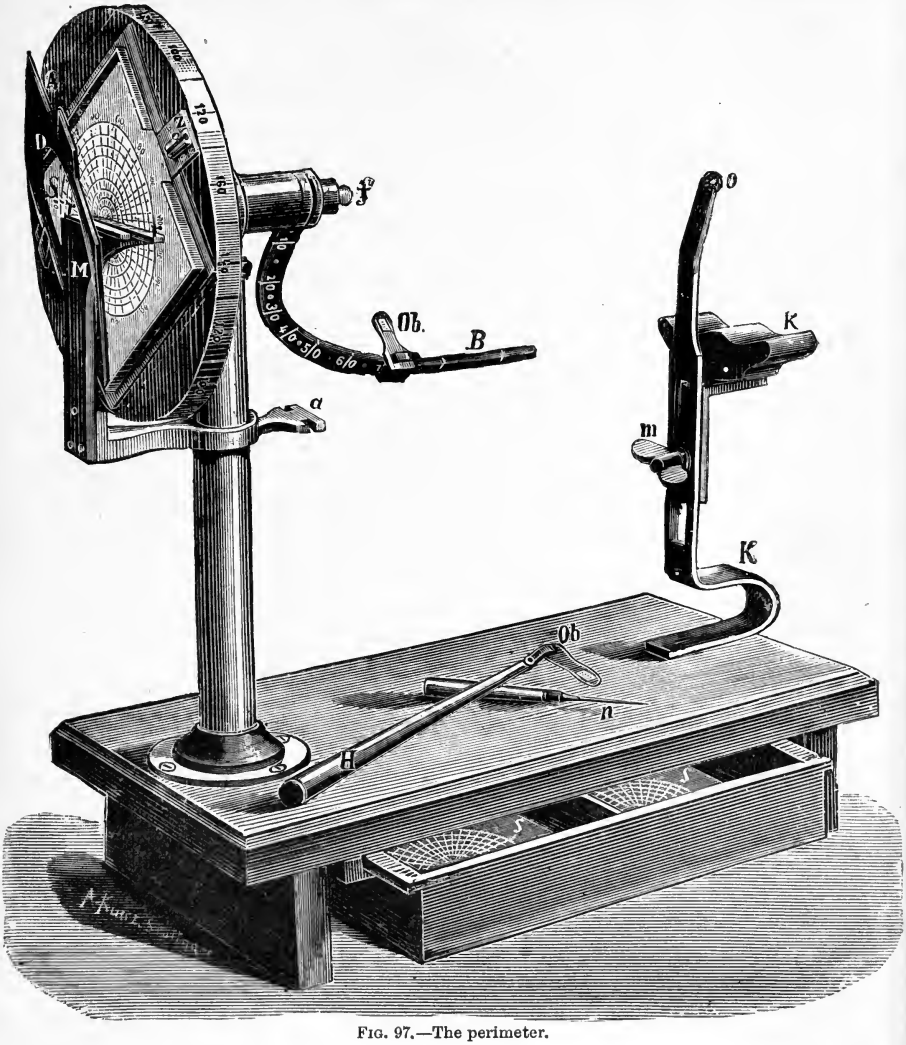


FIG. 97.—The perimeter.

perimetric observations. Starting at the extreme distance the mark *Ob* is gradually moved along the quadrant and at a certain angle the white spot will be just visible. The angle indicates the limit of vision in this meridian and can be recorded on the chart. Similar observations

are made in other meridia. In this manner the limits of vision in the different meridia of the field of vision can be recorded.

It is of course essential that the subject keep his eye fixed on *f* the whole time the spot is being moved.

The area bounded by a line drawn through the limiting points in the different meridia is properly the area of the field of vision. It is, however, often desirable to refer this area to the retina. If the meridia be inverted, the figure traced would then correspond to the sensitive portion of the retina. It will be found that perimeters are generally so constructed that the limiting marks in the different meridia are inverted on the chart, so that the latter becomes a chart of the extent of the sensitiveness of the retina. This is indicated in the figure above.

The Ophthalmoscope.—Prior to the invention of the Ophthalmoscope it was not possible to view the interior of the eye. The reason of this is that when the interior is illuminated an image of the source of illumination is formed in the retina, and the reflected light passing from the illuminated area out again from the eye will be subject to the refracting mechanism of the eye, and form a small image in the line of incidence of the source of light.

The Ophthalmoscope (Fig. 98) is really a contrivance to enable an observer to direct his vision along the axis of the pencil of light illuminating the subject's eye, and thereby to enable him to receive light reflected from the retina of the subject, in other words, to actually see the illuminated retina. The instrument consists essentially of a mirror, in which is a central aperture. The mirror is arranged so as to reflect light from some source through the pupil into the interior of the eye. The observer, looking through the central aperture, is able to view the illuminated posterior wall of the eye.

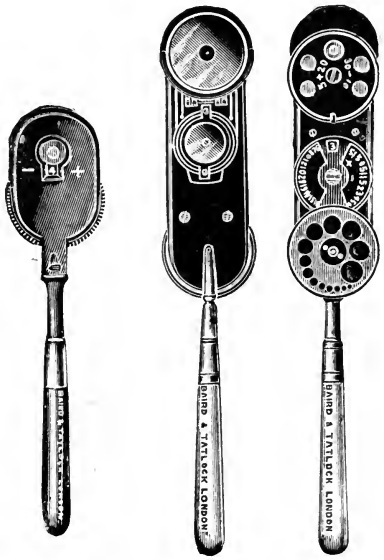


FIG. 98.—Ophthalmoscopes.

Two methods are usually adopted of using the ophthalmoscope, one being known as the **direct**, the other as the **indirect**. In the first case there is obtained an erect view of a small area of the retina, magnified

about thirteen times; in the second case a less magnified and inverted view is obtained of a larger area of the retina.

The Direct Method.—The source of light is placed at the side of the head of the subject, so that no light falls directly on the cornea. The mirror, which is somewhat strongly concave, is held a few inches from the subject's eye, and is so tilted that light is directed into the pupil. The observer uses his left eye to examine the subject's left eye, and similarly his right eye for the subject's right eye. By bringing the light very close to the mirror, and this again close to the eye, the subject will not be able to accommodate for the image of the source of light, and consequently a dispersion circle of light will fall upon the retina. If the observer look through the aperture and the subject's eye be emmetropic he will obtain a clear view of the details of the retina. The reflected light from the subject's retina will issue as parallel rays and thus be in an appropriate state to impinge on the observer's cornea without requiring him to make any effort of accommodation.

The Indirect Method.—In this case a somewhat larger, but less concave or a plane mirror is used. The mirror is held at a distance of about eighteen inches, and if the accommodating power of the subject is intact his eye will accommodate for the source of light or its image formed by the mirror. An inverted image of the illuminated area of the retina will be formed at a certain distance behind the mirror. If the rays issuing from the eye be intercepted by a rather strong convex lens held close to the cornea a new image will be formed, smaller and more brilliant but still inverted. The observer then looks through the aperture of the mirror, and holding a lens as above against the cornea obtains a clear view of a considerable portion of the illuminated retina.

Ophthalmoscopes are generally supplied with a revolving disc of lens of different strengths, which are used to correct any error of refraction in the subject's or observer's eyes.

It is frequently a matter of difficulty to obtain a clear view of the back of the eye or **fundus** in the subject unless some drug previously has been applied which causes dilation of the pupil. For practice in the use of the ophthalmoscope, an albino rabbit, the eye of which has been treated with atropin, can be advantageously substituted for the human subject; or artificial eyes, such as Frost's or Perrin's artificial eyes, may be used. In absence of these, the ocular of a microscope furnishes the material for the construction of an artificial eye. If the lower lens of this be removed and a disc painted to represent the fundus be inserted and blocked behind, an artificial eye is obtained which can be used with advantage.

THE VASCULAR, RESPIRATORY, AND ALIMENTARY SYSTEMS. DEMONSTRATIONS.

CHAPTER XXX.

CIRCULATION OF THE BLOOD.

Proofs of the Circulation of the Blood.—A mammal is anaesthetised with ether and chloroform.

The external jugular vein is exposed and the carotid artery. A clip is placed on the jugular vein. Note the central end of the vein empties, while the peripheral end becomes enlarged. A clip is next placed on the carotid artery, the central end becomes distended and pulsates, while the peripheral end shrinks and ceases to pulsate. The clips are now removed and two ligatures placed in position (but not tied) under each vessel. The vein is pricked. Note the dark blood which flows out from the peripheral end steadily and without force. The vein is then tied above and below the opening. The artery is next pricked. Note the blood spurts out forcibly and in jets from the central end. The artery is then tied above and below the opening.

A tracheal cannula is placed in the trachea and connected with the artificial respiration apparatus. The sternum is divided in the mid-line, and the thorax opened, so as to expose the heart. The pericardum is slit open. Observe the systole and diastole of the auricular appendices and ventricles. Ligatures are now passed under the superior and inferior venae cavae and tightened. The heart quickly empties. On loosening the ligatures observe the immediate filling of the right heart. A ligature is next passed under the aorta and tightened. Observe the engorgement, firstly, of the left, and then of the right heart. On loosening the ligature note the effect. A ligature is next passed under the pulmonary artery and tightened. The right heart becomes engorged while the left empties. On loosening the

ligature note the result. If the origin of the pulmonary artery and aorta be forcibly compressed between thumb and finger, the second sound of the heart disappears. If the index finger be placed behind the aorta and pulmonary artery, and the thumb at the level of the auriculo-ventricular groove, and the heart be thus forcibly compressed, the filling of the heart will be prevented. The first sound continues to be heard feebly, while the second sound ceases.

The heart is now excised, the right ventricle quickly opened. The papillary muscles may be observed contracting synchronously with the ventricular wall. The first sound may be heard in the excised heart.

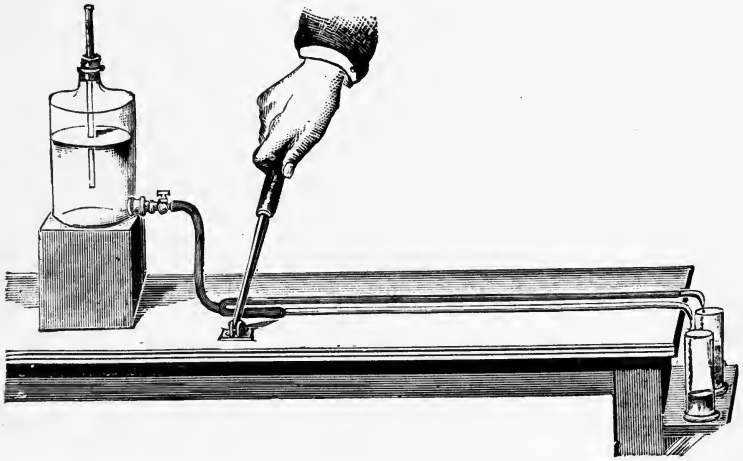


FIG. 99.—Schema to show the flow in rigid and elastic tubes. (Marey.)

The Flow in Rigid and Elastic Tubes.—Arrange an experiment as shown in figure 99. The two tubes are 1 metre long and of the same bore, but one is a rigid tube and the other elastic. The small outflow orifices are made as nearly as possible of the same size. Rhythmically open and shut the compressor. The flow from the rigid tube is intermittent, while from the elastic tube it is continuous. The latter delivers more fluid in one minute than the former. Change the outflow orifices to eliminate this source of error and repeat the experiment. Feel the pulse in the elastic tube. Observe that the outflow from it becomes intermittent when the outflow orifice is enlarged. The increased and continuous flow from the elastic tube is due to the potential energy stored up in the stretched wall of the tube, which maintains the flow during diastole.

The Artificial Schema.—The two ends of a Higginson syringe *B* are connected with a long rubber tube. The middle of the tube divides into two channels; (1) a glass tube filled with shot representing the

capillaries, (2) a rubber tube closed by a screw-clip. The screw-clip represents the muscular wall of the arterioles. A mercury manometer is connected by a ⊥ tube with the artery and another with the vein. A loose cotton wad plug is placed in the open end of each manometer to prevent the mercury being accidentally forced out. The whole system is filled with water. The bulb of the syringe is placed under a hinged board which lies just beneath the shaft of the motor. The shaft is provided with a projecting screw, which depresses the board on each revolution, and compresses the syringe. When the motor is set going the water is pumped by the syringe round

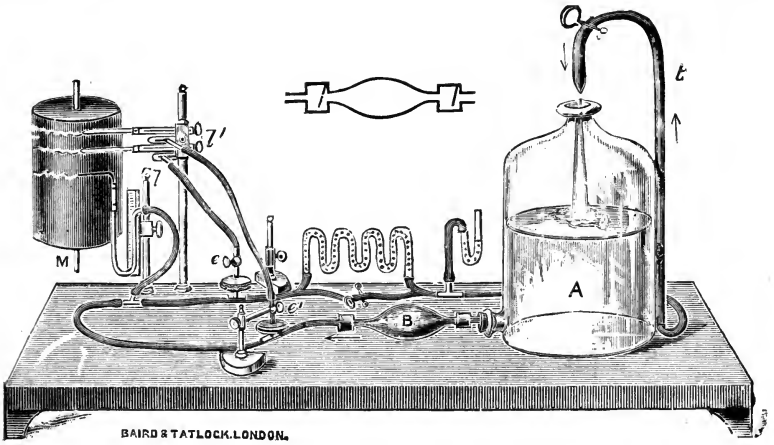


FIG. 100.—Artificial schema of the circulation.

the schema. The valves act as the mitral and aortic valves. When the screw-clip is widely open, there is little resistance to flow. The outflow from the artery into the vein ceases during the diastole of the syringe. The conditions are the same as if the artery were a rigid tube. The diastolic and systolic variations of pressure are very great, and affect both manometers to a like extent. Screw up the clip. The flow, as the resistance increases, becomes less and less intermittent and finally continuous. The mean pressure rises in the arterial and falls in the venous manometer. The systolic and diastolic variations of pressure become greatly reduced in the former. The systolic variation disappears in the latter. When the vascular system is formed of a wide tube free from constrictions, each systolic pulse-wave travels with so great a velocity that the whole system reaches the same pressure before the next systole of the heart occurs. The conditions are otherwise when the clip is screwed up, for the friction of the blood flowing through the narrow channels prevents the blood from passing with anything like

the velocity of the pulse-wave. In the vascular system the pulse-wave travels in the arteries 8 metres per second, while the blood travels $\frac{1}{3}$-metre.

The resistance to flow is chiefly situated in the arterioles, where the velocity is high. It is due to the friction of the moving concentric layers of blood against one another, and against the stationary layer which wets the wall of the blood vessels. It is proportional to the surface area, to the viscosity of the blood—nearly proportional to the square of the velocity of flow, and inversely proportional to the sectional area of the vessel. In the arterioles the velocity is high, the total wall surface wet by the blood great, the sectional area of each arteriole very small.

In the schema the resistance is increased by diminishing the sectional area of the arterioles and increasing the velocity of flow. Owing to the resistance to the outflow the arteries are expanded by each systolic output, and the elasticity of their walls comes into play, causing the outflow to continue during the succeeding diastole of the heart. The larger part of the kinetic energy of the systolic outflow is stored up as potential energy by the stretched arteries, and converted into kinetic energy during diastole.

Remove the bottle A and connect the vein directly with the syringe B. Stop the pump, the pressures in the manometers fall to the same level, *i.e.* to a mean hydrostatic pressure. The amount of this depends on the distension of the system with water. Start the pump again. The fluid is taken from the vein and piled up in the artery, for at each systole a greater quantity of blood is driven into the artery than can escape through the capillaries. With each succeeding systole, therefore, the pressure in the artery rises, and the pressure in the vein falls. Venous pressure cannot really sink below the atmospheric pressure as in the schema, for the flaccid walls of the veins collapse. It is not possible to measure a mean hydrostatic pressure in the vascular system, for the arteries contract when the heart is inhibited and drive the blood into the venous side of the system. The venous side is capacious, and possesses little elasticity. Thus the changes of pressure in the venae cavae, when the heart is arrested or starts beating, are insignificant. After the first few strokes of the pump, a condition of equilibrium is established, as the capillary outflow during the period of the cardiac cycle becomes equal in volume to the systolic output.

The continuous flow of blood thus established through the capillaries is due to the difference between the pressure in the arteries and veins. This difference depends on three factors: (1) the energy of the heart, (2) the elasticity of the arteries, (3) the peripheral resistance. The energy of the heart is spent in overcoming the resistance, and is dissipated into heat.

Vary (1) by lessening the rate of the pump; vary (2) by opening the screw-clip—the difference in pressure diminishes in either case, and the flow becomes intermittent. When the screw-clip is open a very frequent beat of the pump is required to make the flow continuous, and scarcely any fluid passes through the capillary tube. By means of the vaso-motor nerves the arterioles are similarly dilated or constricted, and the current switched on to or off an organ, according to its functional activity.

CHAPTER XXXI.

CIRCULATION—Continued. VELOCITY OF FLOW.

Artificial Schema. Velocity of Flow.—(1) Insert the Ludwig stromühr (Fig. 101) into the artery. It is convenient to fill one side with water, and leave the other full of air. In actual practice one tube is filled with defibrinated blood and the other with oil. Set the pump going, and find the number of times the stromühr must be turned per minute. Turn rapidly the moment the water reaches the mark *X*. Each turn means the flow of the quantity of water contained in one half of the stromühr. Measure the capacity of the stromühr and the diameter of the artery. The capacity of half the stromühr multiplied by the number of revolutions gives the volume, and this divided by the time and the sectional area of the artery gives the mean velocity per second. The sectional area of the artery equals the radius × 3·14.

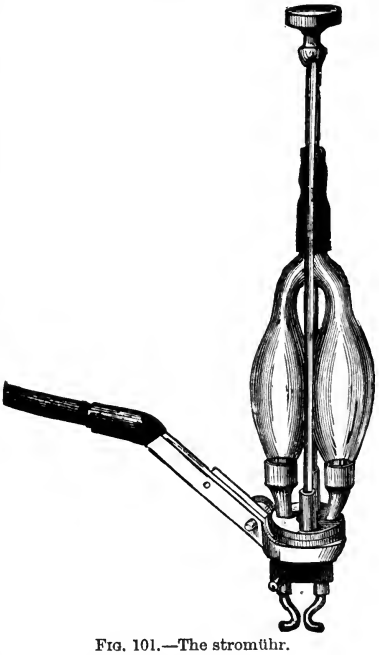


Fig. 101.—The stromühr.

Note the effect on the velocity of (1) opening the clip on the arteriole tube, (2) of increasing the frequency of the pump.

If the energy of the heart is constant, then in proportion as the peripheral resistance increases so the lateral pressure rises and the velocity in the aorta lessens. On the other hand, as the peripheral resistance decreases the pressure falls and the velocity increases. If

the peripheral resistance be constant, then as the energy of the heart increases or decreases both the pressure and the velocity in the aorta together become greater or less. By compensatory changes taking place in the heart and the resistance, the velocity may remain constant while the pressure varies, or the pressure may remain constant while the velocity varies.

The average velocity at any part of the vascular system is inversely proportional to the total cross section at that part. If the total cross section of any one part of the circuit be dilated the velocity becomes slower there, while it proportionately increases in the other parts. This must be so if the blood continues to circulate round the whole system in the same time. Vaso-dilatation in one part is normally compensated for by constriction in other parts.

Velocity in the Capillaries.—Pith the cerebrum of a frog and plug the hole. Lightly curarise the frog, and spread the web over the hole in the web-board. Examine the circulation under the microscope, and with the aid of an ocular micrometer and a clock beating $\frac{1}{5}$ seconds measure the time it takes for a red corpuscle to move through $\frac{1}{100}$ mm. Note in an arteriole that the red corpuscles move the fastest in the axial stream, while the white corpuscles roll slowly along the margin.

Place on the web a drop of hot water (50°-60° C.). The flow at first is accelerated owing to vaso-dilatation, but soon slackens as the red corpuscles clump together owing to the escape of the plasma through the damaged capillary walls.

CHAPTER XXXII.

CIRCULATION—Continued. THE INFLUENCE OF GRAVITY.

Artificial Schema. Influence of Gravity.—The schema is set up as figured (Fig. 102). The lower distensible rubber bag *B* represents the splanchnic vein and capillaries. The upper one *A* those of the head and neck. The skeletal muscles and vaso-motor mechanism are supposed to be paralysed, and therefore the bags are unsupported. Work the Higginson syringe. In the horizontal posture the circulation continues through both bags. In the vertical posture the lower bag fills and becomes distended, owing to the weight of the fluid, the heart no longer fills, and the upper bag is empty and collapsed. Compress the lower bag with your hand, the heart and upper bag fill. This represents the normal action of the skeletal muscles and the vaso-motor mechanism.

The Influence of Gravity on the Circulation of the Snake.—Pith the brain of a grass snake or eel. Fasten the animal on to a board. Expose the heart, which may be seen beating beneath the skin, about 2-3 inches below the mouth. Place the animal head down in the vertical position. Notice the pericardium prevents the over-distension of the heart by the weight of the super-incumbent column of blood. Slit open the pericardium and observe the result. The heart becomes greatly congested. This is especially marked in the eel, when reflexly excited to writhe. Turn the animal head uppermost. The heart gradually empties, and becomes at last pale and bloodless. Slowly tilt the board and observe the blood as it runs up the inferior vena cava and fills the heart. Place the animal again in the vertical posture (head up), and observe that the heart fills (*a*) on compressing the abdomen from below upwards (*b*) on sinking the animal in a bath of water up to the level of the heart. In (*b*) the weight of the water outside tends to balance the weight of the blood within.

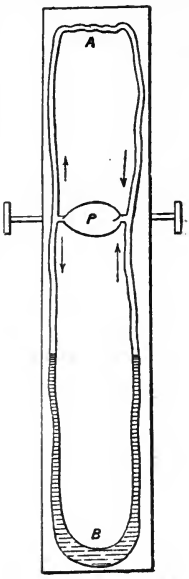


FIG. 102.—Artificial schema.

The vagus nerve may easily be found at the side of the neck in the snake, and the effect of its excitation noted. In the eel reflex inhibition of the heart is very easily brought about by striking the abdomen or gills.

CHAPTER XXXIII.

VASO-MOTOR SYSTEM. CIRCULATION TIME.

Demonstration of Vaso-Motor Nerves.—A white rabbit is chosen, or one with a white ear; the animal is anaesthetised with chloral or urethane; the ear is shaven and fixed by threads to a loop of stout wire. This wire is clamped in front of the lantern, so that the blood vessels in the ear can be plainly seen. The cervical sympathetic is exposed in the neck, where it lies behind the carotid artery, and is traced up to the superior cervical sympathetic ganglion. The thread is tied round the nerve, and the latter is cut. Observe that at this moment the blood vessels in the ear dilate and the ear becomes warmer. The palpebral fissure at the same time becomes narrowed. The change will be much more marked had the ear of the rabbit been previously exposed to cold. The cervical sympathetic exercises a tonic action.

On exciting the peripheral end of the nerve with the faradic current, the vessels in the ear will be seen to constrict, and this will take place to such a degree that all the smaller vessels will disappear from view. The ear will at the same time become several degrees cooler. Note that the latent time is considerable between the excitation and the effect. Note that the pupil also dilates, the nictitating membrane retracts, and the palpebral fissure is widened. The eyeball at the same time projects forwards. The pupillo-dilator fibres arise from the first three thoracic anterior roots, the vaso-constrictor fibres from the second to the fifth, and even to the seventh, in the rabbit. If the superior cervical sympathetic ganglion be painted with nicotine, excitation of the preganglionic fibres will no longer have any effect on the ear, while excitation of the post ganglionic fibres will still be effectual. The sympathetic fibres to the head have their cell-stations in this ganglion.

The Circulation Time of the lesser Circulation.—The carotid artery is exposed in the anaesthetised rabbit. A piece of thin rubber membrane is placed beneath it. Between the membrane and the artery a piece of white paper is inserted. The artery is illuminated by a lamp and condensing lens.

The external jugular vein is exposed on the other side of the neck, and into its central end a cannula is inserted. A burette containing methylene blue (sat. sol. in normal saline) is attached to the cannula, a clip being interposed. No air must be left in the connections. With a stop-watch, or by means of an electric signal and drum, note the time which elapses between the injection of 1-2 c.c. of the solution of methylene blue and its appearance in the carotid artery.

CHAPTER XXXIV.

BLOOD PRESSURE.

Demonstration of Arterial and Venous Pressure by the Method of Stephen Hales.—An incision is made in the mid-line of the neck, from the larynx to the sternum of the anaesthetised animal. The skin-flaps are pulled apart, and the sterno-mastoid and sterno-thyroid muscles separated, so as to expose the carotid artery. With an aneurism needle the artery is freed from the carotid sheath for the space of about an inch. Two ligatures are then placed beneath the artery, and one is tied at the upper end of the exposed portion. On the lower end an artery clip is placed. With sharp scissors an oblique cut is next made into the artery, and the nozzle of the arterial cannula is

inserted, and tied in with the second ligature. Lastly the ends of this ligature are brought round the bulb of the cannula, and tied to make the connection secure.

The arterial cannula is ⊥ shaped and provided with a bulbous enlargement. This shape is chosen both to hinder clotting and to facilitate the washing out of clots. One limb of the ⊥ is fitted with a short piece of rubber tube, and this is closed by a piece of glass rod or a clip. The other limb is connected by a short length of thick rubber tube (pressure tubing) to a long length of fine bored glass tubing. The latter must be at least 5 feet in length, and is held in the vertical position by a clamp.

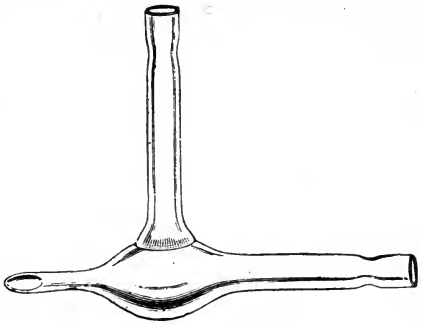


Fig. 103.—Arterial cannula.

The glass tube and cannula are filled with 1 per cent. sodium citrate, and this decalcifies the blood and so prevents clotting. The solution is coloured with methylene blue, and a long strip of white paper scaled in centimetres is placed behind the tube.

By cutting through the attachment of the sterno-mastoid muscle, the junction of the jugular with the subclavian veins is next exposed. The innominate vein is picked up and cleaned with the aneurism needle. Two ligatures are placed under it, and a clip on the part nearest the heart. One of the ligatures is tied round the junction of the jugular and subclavian veins. As the vein is clipped before the ligature is tied, it becomes distended with blood, and this facilitates the introduction of the cannula. The straight vein cannula is connected with a short length (1 foot) of glass tubing. The latter is clamped in the vertical position, and is filled with sodium citrate solution. The cannula is also filled with sodium citrate solution, and to retain the solution a clip is placed on the rubber tube, which connects the cannula with the glass tube. The positive pressure in the glass tube must not be more than 3 to 4 inches of the solution.

The innominate vein is now slit and the cannula introduced. Then the clip on the vein is removed, and the cannula is pushed down into the superior vena cava. The clip on the rubber tube is next opened so as to place the venous cannula in connection with the vertical tube. The fluid in this will now oscillate with each respiration at a level of about 2-3 inches. The clip on the artery is next opened. The fluid in the

arterial tube will oscillate at a height of about 4 to 5 feet. Notice in each tube the cardiac pulsations and respiratory oscillations. The arterial pressure rises in inspiration—the venous in expiration.

1. If the abdomen be compressed the pressures will rise in both the artery and vena, but to a greater extent in the former. The heart is better filled in diastole and the peripheral resistance is increased by the compression of the splanchnic vessels.

2. If the thorax be squeezed so as to compress the heart and prevent its filling, the arterial pressure will fall very greatly, while the venous pressure will rise slightly. The arterial manometer tube must be again filled with sodium citrate solution before the arterial pressure is again allowed to rise.

3. If the administration of chloroform be pushed the arterial pressure falls in a striking manner, while the venous pressure rises slightly. In all these observations notice that the variations in venous pressure are trifling compared with those of arterial pressure.

CHAPTER XXXV.

BLOOD PRESSURE—Continued.

Demonstration. Record of Arterial Pressure, Effect of Excitation

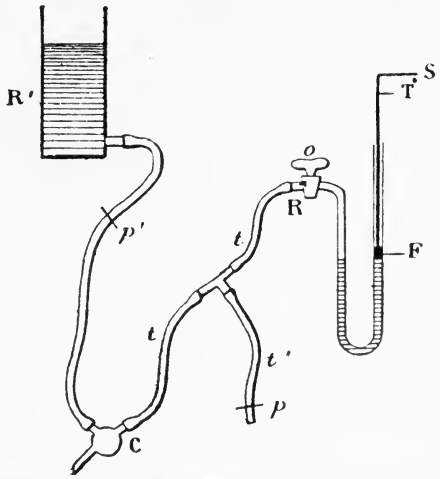


Fig. 104.—Arrangement of cannula, pressure bottle, and mercurial manometer for recording blood pressure. C, cannula; *p*, *p'*, clips; F, float; S, writing style.

of the Vagus and Depressor Nerves. Effect of Gravity. Effect of Asphyxia.—A cat is anaesthetised with ether and chloroform, or

a rabbit is anaesthetised with urethane. The carotid artery is exposed, and a cannula inserted. The cannula is connected to a mercurial manometer by a piece of pressure tubing, a ⊥ piece being interposed. The cannula and tube are filled by means of a pressure bottle or syringe with sodium citrate 1 per cent. solution, and the pressure in the manometer is raised to about the arterial pressure. The vagus nerve is exposed, ligatured in two places, and divided between the ligatures. The depressor nerve is exposed, ligatured, and divided below the ligature. The depressor in the cat runs separately from the vagus on the left side. On the right side it can generally be separated from the rest of the vagus without much difficulty. In the rabbit the depressor runs separately on both sides. In the dog it is bound up in the vago-sympathetic trunk.

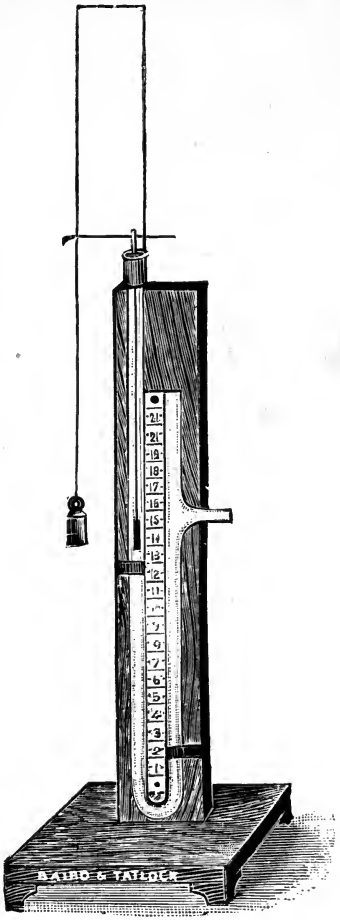


FIG. 105.—Mercurial manometer fitted with float and writing style.

The trachea is opened and a tracheal cannula inserted. This is connected with the anaesthetic bottle and by a side tube with a recording tambour. The writing styles of the manometer float and of the tambour are brought to write on the kymograph exactly beneath one another. A clock marking seconds and an electric signal placed in the primary circuit are also brought to write on the kymograph. The

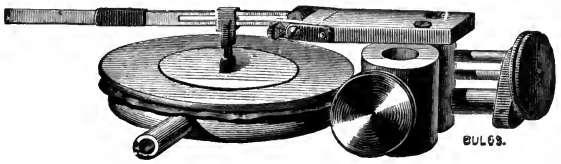


FIG. 106.—Recording tambour.

primary circuit is arranged to give tetanising shocks, and shielded electrodes are connected with the secondary coil by means of a Du Bois

key, and are placed in position under the peripheral end of the vagus nerve. The clip is then removed from the carotid artery and the kymograph started. Note the height of the arterial pressure. the cardiac pulsations, and the respiratory oscillations of arterial pressure. The pulsations are distorted by the momentum of the mercury.

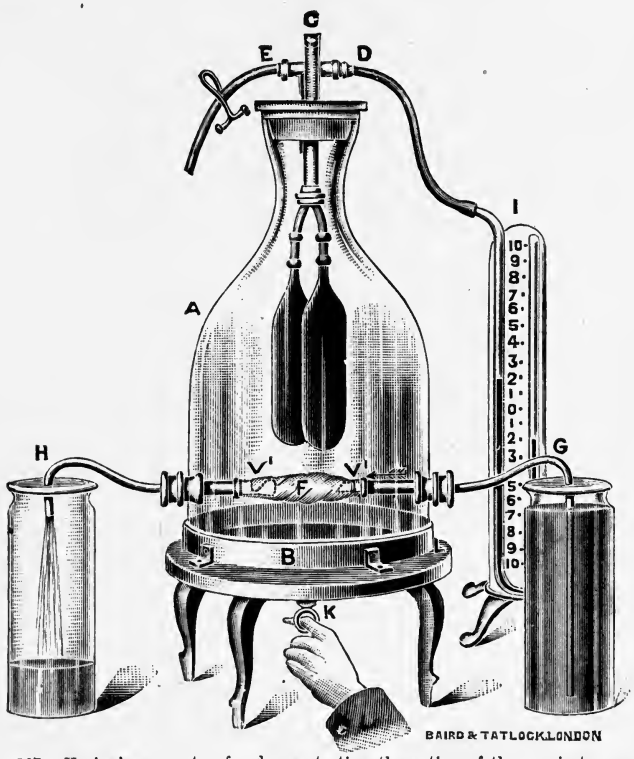


FIG. 107.—Hering's apparatus for demonstrating the action of the respiratory pump. A, Glass bell, thorax; B, air-tight base; K, diaphragm; C, trachea leading to lungs; I, manometer; E, tube opening into A; F, heart with valves V. The action of the diaphragm pumps air in and out of the lungs and water through the heart. The lungs and heart are thin rubber bags.

The arterial pressure, if the respiration is slow, begins to rise during the second third of inspiration, and begins to fall during the second third of expiration. If the respiration is rapid the pressure rises during expiration and falls during inspiration. The inspiratory fall of intra-thoracic pressure aspirates blood into the intra-thoracic veins and thin walled auricles, and dilates the pulmonary vessels. The descent of the diaphragm expresses blood from the liver and abdominal vessels into the right heart. Expiration has the opposite effect in each respect.

When the respiration is rapid the inspiratory dilatation of the

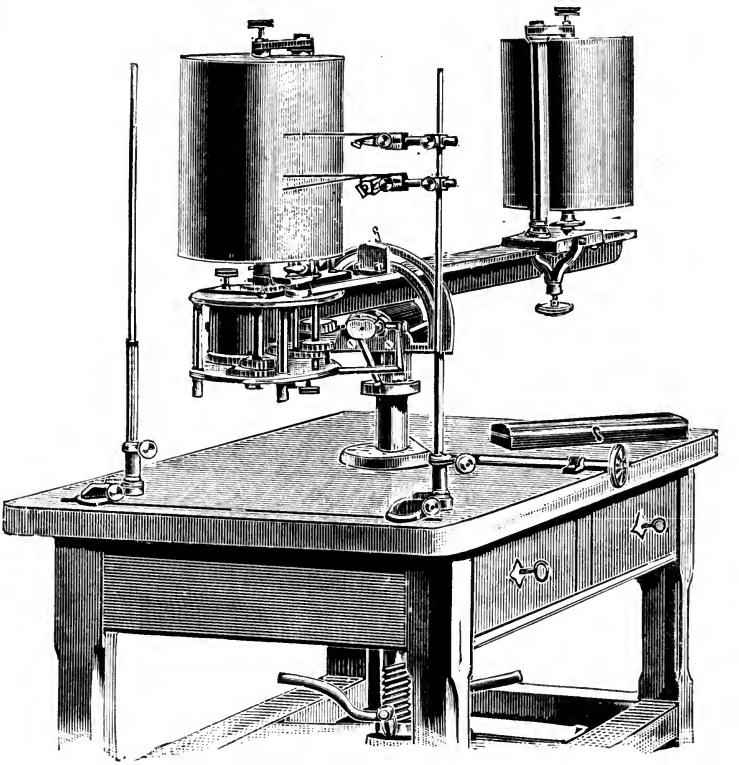
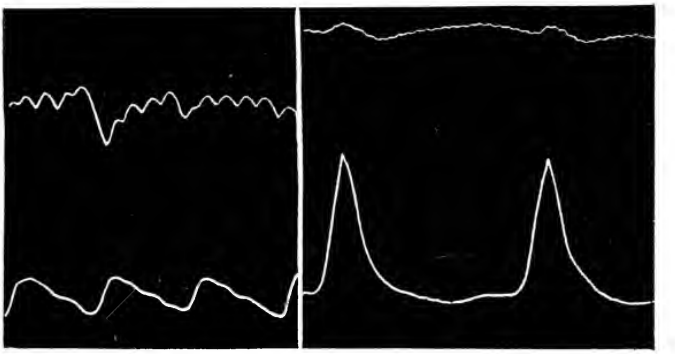


FIG. 108.—The Hürthle kymograph.



A B

FIG. 109.—Record of arterial pressure and respiration (A) before and (B) one minute after dividing the vagi. The upstroke marks inspiration. The arterial pressure rose from 150 to 180 mm., the pulse rate from 110 to 260. Respiration fell from 24 to 10. The expirations became strenuous. (Burdon Sanderson.)

pulmonic system lessens the flow of blood into the left heart, and

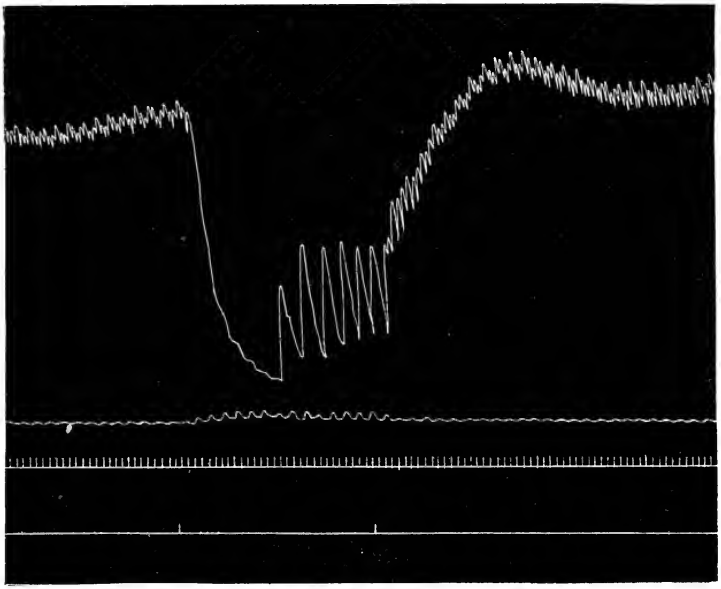
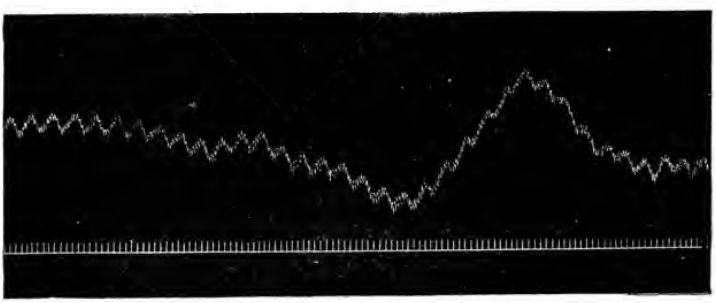


FIG. 110.—The effect of excitation of the peripheral end of the vagus nerve upon the blood pressure in the aorta (top curve) and the vena cava (second curve) of a curarised animal with artificial respiration. Note the inhibition of the heart; the great fall of aortic and the insignificant rise of vena cava pressure; the escape of the heart from the vagus action and the after effect on the aortic pressure. The time is marked in seconds, and the signal line shows the duration of vagus stimulation. (L.H.)

arterial pressure falls. Expiration expresses blood from the pulmonic system into the left heart, and arterial pressure rises. When the rate



A B

FIG. 111.—Aortic blood pressure. A, Effect of exciting the central end of vagus. The effect was depressor. B, On shifting up the electrodes to a fresh unexposed part of the nerve the effect changed to pressor. The time is marked in seconds. (L.H.)

of respiration is slow there is time for the increased or diminished entry of blood into the right heart to exert its effect in the latter stage of inspiration and expiration respectively.

Stimulate the peripheral end of the vagus nerve. The heart is inhibited, and the arterial pressure falls. Complete arrest cannot be

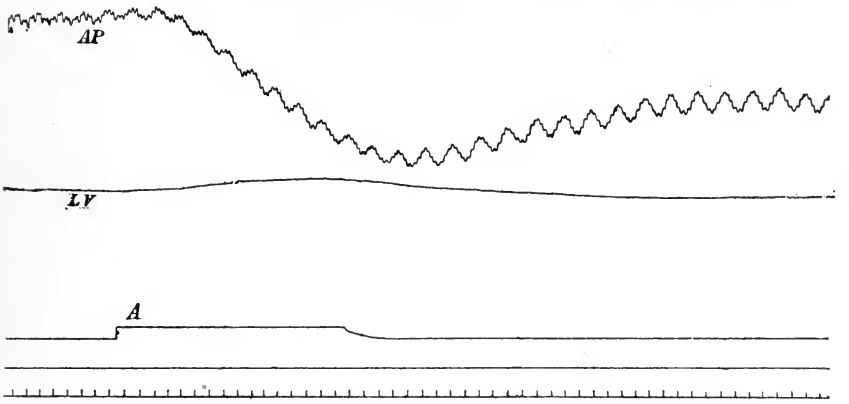


FIG. 112.—Record of arterial pressure (*AP*) and plethysmogram of limb (volume record *LV*). Excitation of the depressor nerve at signal *A*. The limb expanded in spite of the fall of arterial pressure. The time is marked in seconds. (Bayliss.)

obtained in the cat. It is easily obtained in the dog. In the chloroformed dog with *low* blood-pressure, vagus excitation, produced by

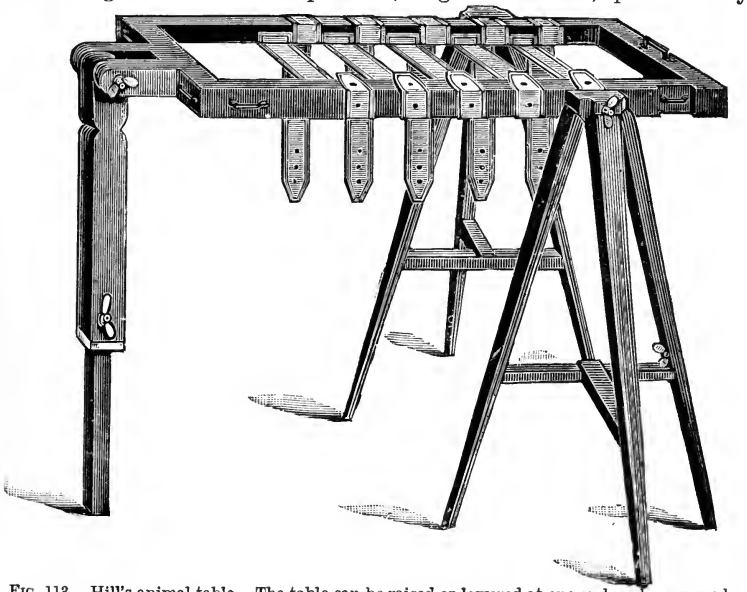


FIG. 113.—Hill's animal table. The table can be raised or lowered at one end, or be reversed.

inhalation of concentrated chloroform vapour, may arrest the heart for so long a period as to kill the animal. This is one cause of chloroform syncope in man. The heart soon escapes from vagus arrest if

the blood pressure is high. The pressure (after vagus inhibition) for a brief space of time rises to a higher level.

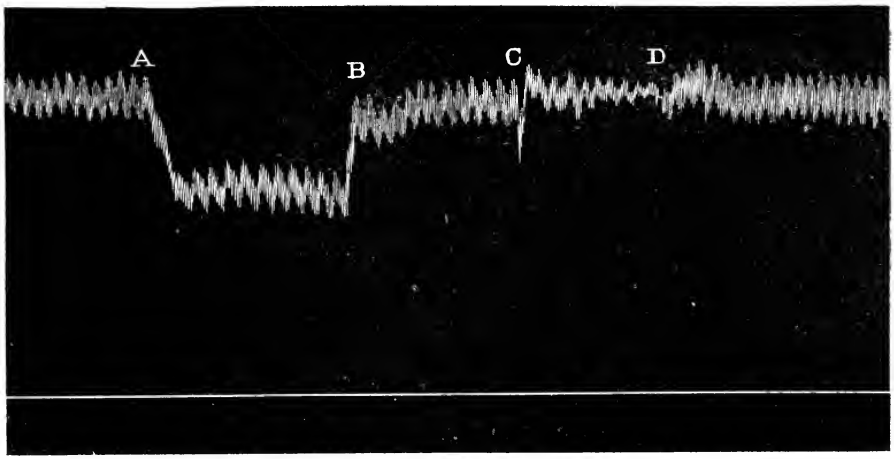


FIG. 114.—Aortic blood pressure. Effect of posture. A, Vertical head up; B, horizontal; C, vertical head down; D, horizontal. (L.H.)

The electrodes are now transferred to the central end of the vagus. Excitation produces either a slight rise (pressor effect) or a slight fall

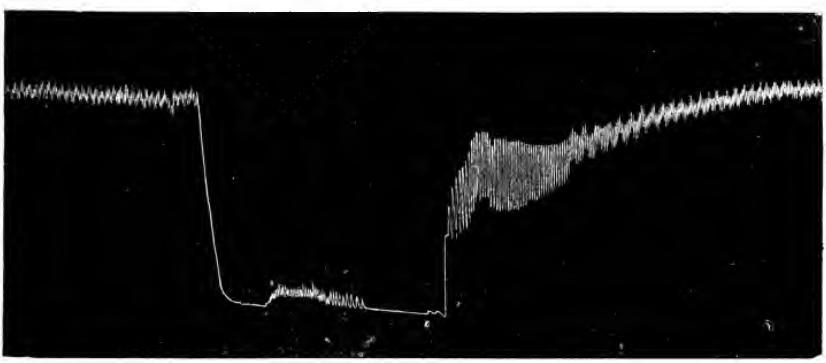


FIG. 115.—Aortic pressure. Spinal cord divided in upper dorsal region. Effect of placing animal in vertical head up posture. The heart emptied. On the return to the horizontal posture the circulation was restored. (L.H.)

(depressor effect) of pressure. The heart rate is reflexedly slowed, and the respiration is stopped with the diaphragm in inspiratory spasm.

The electrodes are next transferred to the central end of the depressor nerve. On excitation the blood-pressure slowly falls, and remains at a lower level so long as the excitation is maintained. The rhythm of the heart is as a rule unaffected. The second vagus nerve

is now exposed and divided. The heart accelerates, and the arterial pressure rises. This is very marked in the morphinised dog. The vagus centre tonically controls the rhythm of the heart.

The Effect of Posture.—The animal is placed on a swing board, with the arterial cannula in the axis of rotation. On dropping the animal into the vertical posture, with the head up, the arterial pressure falls. It may rise again to, or even beyond, the normal level in the cat. In the hutch rabbit the pressure falls, until the medullary centres become

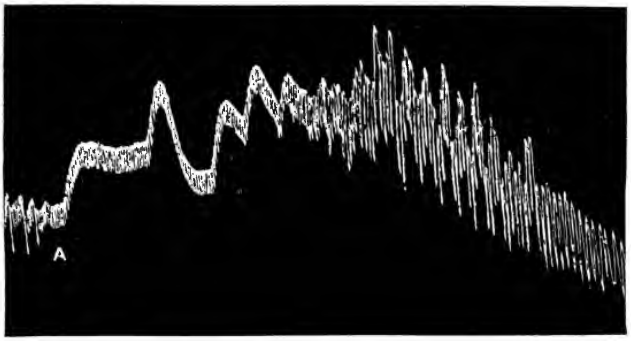


FIG. 116.—Arterial pressure; effect of asphyxia. Animal anaesthetised and curarised. At A the artificial respiration was stopped. The large oscillations are Traube-Hering curves. (L.H.)

paralysed from anaemia. The weight of the blood in the vertical posture is supported by the taut skin, the tone of the skeletal muscles, and the tone of the arterial system.

The blood is largely returned to the heart by the action of the skeletal muscles, aided by the valves in the veins, and the respiratory pump.

If the spinal cord be divided in the lower cervical region, or the administration of chloroform be pushed, these mechanisms are paralysed, and the blood congests in the lower parts, and the heart fails to fill. In such case the circulation is immediately restored by placing the animal in the horizontal posture.

Asphyxia.—The trachea is clamped. Note the sequence of events.

1st stage: Respirations deeper and more ample; heart accelerated and more forcible. In the normal animal loss of consciousness now occurs and convulsive movements.

2nd stage: Respiration convulsive, less frequent; blood presure rising; heart slow. At the end of second minute the pupils dilate and emission takes place of urine and faeces. The veins are congested with black blood.

3rd stage: The inspirations, which have occurred at longer and longer intervals, finally cease. The heart beats slowly and with great force. Finally the heart accelerates, and the blood pressure falls to zero.

CHAPTER XXXVI.

RESPIRATION.

Record of the Respiratory Movements in Man.—A receiving tambour —the stethograph—constructed like a drum (Fig. 117) is fastened by

Fig. 117—Stethograph. A, Metal drum; B, hooks for tapes which pass round neck; C, rubber discs; D, hooks for attaching tapes which are tied round thorax; E, tube leading to the recording tambour.

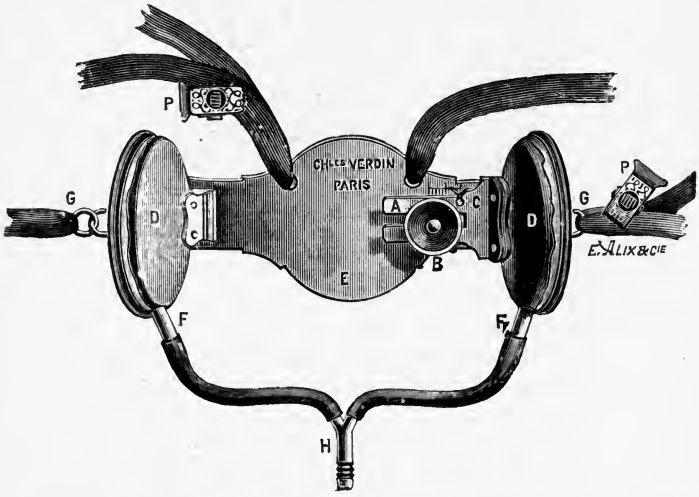


Fig. 118.—A stethograph employed to record the respiration and cardiac impulse of the rabbit or cat. The tambours press on either side of the thorax. The T tube leads to a recording tambour.

tapes to the thorax. One tape passes round the thorax and the other over the shoulder. The 'stethograph' is connected with a recording

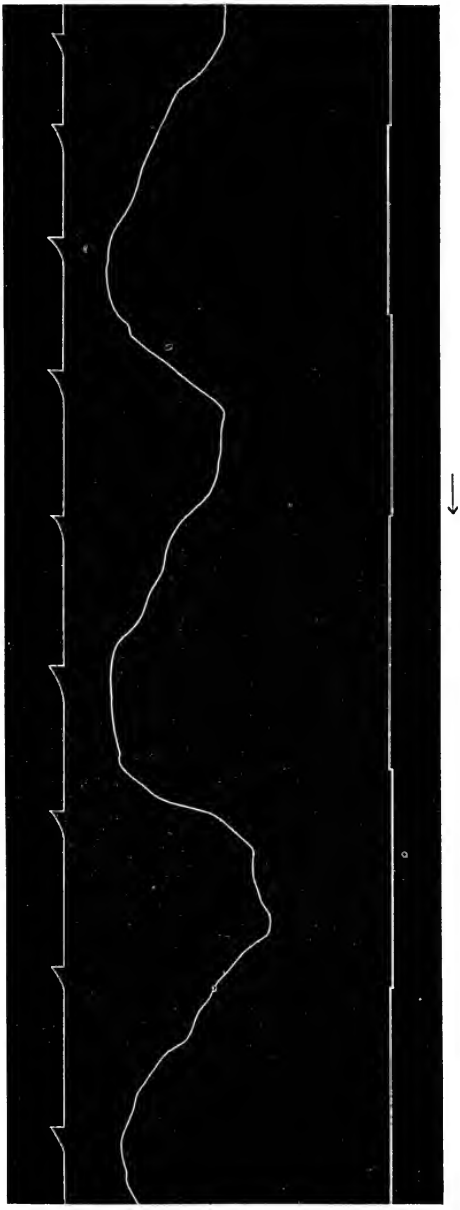


FIG. 119.—Record of respiration. The signal marks the duration of the vesicular sound. Inspiration is marked by the down-stroke. Time marked in seconds. (L.H.)

tambour, and the latter is brought to write on a moderately fast drum. A signal, in circuit with key and battery, is placed to write exactly

beneath the tambour. Listen to the vesicular sounds with the binaural stethoscope, and, by means of the key, signal the moment when the sound begins and ends. Repeat the experiment for bronchial breathing, placing the stethoscope over the 7th cervical spine. Take a time tracing with the seconds clock beneath the respiratory tracing. Count the pulse of the subject, and determine the ratio of the frequency of respiration to that of the pulse. It is about 1 : 4.

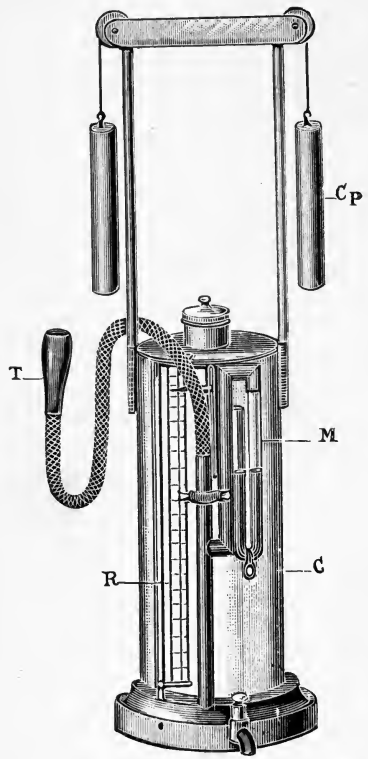


Fig. 120.—Spirometer. T, Mouthpiece; M, manometer; Cp, counterpoise; R, scale.

The Spirometer.—The **vital capacity** is the greatest volume of air that can be expired after the deepest possible inspiration. The **tidal air** is the volume of air breathed at each respiration. These measurements are made by means of a spirometer.

The spirometer is constructed as in Fig. 120. Open the tap, place the spirometer at the mark 1000. Then close the tap. Breathe in and out through the mouthpiece, holding the nose meanwhile. Divert your thoughts, and let another observe the volume of the 'tidal air.' It will be about 200-250 c.c. After breathing out the tidal air, expire as deeply as possible. An additional 1500 to 2000 c.c. will be expired. This is called the **supplemental** air. Breathe in as deeply as possible. About 1500 to 2000 c.c. **complemental** air will be inspired in addition to the tidal air. Place the spirometer at zero and take the deepest possible inspiration. Then make the deepest possible expiration into the spirometer and thus measure the vital capacity, *i.e.* tidal 250 c.c. + complemental 2000 c.c. + supplemental 2000 c.c.

CHAPTER XXXVII.

RESPIRATION—CONTINUED. NERVOUS CONTROL OF RESPIRATION.

Record of the Respiratory Movements of the Diaphragm. Head's Method.—DEMONSTRATION. A rabbit is anaesthetised with urethane or chloral. The skin is divided over the ensiform cartilage. One blade of a pair of scissors is passed carefully under the cartilage, and the latter is severed from the sternum. Two slips of muscle pass from the central

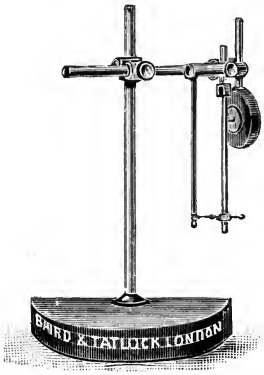


FIG. 121.—Tambour recorder. The muscle is attached to the hook and pulls against a spring. The lever compresses the tambour. This tambour is connected with a recording tambour.

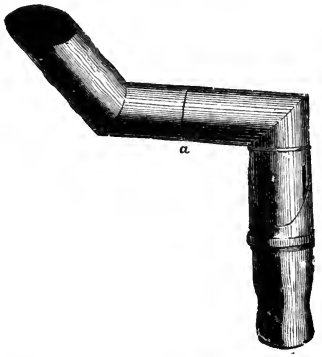


FIG. 122.—Tracheal cannula for artificial respiration. The slit at the side, the size of which can be controlled, allows the air to escape in expiration.

tendon of the diaphragm to the ensiform cartilage. One end of a ligature is tied on to the cartilage and the other end to a lever. The lever pulls against a spring and presses against a receiving tambour. The contraction of the muscle slips can be recorded by connecting the receiving with a recording tambour. A thread, carrying a small flat button, is passed on either side through the diaphragm and the walls of the thorax, between the fifth and sixth ribs. As soon as the two threads are tightened the buttons become pressed against the tendonous ends of the muscle slips and fix them to the anterior wall of the thorax. The threads are knotted together over the front of the sternum.

A tracheal cannula is inserted. Note the effect of blowing air several times in succession into the lung and of sucking air out of the lung. Positive ventilation provokes expiratory spasm, negative ventilation expiratory relaxation of the diaphragm. Next note the effect when the vagi are divided. The division of one vagus has but little effect on the respiration. If the divided central end of the nerve be allowed to fall back into the wound it may be stimulated by its own action-current,

and provoke increased inspiratory movements. To prevent this the vagi may be frozen instead of being cut. When both vagi are divided note that the respiration is both slowed and deepened. The respiratory exchange of gases, however, is not altered. Positive and negative ventilation no longer produce an effect. When the central end of one vagus is tetanised there results inspiratory spasm; with a very weak current expiratory spasm may result. Excitation of the central end of

FIG. 123.—Expiration spasm of the diaphragm produced by weak stimulation of the vagus. The down stroke represents inspiration, the up stroke expiration. The signal line shows the duration of stimulation. (Fredericq and Nuel.)

the superior laryngeal nerve provokes expiratory spasm. Division of the phrenic nerves paralyses the diaphragm. If the tracheal tube be connected with a water manometer or tambour immediately after the death of an animal, and the peripheral end of the vagus be excited, a rise of pressure will be observed, due to the constriction of the bronchial

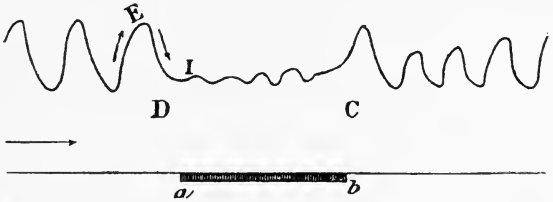


FIG. 124.—Inspiratory spasm of the diaphragm produced by excitation of the vagus during the period shown by the signal *a*, *b*. The down stroke represents inspiration, the up stroke expiration. (Fredericq and Nuel.)

muscles. The effects of positive and negative ventilation, and of vagus excitation and section, may be observed by merely inspecting the respiration of the animal.

The Respiratory and Vasomotor Centres.—The arterial pressure is recorded, and the occipito-allantal membrane is exposed and opened. The upper end of the cervical cord is then divided. After the initial convulsion due to the lesion the respiratory movements of the thorax cease, but those of the alae nasi and mouth continue for a minute or two. The arterial pressure falls. The animal dies of respiratory paralysis. If artificial respiration be established the circulation will continue, but the arterial pressure will be low. The pressure will be raised very greatly by tetanisation of the lower end of the divided cord.

By observing the effect of division of the mid-brain in another animal the respiratory centre and vasomotor centres can be localised to the spinal bulb.

CHAPTER XXXVIII.

INTRA-THORACIC PRESSURE.

Intra-thoracic Pressure.—The thoracic cavity, when opened, is far larger than its contents, for the lungs, owing to their elasticity, collapse so soon as the intra-pulmonary and pleural pressures became equal. The intra-pleural pressure is less than the atmospheric pressure by that amount of the atmospheric pressure which is required to overcome the elasticity of the lungs and distend these organs to the size of the thoracic cavity. The intra-thoracic pressure or elastic traction exerted by the lungs on the thoracic wall varies as follows:

Normal inspiration - - -	about	– 10 mm. Hg.
,, expiration - - -	,,	– 7 ,,
Deep inspiration - - - -	,,	– 40 ,,
,, expiration - - - -	,,	0 ,,
,, inspiration with air-way closed	,,	– 100 ,,
,, expiration ,, ,, ,,	,,	+ 100 ,,

The intra-tracheal pressure varies from – 1 mm. Hg. in quiet inspiration to + 1 mm. Hg. in expiration. During forced breathing with the air-way closed the intra-tracheal pressure is greater than the intra-thoracic pressure by the amount of the elastic traction exerted by the lungs. All the structures, *e.g.* heart and blood-vessels, are affected by the respiratory variations of pressure.

Demonstration. The trachea of a dead rabbit is exposed, and a ligature tied round it. The skin is divided over the thorax on one side, and the ribs exposed. One inch of two adjoining ribs is removed. Note that the lung is in contact with the thoracic wall. The ligature round the trachea is now divided; the air escapes, and the lung, owing to its elasticity, will collapse. On opening the pleural cavity the pressure within and without the lung becomes atmospheric. The elasticity of the distended lung then comes into play and causes its collapse.

Demonstration. In the rabbit anaesthetised with urethane or chloral the skin is divided over an intercostal space. The intercostal muscles are then separated with care, and a piece of rib removed, while the parietal pleura is left quite uninjured. The lung will not collapse so long as the pleural cavity is not opened. On the contrary it will be seen gliding to and fro with each movement of respiration. Note how easily the pleural surface of the lung glides over the parietal pleura. A glass cannula attached to a water manometer is

pushed throughout the intercostal muscles until the end comes to lie in the thoracic cavity. Notice the negative pressure indicated in the manometer, which becomes greater in inspiration and less in expiration. Note the immediate collapse of the lung on opening the pleural cavity.

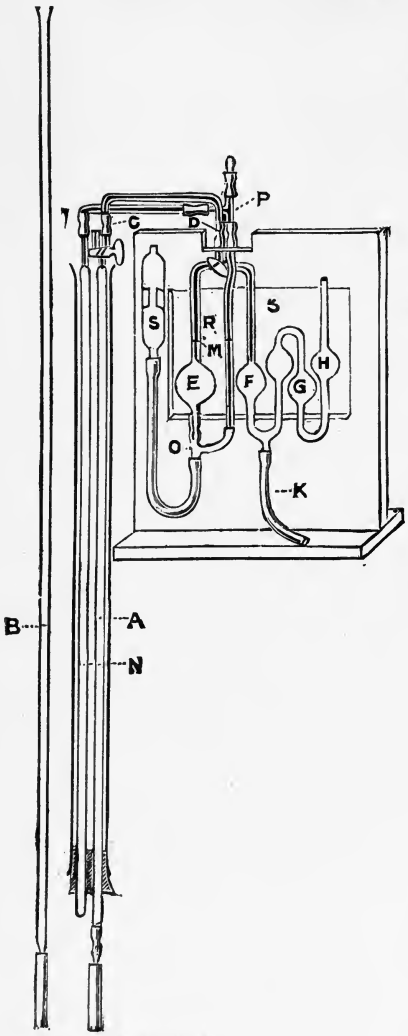


FIG. 125.—Haldane's gas analysis apparatus.

CHAPTER XXXIX.

THE CHEMISTRY OF RESPIRATION.

The Estimation of the Gases in Inspired and Expired Air by Haldane's Apparatus.—The gas is measured in the graduated gas-burette A, provided with a three-way tap. Surrounding the gas-burette is a water-jacket. The whole is supported by a clamp and retort stand. The gas-burette is connected by pressure tubing to the levelling tube B, which is held by a spring clamp attached to the retort stand. A and B contain mercury, and by raising or lowering B gas can be expelled from or drawn into A. One of the connections of the three-way tap is used for taking in the sample, the other connects the burette with an absorption apparatus arranged as in the figure.

The bulb E, filled with 20 per cent. caustic potash, absorbs CO_2. The bulb F, filled with alkaline pyrogallic acid solution,[1] absorbs O_2. The

[1] Dissolve 100 grms. of stick caustic potash in 50 c.c. of water. Add 10 grms. of pyrogallic acid to this solution. The pressure in the burette is adjusted by using the potash pipette as a pressure gauge and bringing the potash before every reading of the burette to the mark M. In order to make the reading of the burette

water in G and H protects the pyro solution from the air. F is emptied and refilled through K. The tap on the absorption pipette places either E or F in connection with the gas-burette.

A sample of expired air is obtained by breathing through the tube into the burette B (Fig. 126). A and B are filled with acidulated water, and B is controlled by a clip.

The portion of B which lies beyond the clip is squeezed empty of air before it is inserted over the entrance tube of the Haldane gas-burette. The sample is then taken over by lowering the levelling tube and opening the clip.

The Respiratory Exchange of Gases.—Atmospheric air measured dry at 0° C. and at 760 mm. pressure has the following composition:

	O_2.	N_2.	CO_2.
Atmospheric air,	20·95	79·02	0·03
Average expired air,	16·03	79·59	4·38

Expired air is warmed nearly to body temperature, and from 50 to 100 kilo-calories of heat are lost per diem in this way. It is saturated with water vapour. One cubic metre of air takes up 42·2 grms. of water at 37° C. The total loss per diem varies from 250 to 500 grms. The heat lost by evaporation of this water equals 145 to 290 kilo-calories.

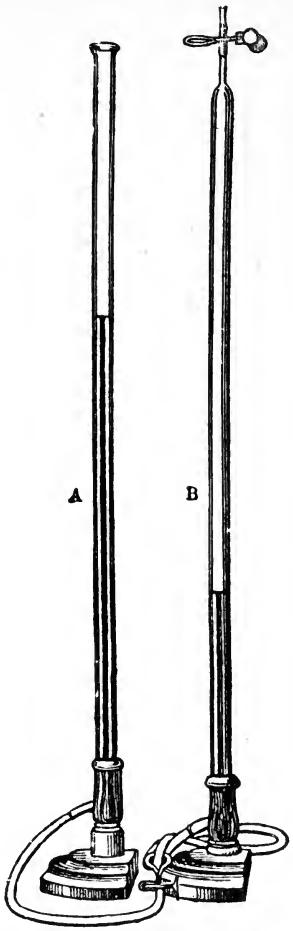


FIG. 126.—Hempel's burette for collecting a sample of expired air.

One third of the 250 c.c. tidal air is breathed out from the large air tubes at the following expiration, the rest (170 c.c.) mixes by diffusion with

independent of changes in temperature and barometric pressure during analysis a control tube N is employed. N is connected with the potash solution by means of a T-tube O. The tap at P makes it possible to render the pressure in N equal to that of the atmosphere. At the beginning of the experiment the potash is adjusted to the mark R by altering S, P being open. P is then closed, and not opened again till the analyses are complete. The barometer and temperature of the water-jacket are read. Each time a reading of the burette is made the potash is brought to the mark R by altering S, and to the mark M by means of the levelling tube B. As the control tube and the gas-burette are kept moist, variations in the tension of aqueous vapour in the burette are also corrected by the control tube

the residual and supplemental air (3500 c.c.). The composition of the alveolar air is thus altered by only about one-twentieth at each breath. These facts are determined by taking an inspiration from a gasometer containing pure hydrogen, and analysing the amount of hydrogen in the air expired.

The Tension of Carbon Dioxide and of Oxygen in the Alveolar Air of Man.—Haldane determines the tension of the gases in the alveolar air by an analysis of the last portion of the air expired in an ordinary expiration. The experiment may be performed in the following way. An anaesthetic mask is connected by a T piece to a piece of tubing 80 cm. long and 1·8 cm. internal diameter; to the free end of the T

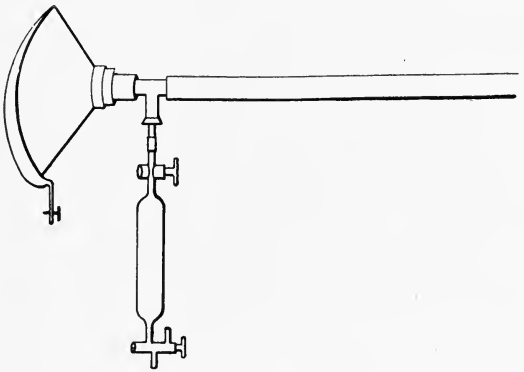


Fig. 127.—Apparatus for collection of a sample of Alveolar Air.

piece is connected (Fig. 127) a gas-sampler with a capacity of 50 cubic centimetres. The subject of the experiment fits the mask to his face and makes an ordinary expiration; as soon as the expiration ceases, the tap of the gas-sampler, the air of which has previously been removed by a vacuum-pump or gas-pump, is opened and a sample of the last portion of the expired air is collected before the mask is removed from the face. The analysis of the air is performed in the manner already described. The percentage composition is about 5·5 carbon dioxide, 14·5 oxygen and 80 nitrogen.

It is an advantage to determine the volume of each expiration by a spirometer attached to the end of the tubing, and it is important that the subject of the experiment should by a little practice with the apparatus learn to breathe naturally, otherwise a fair sample will not be obtained.

The respiratory exchange of gases can be determined in man by the Haldane-Pembrey or Zuntz apparatus. In an average man weighing

70 kilos, the mean daily output of CO_2 equals 800 grms., and the mean intake of O_2 equals 700 grms. A half-metre cube roughly represents the volume of the intake of O_2 or output of CO_2. Note the size of this cube. In rest, walking three miles an hour, and on the tread-mill the production of CO_2 is roughly in the proportion 2, 3, and 6.

The warm-blooded animal responds to a rise or fall in external temperature with a decrease and increase respectively of CO_2 output. The normal response occurs only so long as the body temperature is normal. A man deeply anaesthetised, or one in whom the spinal cord in the upper dorsal region has been divided, or a new born babe, responds (like a cold-blooded animal) to a fall of external temperature by diminished metabolism and fall of body temperature. The surface area of the body, in proportion to the mass, is much greater in the child than in the adult, and in the tall, thin man, than in short, fat man. The respiratory exchange is therefore greater in the child and the tall thin man.

The respiratory quotient $\frac{CO_2}{O_2}$ indicates the amount of oxygen used in the oxidation of carbon. On a carbohydrate diet the R.Q. is 1 for $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$, and by Avogadro's principle a molecule of O_2 occupies the same volume as a molecule of CO_2. The R.Q. is less than 1 on a fat or proteid diet, for a certain amount of O_2 is used up in the oxidation of H_2 and of the nitrogenous elements of the food.

In the case of a fat diet (olein) the final stage of the metabolic process has been represented thus:

$C_3H_5(C_{18}H_{33}O_2)_3 + 80O_2 = 57CO_2 + 52H_2O$, and the R.Q. is $\frac{57}{80} = 0{\cdot}71$. On an ordinary mixed diet R.Q. $= 0{\cdot}8$, while on a strict diabetic diet it may fall to 0·6. On the latter diet the O_2 intake may be almost doubled. When an animal is rapidly putting on fat the R.Q. may become greater than 1, for the CO_2 output is increased owing to the conversion of carbohydrate into fat; it appears that, in addition to the ordinary combustion which results in a quotient approaching unity, there is a discharge of a further quantity of carbon dioxide, the oxygen of which is derived from the intramolecular oxygen of the food.

CHAPTER XL.

RESPIRATION APPARATUS.

The Haldane-Pembrey Respiration Apparatus for the Mouse.—The apparatus is constructed as in Fig. 128. The corks are soaked in melted paraffin before insertion. Each double absorption tube is fitted

with a wire loop, so that the glass need not be touched with the hand. The animal chamber—a light beaker—is provided with a thermometer and is also fitted with a wire loop. The moisture given off by the animal is absorbed by pumice saturated with sulphuric acid in the tubes AB. The pumice is heated red-hot before it is thrown into the acid. The carbon dioxide is removed by soda lime C, the water given off by the soda lime is caught by the sulphuric acid tube D.

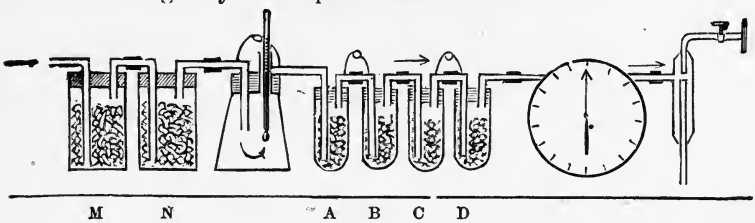


FIG. 128.—The Haldane-Pembrey respiration apparatus.

The animal is weighed in the beaker (with the tubes closed) before and after the experiment. A dummy beaker is placed in the opposite scale pan. The tubes AB and CD are also weighed against a dummy pair of tubes. During the weighings the exit and entrance tubes are left unstoppered. By these means errors due to condensation of water and changes of barometric pressure or temperature are avoided, and the weighings can be carried out to less than a milligramme.

The air entering the chamber is freed from CO_2 and H_2O by soda-lime in M and sulphuric acid pumice in N. The amount of H_2O and CO_2 given off in 15 minutes is determined by the increase in weight of AB and CD respectively. The amount of oxygen absorbed is found by subtracting the loss in weight of the animal weighed in the chamber from the total loss of CO_2 and H_2O.

$$\text{The ratio } \frac{CO_2 \text{ grms.}}{O_2 \text{ grms.}} \times \frac{32}{44} = \frac{CO_2 \text{ by volume}}{O_2 \text{ by volume}} = \text{respiratory quotient.}$$

The effect of external temperature upon the respiratory exchange may be studied with this apparatus.

EXAMPLE. The beaker containing a full-grown mouse was placed in water bath at 9·5° C., and then in water bath at 30° C. At 9·5° C. the mouse gave off from 250-315 decimgrms. of CO_2 per 10 minutes and was *active*.

At 30° C. it gave off 103-116 decimgrms. CO_2 per 10 minutes and was *quiet*. The rectal temperature of the animal scarcely varied during the experiment. New-born mammals in an immature condition behave like cold-blooded animals, and are unable to compensate for low external temperature by increased metabolism. The CO_2 output in them sinks with the body temperature.

A mouse, owing to the greatness of its surface exposure in relation to its mass, gives off 20 times as much CO_2 per kilo of body weight as a man. The mass of a man is about 3000 times the mass of a mouse, but

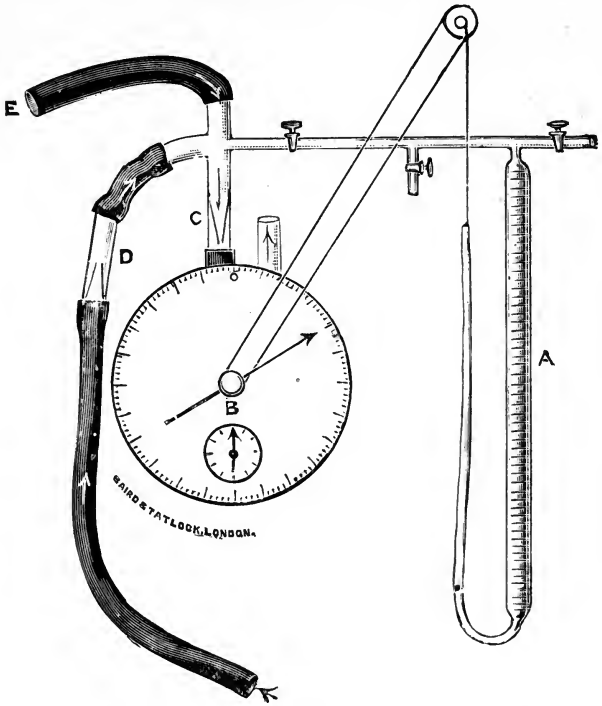


FIG. 129.—Zuntz respiration apparatus. The subject expires through the meter. The inlet and outlet tubes are controlled by valves D and C, made of pieces of intestine which have been soaked in glycerine. A small sample of the expired air is steadily drawn off into the burette A by the escape of mercury from the tube which is lowered by the revolution of the meter B. The meter gives the total volume of air breathed. The measured sample in the burette is analysed by Haldane's gas apparatus.

the external surface of the man is probably only about 150 times that of the mouse. A mouse weighing 25 grms. produces in one hour at ordinary temperatures 0·25 grms. of CO_2. A man weighing 70 kilos produces 40 grms. in the same time. A mouse thus produces about 10 grms., and a man only $\frac{1}{2}$ grm. per kilo of body weight. A mouse is poisoned by CO in $\frac{1}{20}$ the time of a man.

When the Haldane-Pembrey apparatus is applied to a man, air is drawn through the respiration chamber and the total volume measured by a large meter. A small sample of the air entering and leaving the chamber is drawn through two sets of absorption tubes for the determination of the water and carbon dioxide and is then measured

by small meters. The following figures show the effect of diet, rest, and work on the respiratory exchange of man.

	CO_2.		O_2.	
	Day.	Night.	Day.	Night.
Fasting: Rest, - - - -	403 grms.	314 grms.	435 grms.	326 grms.
,, Work for nine hours,	930 ,,	257 ,,	922 ,,	150 ,,
Moderate diet: Rest, - -	533 ,,	395 ,,	443 ,,	449 ,,
,, Work for nine hours,	856 ,,	353 ,,	795 ,,	211 ,,

CHAPTER XLI.

GASES OF THE BLOOD.

Analysis of the Gases of the Blood by Hill's Pump.[1]—The pump consists of a mercury reservoir A, which is connected with a second reservoir B by means of pressure tubing. The upper end of B is closed by a three-way tap. By means of this tap B can be put in connection with either the tube E leading to the blood-receiver F, or with the tube C leading to the eudiometer H. The blood-receiver F is constructed of three bulbs, so as to prevent the blood frothing over into B during the extraction of the gases. To either end of F is fixed a piece of thick small-bored pressure tubing provided with a clip.

In using the pump the manipulations are as follows: F is placed in the position indicated by the dotted line. A is raised and B is put in connection with F, and F is filled with mercury. The screw clip on the rubber tube at the upper end of F is then closed, and A lowered until F is exhausted, except for 2 or 3 c.c. of mercury which are purposely left within.

The screw-clip on the lower end of F is next closed, and F is then detached from the pump and weighed. A sample of blood is collected in the following way: The arterial or venous cannula is filled with blood, and immediately pushed into the rubber tube at one end of F. Before the insertion of the cannula the end of the rubber tube is compressed with the fingers to exclude the air within it. A sufficient quantity of blood is now withdrawn by opening the screw-clip, and the clip placed on the vessel of the animal. The blood is defibrinated by shaking it with the mercury left within F for the purpose. F is then again weighed, and the weight of the sample obtained. F is next affixed to the tube E, and E is exhausted. Finally the screw-clip between E and F is opened, and the gases are withdrawn and collected in the

[1] Many blood-gas pumps have been contrived. Pflüger's is one of the best. A very accurate pump is that of Barcroft (cp. *Journ. of Physiol.* xxv. 265).

eudiometer. To facilitate the escape of the gases F is placed in warm water and shaken. If the blood froths too violently the frothing can be allayed by pouring some warm water on the tube E. The tap is so manipulated that the gases only, and not the water which condenses in B, are driven over into the eudiometer. The water is returned back into F. Three or four exhaustions are sufficient to extract the gases. The eudiometer tube is filled with mercury and surrounded with a water jacket to keep the temperature constant. The eudiometer is transferred to a vessel of mercury and the volume of gas read, the level of mercury inside and outside the eudiometer being the same. The temperature of the water in the jacket of the eudiometer is also read and the barometric pressure. Potash solution 20 per cent. is then introduced into the eudiometer by means of a pipette provided with a bent end. The CO_2 is thus absorbed and

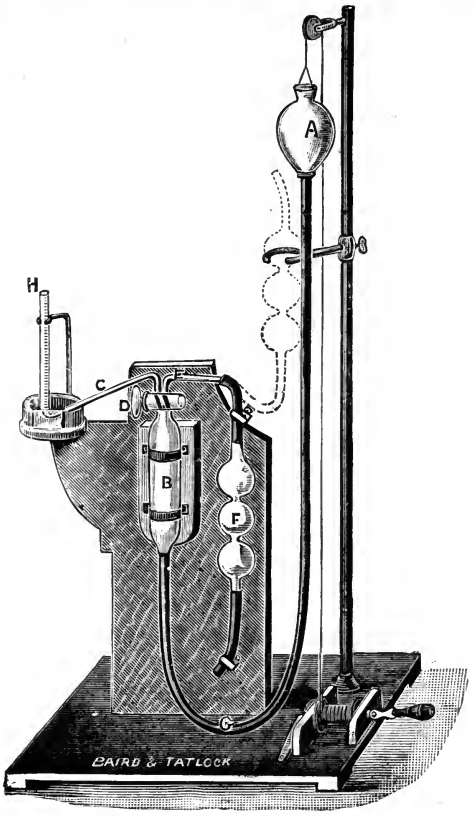


FIG. 130.—Hill's blood-gas pump.

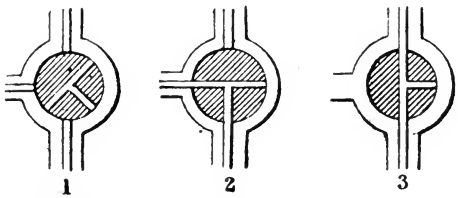


FIG. 131.—The three-way tap of the mercury pump.

the difference in volume read. Pyrogallic acid is then introduced and the O_2 absorbed. The remainder is N_2. The temperature of the water jacket is kept constant by adding cold water during the estimation.

To correct the volume of gas to 0° and 760 mm. the following formula is employed:

$$V = \frac{V'}{1 + t.\,0{\cdot}00367} \cdot \frac{H - f}{760}$$

where H = the observed pressure, f the tension of aqueous vapour at the observed temperature t. The value of $1 + t.\,0{\cdot}00367$ and of f are obtained from tables (cf. Sutton's *Volumetric Analysis*).

In determining the metabolism of an organ simultaneous samples are taken from an artery and from the vein leaving the organ. The samples can conveniently be collected in glass bulbs fitted with short lengths of pressure tubing and clips; the bulbs hold about 10 c.c. and are rinsed out with oil. From these the blood is rapidly transferred to the blood-receiver, half-a-dozen of which are exhausted and weighed ready for an experiment.

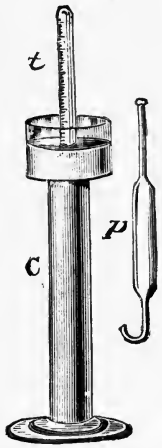


FIG. 132.—*c*, Mercury vessel; *t*, eudiometer; *p*, potash pipette.

The average amount of blood gases obtained is about 60 vols. per cent. Arterial blood contains approximately:

N_2, 2 vols. O_2, 20 vols. CO_2, 40 vols.

Venous blood contains approximately:

N_2, 2 vols. O_2, 12 vols. CO_2, 48 vols.

The following is an example of the differences found between the arterial going to, and the venous blood coming from, the thigh muscles of the dog:

	Rest.	Tetanus.
CO_2	+ 8·76	+ 13·90
O_2	− 12·92	− 13·75

As the flow of blood through the muscles in activity is 3-5 times greater, the results obtained during activity must be multiplied by 3 to 5. The exact increase in rate of flow can be found by noting the time in which the blood-collecting bulbs fill. The differences in the blood gases in the arterial blood going to, and in the venous blood coming from the brain may be contrasted with the above. They were as follows. An epileptic fit was excited by injection of oil of absinthe.

	Rest.	Activity.
CO_2	+ 3·87	+ 4·06
O_2	− 3·42	− 4·95

Here again the rate of blood flow was increased 3-5 times during the fit.

CHAPTER XLII.

THE MOVEMENTS OF THE ALIMENTARY CANAL.

The Movements and Innervation of the Frog's Stomach.—In a freshly caught frog pith the brain and cord, open the abdomen and remove the sternum. Tie the pylorus and open the oesophagus, and by gently compressing the stomach empty it of its contents. Then tie in a tube connected by a ⊥-piece with (1) a recording tambour, and (2) a tube containing salt solution at a pressure of 10-20 cms.

The spontaneous movements of the stomach are localised rings of contraction. These movements are not paralysed by painting the outside of the stomach with ·3 per cent. nicotine or with cocaine. They are therefore myogenic contractions.

Pull the viscera over to the left side, and remove the liver, ovary, and oviducts. Incise the peritoneum and expose the rami communicantes of the spinal nerves. Place the electrodes under the 4th ramus and tetanise it. After a latent period of some seconds the tonus of the stomach is increased. The increase lasts 5 or 6 minutes. The vagus on excitation inhibits the tonus and augments the spontaneous movements. (Dixon.)

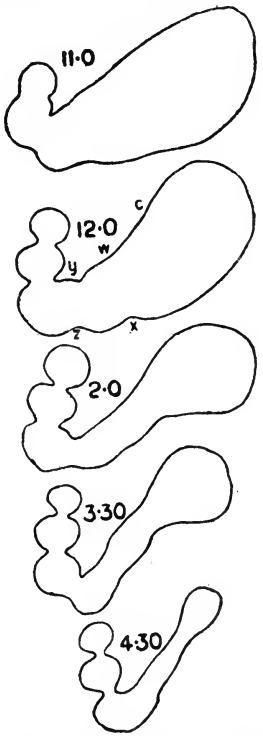


Fig. 133.—Contractions of the cat's stomach, as seen by the Röntgen ray method, after a meal containing bismuth subnitrate. Similar bead-like contractions are seen in the intestine. (Canon.)

Demonstration. **The Movements of the Cat's Stomach and Intestines.**—A tame cat is given a meal of tinned salmon to which 25 per cent. of bismuth subnitrate has been added. The bowels of the animal should previously have been emptied by the administration of three teaspoonfuls of castor oil. The cat is gently placed on its back above the Röntgen light. The animal must not be frightened. The movements of the stomach and intestines during the digestion of the meal can now be observed on a fluorescent screen (Canon). The movements of the stomach begin a few minutes after the meal. They consist of constrictions which appear in the middle of the stomach and run towards the pylorus. Each wave takes about 30 sec. to

reach the pylorus, and the waves occur about every 10 sec. The fundus acts as a reservoir for the food, while the pylorus mixes, triturates and expels the food into the duodenum. In the intestine rhythmic segmentation of the food is brought about by constrictions of the gut which continually occur at ever-varying points. There may be 30 such segmentations per minute. Such movements mix the food and juices, and express the contents of the venous and lymphatic radicles. Peristaltic movements occur in addition to the segmentation movements, and drive the contents onwards. Anti-peristaltic movements are frequent in the large intestine. This increases absorption. The ileo-caecal valve is normally competent, and prevents the antiperistalsis returning any of the contents of the large

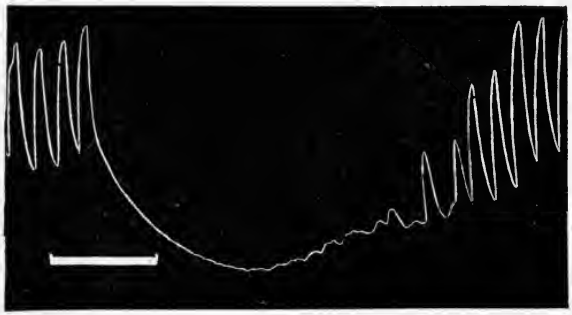


Fig. 134.—Pendulum movements of the intestine inhibited by excitation of the splanchnic nerve during the period marked by the white line. (Starling.)

into the small intestine. With the accumulation of material in the colon deep tonic constrictions appear one after another and carry the food into the descending colon. Fear or rage entirely inhibits the movements of the intestines.

Nutrient enemata (mixed with bismuth), when injected into the large intestine under pressure, may be carried by the antiperistalsis of the colon into the small intestine.

In the anaesthetised animal placed in a bath of warm saline, the bowels may be exposed, and a small indiarubber bag inserted in the small intestine. The bag is connected with a recording tambour. The splanchnic nerves are divided to prevent the reflex inhibition of the movements which otherwise would result. The gut exhibits rhythmic swaying movements caused by waves of constriction which occur every 5-6 secs., and travel 2-5 cm. per sec. These movements are myogenic, for they occur in the enervated intestine. If a bolus of cotton wad and vaseline be introduced, or the gut be pinched below the tambour it excites contraction above and dilatation below the

stimulated point. The contraction preceded by the dilatation slowly passes as a peristaltic wave down the intestine and pushes the bolus onwards. This peristalsis can be excited after section of both splanchnic and vagus nerves, but not after injecting nicotine or painting the intestine with cocaine. It is a co-ordinated reflex carried out by the peripheral nervous mechanism—Auerbach's plexus (Bayliss and Starling).

The small intestine receives nerve-fibres from the splanchnics which pass through the semilunar and superior mesenteric ganglia, and along the mesenteric arteries. It also receives fibres from the vagi through the continuation of the right vagus. The splanchnic nerve inhibits the intestine, while excitation of the vagus after injection of atropin (to paralyse the cardiac inhibiting fibres) augments the tone and swaying movements.

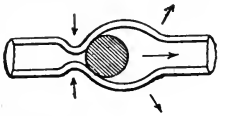


FIG. 135.—Diagram showing peristaltic contraction of intestine.

The colon receives fibres from inferior mesenteric ganglia via the lower dorsal and upper lumbar nerves, and from the second and third sacral nerves (pelvic nerves). The former inhibit and the latter augment the movements.

CHAPTER XLIII.

SALIVARY SECRETION.

Salivary Secretion.—The submaxillary gland is situated within and a little behind the posterior angle of the lower jaw bone.

The animal anaesthetised with ether and chloroform is placed on its back, and its head extended. An incision is then made along the internal border of the jaw bone. The internal border of the digastric muscle is thus exposed. This is pulled aside by a hook so as to expose the transverse fibres of the mylohyoid muscles.

The mylohyoid is carefully severed following the line of the digastric muscle. The submaxillary and sublingual ducts crossed by the lingual nerve are now exposed in the depth of the wound. Wharton's duct is the larger and external to the sublingual duct. Just where the lingual nerve crosses the ducts it gives off a small branch—the chorda tympani. In the angle formed by the origin of the chorda tympani from the lingual nerve there lies the sublingual ganglion (it is erroneous to term this ganglion "submaxillary"). A ligature is placed beneath the lingual nerve central to the origin of the chorda tympani, and the lingual nerve is divided central to the ligature. Two ligatures are

passed under Wharton's duct, and one is tied. The chorda tympani is then tetanised and the duct filled with saliva. A V-shaped slit is

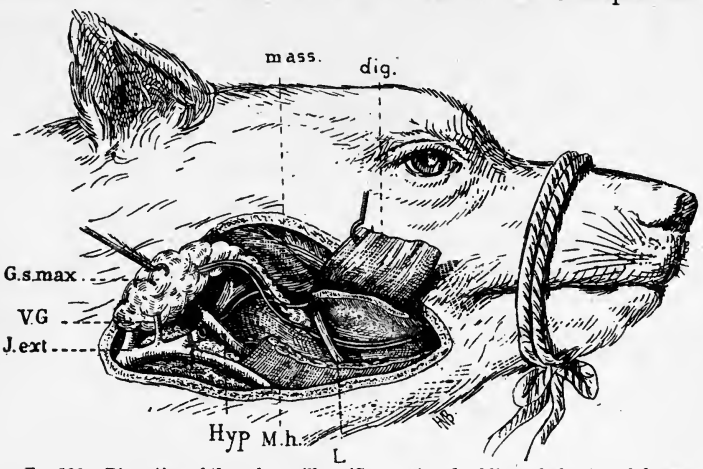


FIG. 136.—Dissection of the submaxillary (G.s.max) and sublingual glands and ducts and the lingual nerve L. The chorda tympani leaves the lingual and runs along the ducts. J.ext, external jugular vein; V.G, branch of vein to gland; Hyp, hypoglossal nerve; M.h., mylehyoid; dig., digastric; mass., masseter muscle. (Bernard).

then made into the duct, and a fine glass or silver cannula inserted and tied in.

The sympathetic fibres run into the gland with the arteries. To expose these the digastric muscle is divided close to its insertion on the

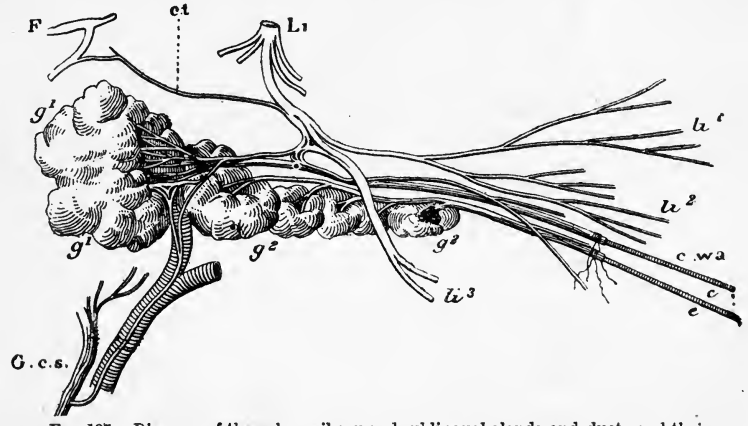


FIG. 137.—Diagram of the submaxillary and sublingual glands and ducts and their nerve supply from chorda tympani and sympathetic. F, facial; Li, lingual; ct, chorda tympani; C.c.s., cervical sympathetic nerve; g^1, submaxillary; g^2, sublingual gland; c.wa, e.c ducts of glands. (Bernard.)

jaw bone, and the posterior end of the muscle hooked back. A triangular cavity is thus exposed. The carotid artery with the nerves lie in the lower part of this, while Wharton's canal and the artery of the

gland appear in the upper part. The gland itself lies a little more to the back.

On exciting the cervical sympathetic, or the sympathetic nerve filaments which accompany the artery of the gland, the gland will pale owing to vaso-constriction. A little thick secretion will at the same time appear in the cannula. On exciting the chorda tympani, an abundant secretion of thin watery saliva appears. At the same time the gland becomes red and turgid. The same effect may be produced reflexly by excitation of the central end of the lingual nerve.

The submaxillary gland is enclosed in a firm capsule. It is fed by a branch of the external maxillary artery which enters the hilus of the gland. The gland also receives small branches from the great or posterior auricular artery. The veins are usually two, but are variable. One enters the internal and the other the external maxillary vein close to where these veins join to form the external jugular vein. The blood coming from the salivary gland can be collected by tying a cannula in the external jugular vein and ligating all branches excepting those coming from the gland. The exchange of blood-gases in the gland can thus be determined.

Nicotine, 30-40 mgrms. in dog, 10 mgrms in cat, injected intravenously, paralyses the preganglionic fibres of the chorda tympani for about 15 minutes. The ganglion cells of the submaxillary gland are in or near the hilus of the gland.

Atropine sulphate, 10-14 mgrms. in dog, 5-15 mgrms. in cat, injected into the blood paralyses the secretory fibres of the chorda tympani, while it leaves the vaso-dilator fibres untouched. Pilocarpine nitrate, 1-2 mgrms, produces prolonged and plenteous secretion. The antagonism may be shown by injecting atropine into the blood and then injecting a little 2 per cent. solution of pilocarpine into the gland by way of the duct cannula.

If the duct cannula is connected with a mercury manometer and the chorda tympani stimulated, the secretory pressure will be observed to rise higher than the pressure in the carotid artery.

The submaxillary gland can be placed in a plethysmograph and its volume recorded (Bunch). Stimulation of the cervical sympathetic causes very considerable diminution in volume and a scanty secretion. Excitation of the chorda tympani is followed by diminution in volume in spite of vaso-dilatation. This is due to the copious secretion. After injection of atropine the volume is increased by chorda excitation.

If a cannula be placed in the cervical lymphatic just above where it enters the thoracic duct the effect of stimulating the salivary gland on the outflow of lymph can be observed (Bainbridge).

Stimulation of the chorda or injection of pilocarpine increases the outflow of lymph 2½ times. If Wharton's duct be obstructed the lymph flow is not so great. After injection of atropine no such increase is found. Stimulation of the sympathetic also increases the flow of lymph.

When a permanent salivary fistula is made, and the duct cannula is arranged to empty into a vessel attached to the dog's neck, it is found that the character of the secretion varies with the nature of sensory excitation (Pawlow). Stones placed in the dog's mouth are rejected without flow of saliva. Sand is washed out by watery saliva which contains almost no solid or ferment. Food provokes the secretion of saliva rich in ferment. The reflex and sub-conscious nervous mechanism which controls the secretion of saliva thus carries out actions which are similar to voluntary or willed actions.

CHAPTER XLIV.

GASTRIC AND PANCREATIC SECRETION.

The Gastric Secretion is obtained by making an incision in the stomach as in Fig. 138, and then reflecting and suturing the mucous membrane of the stomach, so as to make a separate secreting sac which is still in muscular and nervous continuity with the rest of the stomach. The mouth of the sac is sewn to the opening in the abdominal wall (Pawlow). The vagus is exposed ligatured and divided. Three days later the peripheral end of the vagus is excited in the unanaesthetised animal and the juice collected.[1] Anaesthesia or operative procedures easily inhabit the gastric secretion. Pawlow opens the gullet in the neck, and stitches the ends of the gullet to the opening. The dog is then given meat. He eats it, and the meat falls out of the opening in the gullet. This fictitious feeding, which the dog will enjoy for an hour or so, reflexly excites secretion of gastric juice. Mechanical excitation of the mucous membrane of the stomach does not provoke secretion.

The Pancreatic Secretion.—In the anaesthetised dog the duct of the pancreas is exposed where it lies on the duodenal wall and a cannula inserted. A piece of the jejunum is excised, chopped up,and submitted to the action of 0·4 per cent. HCl. This acid extract of jejunum on being injected intravenously excites a copious secretion of the pancreatic juice. Normally the acid of the gastric juice on coming in contact

[1] This experiment cannot be demonstrated.

with the intestinal wall changes a substance called *prosecretin* into *secretin*.

The secretin is absorbed into the circulation and passing to the pancreas excites the pancreatic secretion. The effect of secretin can be obtained equally well after enervation of the solar plexus or injection of atropine. Prosecretin is inactive. It can be obtained in 0·9 NaCl extract of jejunum, and can be changed into secretin by boiling. No secretin can be obtained from the ileum. The pure pancreatic juice which is obtained in the above manner, has practically no

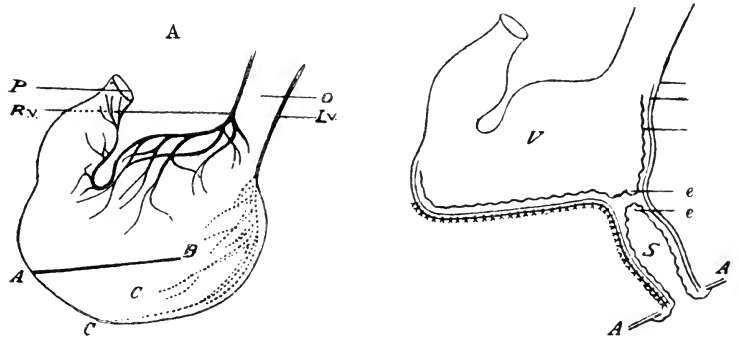


FIG. 138.—Pawlow's method of establishing a gastric fistula. *A*, *B*, Incision; *S*, segment of stomach separated off; *A*, abdominal wall; *e*, mucous membrane; *P*, pylorus; *O*, œsophagus; *Rv*, right; *Lv*, left vagus nerve.

action on fibrin, but is active towards starch. The juice is rendered active towards fibrin by the addition of succus entericus (Bayliss and Starling). The substance in the succus entericus which activates the trypsinogen has been called entero-kinase (Delezenne). Pawlow excited the flow of pancreatic juice by stimulating the vagus. It is possible that the flow is indirectly excited, for stimulation of the vagus causes the passage of the acid gastric juice into the duodenum.

Pawlow makes a permanent pancreatic fistula in the following way. A small piece, including the opening of the pancreatic duct, is cut out of the duodenum. The duodenum is sewed up without appreciable reduction of its lumen. The excised piece is sewed into the abdominal wall with the mucous surface outwards. The animal cage is kept well covered with sand, so that the juice is absorbed when the animal lies down, and does not irritate the edges of the fistulous opening.

Pawlow finds that the activity of the ferments in the pancreatic varies with the diet. The juice is richer in trypsin on a milk diet, in amylopsin on a bread diet. There is more steapsin on a milk than on a bread diet.

CHAPTER XLV.

ABSORPTION.

Absorption of Fat.—A cat is fed on milk, and three or four hours later is killed. Open the abdomen and expose the mesentery. The lacteals will appear as white cords, and the mesenteric lymphatic glands turgid. Expose the thoracic duct at its entrance at the junction of the left jugular and subclavian veins. Open the duct; a milky white fluid will escape. Fix some small pieces of jejunum in osmic acid, 1 per cent. sol., and make teased preparations of the columnar cells in dilute glycerine. The cells will appear full of fat droplets stained black.

Absorption (Reid's Experiment).—The abdomen is opened in the anaesthetised animal. Two loops of small intestine are chosen of equal length and ligated at either end. One loop, A, is washed out with isotonic NaCl solution, the other, B, with distilled water or isotonic saline + 0·1 per cent. sodic fluoride.

An equal measured amount of the animal's own serum is then placed in each loop. The serum is obtained by drawing off some blood from the carotid artery. The blood is whipped and centrifuged. After one hour 50 per cent. of the water may be absorbed from A and none from B, in which the columnar epithelium is destroyed by the reagents used to wash it out.

Ten minutes' anaemia induced by clamping the mesenteric arteries has the same effect in preventing absorption. The water, organic solids and salts, are taken up by the intestinal wall from the animal's own serum which has the same osmotic pressure as the blood in the mesenteric vessels. This occurs when the hydrostatic pressure of the gut is below that of the blood in the mesenteric veins, and when the lacteals of the loop are tied. Osmosis and filtration are thus excluded in this experiment, and absorption must be ascribed to the selective activity of the living columnar epithelium.

PART II.

PHYSIOLOGICAL CHEMISTRY.

ELEMENTARY COURSE.

INTRODUCTION.

PHYSIOLOGICAL Chemistry or Chemical Physiology is the subject which treats of the chemical processes connected with life. It comprises a study of the chemical constitution of the various tissues and of the chemical nature of the constant interchanges undergone by the food-stuffs in their passage through the organism.

Plants, under the influence of the sun's rays, have the power of combining the various simple substances absorbed by the roots and leaves into complex organic compounds. Their chemical processes are, therefore, of the nature of '*syntheses*.' The complex bodies thus produced have, locked up in their molecules, a large amount of potential or latent energy. Animals eat the products of plant life in order to obtain this energy. This they accomplish in their tissues where the complex molecules are resolved into simpler ones and the potential energy becomes liberated as actual or kinetic energy, which is then used for the processes of life. Their chemical interchanges are therefore of the nature of '*analyses*.'

All the food-stuff, however, is not thus decomposed by the animal, a certain amount of it being used in order to build up the tissues themselves (*e.g.* muscle, glands, etc.), and a certain amount being laid aside as storage material (*e.g.* fat) available to the organism as food, should the amount of this latter supplied from without be insufficient for the needs of life.

It will be seen, therefore, that the study of physiological chemistry includes the chemical composition of the various food-stuffs and of the tissues, as well as the nature of the chemical interchanges which these food-stuffs undergo in the tissues.

The chemical substances which exist in the food-stuffs and tissues may be divided into *inorganic* and *organic*, the former include water and the mineral salts, and the latter consist of organic compounds containing the elements carbon, oxygen, hydrogen, and, in a certain class of them, nitrogen. The organic substances are divided into two groups depending on whether they contain nitrogen or not. The *nitrogenous food* stuffs include proteid, which is the most important constituent of the tissues, and without which, as a food-stuff, animal life is impossible. The *non-nitrogenous* include the fats and carbohydrates, the latter being pre-eminently the combustion material, and the former the storage material of the animal body.

The chemical composition of fats and carbohydrates is accurately known, but with regard to the structure of the proteid molecule we know next to nothing. Much less, therefore, do we know of the chemical constitution of living protoplasm of which proteid is the chief constituent. Living matter cannot be analysed, for the mere process of analysis necessarily kills it, and the results obtained show only the decomposition products of dead matter.

These bodies, fats, proteids, and carbohydrates, really represent the elementary constituents of the organism, so that they are frequently called the '*proximate principles.*'

We shall first of all study the chemical nature of the proximate principles, then the variety and amount of these contained in the various tissues and foods.

We shall then be in a position to investigate the nature of the chemical interchanges in the organism, and in order to do this we shall require to study the chemical composition of various excretory bodies given off in the urine and other excreta.

CHAPTER I.

CARBOHYDRATES.

Chemical Relationships.—These are compounds of carbon, hydrogen, and oxygen, in which the latter two elements exist in the same proportion as in water. Their general formula is therefore $C_mH_{2n}O_n$, where m is usually of the same value as n, and in the case of the simple carbohydrates is almost invariably 6 or some multiple of it.

Carbohydrates are found chiefly in vegetables, but may also occur in animal tissues. They form very important foodstuffs, for they are easily digested and assimilated, and moreover are much cheaper than proteids and fats. (See Diet.) The simplest form of carbohydrate is called a monosaccharide, and all other carbohydrates are derived from this by the condensation[1] either of two molecules of a monosaccharide (disaccharides), or of several (polysaccharides).

I. Monosaccharides.

Chemically, monosaccharides are either aldehydes or ketones, the former are called *aldoses*, the latter *ketoses*.

Aldoses.—An aldehyde is the first oxidation product of a primary alcohol,[2] and it contains the end group – CHO.

[1] *Condensation* is a chemical process whereby several molecules of the same body (or even of different bodies) come together with the loss of one or more molecules of water or some other stable body.

[2] A primary alcohol is one in which the 'OH' or *hydroxyl* group is attached to the last C atom of the molecule—as in primary propyl alcohol,

$$CH_3 - CH_2 - CH_2OH,$$

and it contains the end group $-CH_2OH$. If, on the other hand, the hydroxyl group be attached to a central C atom—as in secondary propyl alcohol,

$$CH_3 - CHOH - CH_3,$$

—the alcohol is called secondary, and contains the group – CHOH.

Thus, if ethylic alcohol be heated with potassium bichromate and sulphuric acid, it is oxidised and acetic aldehyde is formed:

$$\underset{\text{Ethyl alcohol.}}{CH_3 - CH_2OH} + O = \underset{\text{Acetic aldehyde.}}{CH_3 - CHO} + H_2O.$$

This group $-CHO$ is, however, not a stable one, but very readily undergoes further oxidation to produce the acid radicle $-COOH$,

$$\underset{\text{Acetic aldehyde.}}{CH_3 - CHO} + O = \underset{\text{Acetic acid.}}{CH_3 - COOH}.$$

Aldehydes are consequently strong reducing agents, and it is this property which constitutes one of their most important group reactions, for the reaction is frequently accompanied by a visible change in the colour of the solution. Thus, in the above experiment, reduction causes the yellow chromate to be changed into the green chromate.

Their power of reducing cupric hydrate, which is blue in colour, to cuprous hydrate, which is red, and of reducing argentic nitrate to metallic silver, is of especial value as a test. Similar reactions are obtained with certain bismuth and mercury salts. In order to produce these reactions it is necessary that the solution be alkaline in reaction.

Experiment I. Demonstrate the reducing power of a simple aldehyde, such as *formalin*, using cupric hydrate as the metallic salt. Place one drop of a weak solution of cupric sulphate in the test tube. Add about ten drops of formalin (or aldehyde), and then, drop by drop, a strong solution of caustic potash. The first drop or so of the latter produces a precipitate of cupric hydrate, but it afterwards becomes redissolved, as aldehydes have the power of dissolving cupric hydrate in alkaline solution. Now boil and note that a reddish-yellow precipitate of cuprous oxide is produced. The chemistry of the reaction is illustrated by the following equations:—

1st Stage. $$\underset{\text{Cupric sulphate + caustic potash.}}{CuSO_4 + 2KOH} = \underset{\text{Cupric hydrate + pot. sulphate.}}{Cu(OH)_2 + K_2SO_4}.$$

The cupric hydrate is kept in solution by the aldehyde to form clear blue solution.

2nd Stage. $$\underset{\text{Cupric hydrate + aldehyde.}}{2Cu(OH)_2 + RCHO} = \underset{\text{Cuprous hydrate + acid.}}{Cu_2(OH)_2 + RCOOH} + H_2O.$$

By heating the cuprous hydrate loses a molecule of water and changes into the oxide: $Cu_2(OH)_2 - H_2O = Cu_2O$.

Experiment II. Demonstrate the reduction of silver nitrate.

Place about 5 c.c. of an ammoniacal solution of silver nitrate (prepared by adding ammonia to a solution of silver nitrate till the preci-

pitate formed just redissolves) in a test tube and add to it about ten drops of formalin. Boil. Reduction takes place, and the metallic silver is deposited on the wall of the test tube as a mirror.

Ketoses.—As mentioned above, some carbohydrates belong to the group of substances called ketones. A ketose is the oxidation product of a secondary alcohol, and contains the group CO which, instead of being at the end of the chain, is situated in the middle of it.

Ketones also form compounds with phenyl hydrazine, but only some of them reduce metallic oxides in alkaline solution. Those ketones which belong to the carbohydrates, manifest this reducing power. The only well-known ketose is *Laevulose.*

Reactions of Monosaccharides depending on their Chemical Nature.

I. Their Reducing Power.—If, in the above reactions, we take instead of ethylic alcohol, the hexatomic[1] alcohol, sorbite, and carefully oxidise it as above we obtain its aldehyde which is *dextrose,*

$$\underset{\text{Sorbite.}}{CH_2OH-(CHOH)_4-CH_2OH}+O=\underset{\text{Dextrose.}}{CH_2OH-(CHOH)_4-CHO}+H_2O.$$

Since it is an aldehyde it will manifest strong reducing powers on metallic oxides in alkaline solution.

Experiment III. Demonstrate the reducing power of a carbohydrate, such as dextrose on cupric hydrate.

Place three drops of a weak solution of cupric hydrate in a test tube; add about 5 c.c. of a 1% solution of dextrose, and then, drop by drop, a 20% solution of caustic potash (KOH) until the precipitate of cupric hydrate at first formed redissolves *and a clear blue solution is obtained.* Boil. Reduction is effected, a *red precipitate* of cuprous oxide resulting.

Experiment IV. Demonstrate that dextrose also reduces silver nitrate to metallic silver.

Repeat Expt. II., using a 1% solution of dextrose instead of formalin.

II. Like other Aldehydes they form Compounds called Osazones, with Phenyl Hydrazine.—The compounds are very useful in identifying the various forms of sugars, as each sugar forms a slightly different compound (see p. 417).

Reactions peculiar to Carbohydrates.

There are, however, other reactions of carbohydrates which are peculiar to them alone, and do not depend on their chemical constitution. The most important of these are:—

[1] A hexatomic alcohol is one which contains six OH groups. Glycerine is called tri-atomic because it contains three such groups. Ethylic alcohol is mon-atomic because it contains one.

I. Fermentation with Yeast.—By allowing yeast to grow on a solution of dextrose, the latter is split up into alcohol and carbon dioxide,

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

Dextrose. Ethylic alcohol + carbon dioxide.

All carbohydrates do not give this reaction. It is of great value as a test for the presence of dextrose in the urine. Commercially it is the agency employed in the preparation of alcoholic beverages.

To ascertain whether the addition of yeast to any solution produces fermentation, the process should be allowed to proceed in an inverted tube, so that any carbon dioxide gas which develops may be collected, and if necessary tested for (see p. 274).

II. Rotation of Polarised Light.—All simple carbohydrates (monosaccharides) rotate light to the right except laevulose, which rotates it to the left. (See Advanced Course.)

III. Moore's Test.—When heated with caustic potash a dark substance called caramel is produced. This is also produced when sugar is burnt. Caramel contains several chemical bodies, the most important of which is an acid called levulinic acid ($CH_3 - CO - CH_2 - CH_2 - COOH$).

Experiment VI. Mix equal quantities of a 1% solution of dextrose and 40% NaOH in a test-tube; heat. A yellow to brown colouration results, and a smell of burnt sugar (caramel) is evolved, especially on adding weak H_2SO_4.

The Chief Monosaccharides are dextrose, laevulose, and galactose.

Dextrose, Grape Sugar or Glucose ($C_6H_{12}O_6$) is found in many fruits and is an important food stuff. In the healthy animal body it occurs in minute traces in blood and muscle, and in disease its percentage may rise in the blood, and it then also appears in the urine (see p. 271).

It is soluble in water and in alcohol. It has only a slightly sweet taste. It rotates polarised light to the right.

Laevulose ($C_6H_{12}O_6$) is found along with dextrose in fruits, and results from the hydrolysis of cane sugar (see disaccharides). It is very rarely found in animal tissues. It is crystallisable with great difficulty, being usually obtained as a putty-like mass. It is laevo-rotatory.

Galactose ($C_6H_{12}O_6$) is a dextro-rotatory sugar produced, along with dextrose, by hydrolysing lactose (see Disaccharides). Certain gums also yield it on hydrolysis. It differs but slightly from dextrose in its reactions.

II. Disaccharides.

Chemically each molecule of a disaccharide consists of two molecules of a monosaccharide minus one molecule of water.

$$2C_6H_{12}O_6 - H_2O = C_{12}H_{22}O_{11}.$$

Their structure can be demonstrated by *hydrolysing them*, *i.e.* by causing them to take up a molecule of water, in consequence of which they split up.

The chief means of hydrolysing include boiling with a weak acid, the action of the succus entericus and of certain organised ferments.

The members of this class are cane sugar, maltose, and lactose, and of these cane sugar does not reduce metallic oxides in alkaline solution, nor does it form an osazone, whereas lactose and maltose give both these reactions. With yeast they are first hydrolysed, and the monosaccharides thus produced then undergo alcoholic fermentation.

Cane Sugar ($C_{12}H_{22}O_{11}$) is the common sugar obtained from sugar cane, beet root, etc. It is very soluble in water, and has a sweet taste. It does not reduce metallic oxides in alkaline solution.

EXPERIMENT VII. Perform Trommer's test with some cane sugar solution. Notice that, although no reduction occurs, the cane sugar is capable of holding the cupric hydrate in solution, so that a clear blue colour is produced as it was with dextrose. By hydrolysis, reducing sugars are developed.

EXPERIMENT VIII. Boil some cane sugar solution with a few drops of 25% sulphuric acid. Now neutralise and apply Trommer's test and notice that reduction occurs. The monosaccharides developed are dextrose and laevulose.

DEMONSTRATION III. A solution of cane sugar is dextro-rotatory, but after hydrolysis it is laevo-rotatory, the laevo-rotatory power of the laevulose being stronger than the dextro-rotatory power of the dextrose formed. On this account the process of hydrolysis is sometimes called *Inversion*, and the hydrolysing agent in the succus entericus is called an *inverting ferment*. Yeast also contains an inverting ferment.

Lactose ($C_{12}H_{22}O_{11}$) is the sugar found in milk, and it has been detected in the urine of nursing mothers.

It is not very soluble in water, and is quite insoluble in alcohol and ether. It has only a slightly sweet taste. It does not readily ferment with yeast, but it undergoes a special fermentation with another organism which results in the production of lactic acid.

$$C_{12}H_{22}O_{11} + H_2O = 4CH_3 - CH\begin{matrix}OH\\COOH\end{matrix}$$

Lactose. Lactic acid.

It reduces metallic oxides in alkaline solution. It is dextro-rotatory.

Maltose ($C_{12}H_{22}O_{11}$) is important physiologically because it is the sugar produced from starch by the action of *ptyalin*, the ferment of the

saliva, or of *amylopsin*, a ferment in the pancreatic juice. It is also produced by the action of malt diastase, and to a certain extent by ordinary hydrolysing agencies acting on starch.

By moistening barley and allowing it to germinate in heaps at a constant temperature, the starch which it contains is converted into dextrose (see below) and maltose. This change is brought about by the ferment called diastase which exists in barley. The product when dried is called *malt*. If this be dissolved in water and the yeast plant allowed to grow on the solution, *malted liquors*, such as beer and ale, are obtained.

Maltose readily ferments with yeast, being first of all inverted into two molecules of dextrose by the inverting ferment contained in the yeast.

It reduces metallic oxides in alkaline solution.

CHAPTER II.

CARBOHYDRATES—Continued.

III. Polysaccharides.

A POLYSACCHARIDE is the condensation product of more than two monosaccharide molecules, and has accordingly the general formula, $(C_6H_{10}O_5)_n$, where n stands for a variable number.[1] They can be hydrolysed, the ultimate products being monosaccharides; polysaccharides (dextrines) of lower molecular weight (*i.e.* with n of less value), and several disaccharides being developed as intermediate products.

Thus, when starch is boiled with a weak acid, or is acted on by the ferments ptyalin or amylopsin, it yields at first dextrine—a lower polysaccharide—and maltose—a disaccharide. The former of these is then further hydrolysed to form maltose. Boiling with an acid further produces dextrose.

The most important members of this group are starch, dextrine, glycogen, cellulose, and gums. They are very widely distributed in vegetables and constitute a most important class of food-stuffs.

General Characters. They do not form crystals, nor, with few exceptions, are they soluble in cold water. Few possess any sweet taste. They do not reduce metallic oxides in alkaline solution, they do not form osazones, and they cannot be fermented with yeasts. They are precipitated when their solutions are saturated with certain neutral salts, such as ammonium sulphate. They may be sub-divided into three sub-groups, the starches, the gums, and cellulose.

[1] It is impossible to give a definite value to n because the molecular weight is unknown.

1. **The Starches.** These include *ordinary starch* and *glycogen* $(C_6H_{10}O_5)_n$. Starch is the most widely distributed carbohydrate in the vegetable kingdom, for it is in this form that plants store up their excess of nutriment. We store ours chiefly as fat. In plants, dextrose is formed in the leaf by the chemical energy of the sun's rays acting on the crude chemical substances absorbed by the roots. If the amount of dextrose thus produced be in excess of the present needs of the plant, it is stored up as starch. These starch grains may be seen in various parts of the plant. They show under the microscope concentric markings, produced by alternating layers of starch granulose and starch cellulose. The former of these is more easily hydrolysed than the latter, so that intact starch granules are less easily hydrolysed than those which have been ruptured by boiling or some such agency.

The exact shape of starch grains varies according to the plant from which they are obtained. In this connection they may be divided into two groups: (1) a group in which their contour is even, such as wheat, barley, arrowroot, potato; (2) a group in which the contour is marked by facets, either completely as in oats and rice, or only partially so, as in tapioca and sago.

EXPERIMENT IX. Examine some grains of wheat, a scraping of potato, and some ground rice under the microscope. To do this, mix the flour, etc., with a drop of water on a slide.

Starch, like most other polysaccharides, is insoluble in cold water, but it swells up in hot water, an opalescent solution being formed. This is not a true solution as only a trace is actually dissolved, the rest being swollen up into transparent clumps which run together.

EXPERIMENT X. Place some powdered starch in a test tube, and half fill up with cold water—no solution occurs—now boil, when an opalescent solution will be produced, and, if of sufficient concentration, this will gelatinise on cooling. Try Trommer's test with this solution —no reduction occurs. By boiling, the cellulose layers of the granule are ruptured, the granulose being liberated.

EXPERIMENT XI. Place some of the solution in the mouth, and after a minute or so transfer it again to the test tube: now apply Trommer's test—reduction occurs.

Try the same experiment with some unboiled starch, and note that with Trommer's test no reduction is effected (*i.e.* the resistent cellulose layers have not been hydrolysed).

The standard test for starch is with iodine solution.

EXPERIMENT XII. To an opalescent solution of starch add a drop

or two of liquor iodi:—a blue colour results which disappears on heating, and returns again on cooling. Excessive heat must be avoided, since the iodine is volatile.

Starch granules also give this reaction under the microscope, as does the cut surface of a potato.

Hydrolysis can be effected by boiling with a weak acid or by the action of ferments ptyalin and amylopsin (see above), and malt diastase (see Advanced Course).

Experiment XIII. Place some starch solution in a flask and add to it a few drops of 25% sulphuric acid: boil for about a quarter of an hour. Now apply the iodine test and notice that instead of a blue a port wine colour is produced (due to dextrine). Apply Trommer's test, and note that reduction is effected.

The sugar produced by hydrolysing with an acid is dextrose, whereas that produced by ferment action is maltose (see Advanced Course).

Glycogen $(C_6H_{10}O_5)_n$.—Just as plants store up excess of carbohydrate in the form of starch, so do animals store it in the form of glycogen. The chief seats of this storage are the liver and muscles, but in the embryo it is more widely distributed.

Experiment XIV. A simple method for the preparation of glycogen is that introduced by Fränkel. It consists in grinding up the fresh liver in a mortar with about three times its volume of a 3 per cent. solution of tri-chloracetic acid. This reagent coagulates the proteids. The glycogen contained in the extract is precipitated by 90 per cent. alcohol. Filter. Dissolve some of the glycogen in water and notice that the solution is opalescent. Add to this a drop or two of iodine solution. A port-wine colour results, which disappears on heating, and returns on cooling.

Experiment XV. Add some basic lead acetate; a precipitate results.

Experiment XVI. Try Trommer's test; no reduction occurs, but the $Cu(OH)_2$ is kept in solution.

Experiment XVII. To some of the solution add a few drops of 25% H_2SO_4, and boil for about ten minutes; dextrose is produced, and reduction now occurs.

Dextrine $(C_6H_{10}O_5)_n$.—During the hydrolysis of starch dextrine is formed as an intermediate product. British gum is dextrine produced by heating starch to 200° C. This substance is much employed in the manufacture of envelopes.

Dextrine is a fawn-coloured amorphous powder, soluble in cold water and forming a *clear solution* with which the following reactions can be obtained:

EXPERIMENT XVIII. Add some iodine solution; a port-wine colour, like that obtained with glycogen, results, which disappears on heating and returns on cooling. It is only one form of dextrine—*erythro-dextrine*—which gives the reaction, the other variety—*achroö-dextrine*—does not.

EXPERIMENT XIX. Try Trommer's test: no reduction is obtained, but $Cu(OH)_2$ is held in solution.

EXPERIMENT XX. Hydrolyse, and a reduction will be obtained.

CHAPTER III.

PROTEIDS.

OF all the chemical substances occurring in the animal organism proteids are the most important.

With the exception of the urine, the sweat, and the tears, there is no secretion or excretion which does not contain them, and they form the chief constituent of the vital tissues. They are also indispensable as foods, their absence from the diet being sooner or later followed by death, for it is impossible to replace them by any other food-stuff.

Chemical Nature.—As to the chemical constitution of the proteid molecule we know very little. By elementary analysis it is found to contain the elements carbon, hydrogen, oxygen, nitrogen, and, nearly always, sulphur. The percentage amount of each of these in the various forms of proteid is so much alike that a determination of the elementary composition helps us very little in distinguishing one proteid from another. The following is the average percentage composition for all proteids:—

C	-	-	50·6	-	54·5.
H	-	-	6·5	-	7·3.
N	-	-	15·0	-	17·6.
S	-	-	0·3	-	2·2.
O	-	-	21·50	-	23·50.

The nitrogen and sulphur are each contained in the molecule in two forms, the one loosely combined, the other firmly combined. The loosely combined portion of each of these elements can be split off from the rest of the proteid molecule by boiling with caustic alkali.

EXPERIMENT I. **The loosely combined Nitrogen.**—To about five cubic centimetres of diluted egg-white add a few drops of 20 per cent. caustic potash; warm slowly, and hold a piece of moistened red litmus paper over the mouth of the test tube. The litmus turns blue, showing that ammonia gas is being evolved. The ammonia may

also be detected by its smell, or by holding the stopper of the concentrated hydrochloric acid bottle over the mouth of the test tube when fumes of ammonium chloride are formed.

Experiment II. **The loosely combined Sulphur.**—To about five cubic centimetres of 20 per cent. caustic potash add two drops of lead acetate solution; a precipitate at first forms, which, however, redissolves on shaking. When clear, add some solution of egg-white and boil, when a brown to black colour will be developed, due to the lead sulphide which is formed.

By studying the decomposition products resulting from their hydrolysis, many attempts have been made to construct the constitutional formula of proteids, and, although this has not been attained, still much knowledge has been gained of their chemical nature.

Recently Emil Fischer and his pupils have succeeded in linking various amido acids together, the resulting bodies being named *peptides*. This discovery justifies the hope that in the near future proteid synthesis will be possible.

The basis of construction of all proteids is, according to Kossel, a body called *protamin* ($C_{30}H_{57}N_{17}O_6$), which yields on hydrolysis three basic substances, lysin, histidin, and arginin, each containing six carbon atoms, and hence called **hexone bases**. Protamin has been found loosely combined with nucleic acid (see p. 428) in the spermatozoa of certain fishes. In the proteid molecule it is firmly combined with amido-acids (*e.g.* leucin, glycin, etc.), and usually with aromatic bodies (*e.g.* tyrosin, etc.), and inorganic elements, (*e.g.* sulphur and phosphorus). The nature and the amount of the decomposition products yielded by different forms of proteid varies, and even in the case of protamin there is no doubt that the molecule is of enormous size, though the accurate determination of this is impossible on account of the peculiar physical properties of proteids.[1]

In the animal body the ultimate decomposition products of proteids are urea, carbon dioxide gas, water, ammonia, and sulphuric acid; the intermediate bodies, such as hexone bases, amido-acids, aromatic bodies, etc., being produced during digestion in the intestine, in connection with which they will be more closely studied.

Physical Properties of Proteids.

I. **Diffusibility.**—Proteids belong to the class of bodies called *Colloids*, which do not diffuse through animal membranes or parch-

[1] The decomposition products also vary with the nature of the decomposing agency employed, but the subject is too complicated for study here. Attempts at the synthesis of proteids have as yet proved futile.

ment paper. In this they are unlike *Crystalloids*, such as inorganic salts, which readily diffuse.

Various forms of dialyser are used, but the simplest consist, either of a bell-shaped glass vessel closed below by a tightly stretched piece of parchment or membrane and open above (Fig. 139), or of a tube of parchment.

EXPERIMENT III. Place a mixture of diluted egg-white and of a 10 per cent. solution of NaCl in a dialyser (the tubular dialyser is the best to use), and place the latter in a large basin or beaker filled with distilled water. Before inserting the dialyser, test a sample of the water for chlorides with argentic nitrate solution, and note that no haze results. Allow the dialysis to proceed for a day, then test a sample of the water again, when a white precipitate of $AgCl_2$ will result.

FIG. 139.—Dialysers.

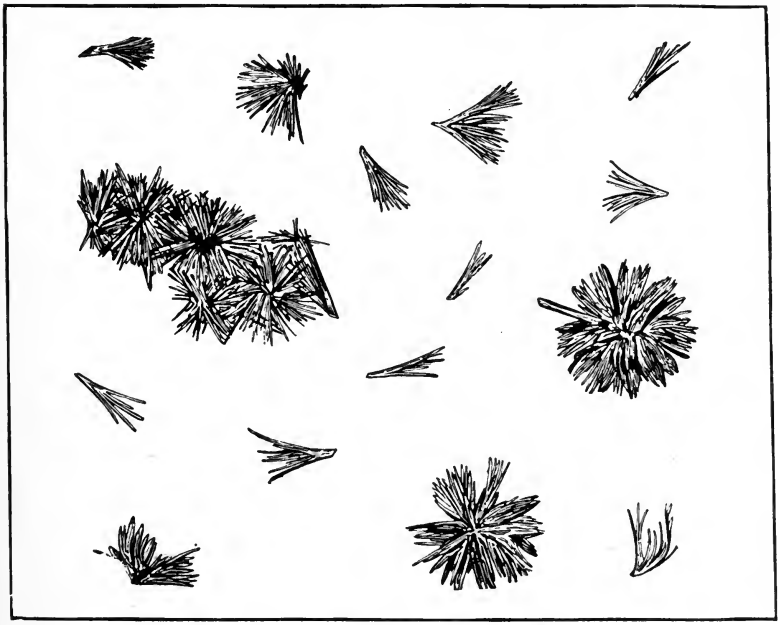


FIG. 140.—Crystallised albumin. × 600.

The NaCl, being a crystalloid, has diffused out, but no proteid has passed out, as can be ascertained by applying the proteid tests (see below).

II. **Crystallisation.**—Proteids usually exist when dried as amor

phous masses or powders, but some of them, *e.g.* haemoglobin, are easily obtainable as crystals, and of the others many can now be made to crystallise. Thus egg-albumin can be made to crystallise by allowing a solution of it, in which the solvent just barely dissolves the albumin, to stand exposed to the air (Fig. 140). Slow evaporation of the water takes place, in consequence of which the albumin is no longer held in solution, and is precipitated as crystals (see Advanced Course).

III. **Rotation.**—All proteids are laevo-rotatory.

Chemical Properties and Reactions.

I. **Colour Reactions.**—These are very important as tests for proteids. They are:—

(1) **Biuret Reaction** (Piotrowski's test).

Experiment IV. Place a trace of a weak solution of $CuSO_4$ in a test tube (this is best done by placing some of the solution in the test tube and then pouring it out, sufficient of it remaining adherent to the glass). Add to this about 5 c.c. of a weak solution of egg-white, and then, drop by drop, a 20 per cent. solution of KOH until a violet colour is produced.

Experiment V. Repeat this experiment with a solution of peptone. A rose-pink colour is developed.

This violet or pink colour is given by all proteids, and it seems to depend on the presence of a HCNO group. It is called the biuret reaction, because biuret (the substance left after heating urea crystals in a dry test tube) gives it (see "Urea"). The chemical equation of the reaction is unknown.

(2) **Xanthoproteic Reaction.**

Experiment VI. To about 5 c.c. of a solution of egg-white add a drop or two of HNO_3 (con.); a white precipitate results. Warm this, and the white precipitate changes to a yellow curd. Cool under the tap, and then add a few drops of strong ammonia, when the yellow will change to a brilliant orange.

(3) **Millon's Reaction.**

Experiment VII. Millon's reagent consists of a solution of mercurous and mercuric nitrates in concentrated nitric acid. Add a few drops of it to a solution of diluted egg-white; a white coagulum results, which on boiling changes into a brick-red curd.

Both these reactions (viz. xanthoproteic and Millon's) seem to depend on the presence of some hydroxyl derivative of benzene in the

proteid molecule.[1] Consequently protamin, which does not contain an aromatic radicle, gives neither test, and gelatine, in which there is only a trace of an aromatic body gives only a very feeble colouration.

II. **Precipitants of Proteids.**—Certain reagents have the power of throwing proteids out of solution without in any way changing their chemical nature, *i.e.* the precipitated proteid is still soluble in its original solvents. These reagents are:—

(1) **Neutral Salts** (*i.e.* neutral salts of the alkalies and of certain of the alkaline earths).

(*a*) **Ammonium Sulphate.**

EXPERIMENT VIII. Half fill a test tube with diluted egg-albumin, and add to this crystals of ammonium sulphate till no more will dissolve (*i.e.* until the fluid is completely saturated). A precipitate of proteid is produced. Filter. Test the filtrate by any of the colour reactions described above, when it will be found that no proteid is to be detected.

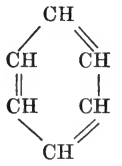
Add water to the precipitate on the filter paper; by so doing a weak saline solution is produced (the ammonium sulphate adherent to the filter being dissolved), and in this the precipitate dissolves, the resulting solution giving the colour reactions described above.

EXPERIMENT IX. Repeat Experiment VIII. with a solution of Witte's peptone (a mixture of albumoses and peptone). In this case the filtrate gives the colour reactions. To obtain the biuret reaction a large excess of KOH is, however, necessary, as the Am_2SO_4 present in the filtrate at first reacts with it forming K_2SO_4. The colour produced by this latter test is rose-pink, showing that the filtrate contains peptone. *Saturation with ammonium sulphate precipitates all proteids except peptone.* Anhydrous sodium sulphate at 30° C. possesses the same precipitating properties as ammonium sulphate (see Advanced Course).

(*b*) **Magnesium Sulphate.**

EXPERIMENT X. Saturate some egg-white solution with crystals of $MgSO_4$—a precipitate (of globulin) falls down—filter—the filtrate gives the colour reactions. The precipitate, if dissolved by adding

[1] The formula for benzene is



and if one of the 'H' atoms be replaced by 'OH,' *Phenol*, a hydroxy-benzene, results. The radicle C_6H_5 is often called Phenyl.

water, also gives the colour reactions. Magnesium sulphate in saturated solution precipitates *certain* proteids (globulins). It does not precipitate albumins.

(*c*) **Sodium Chloride, Ammonium Chloride.**—These salts in their action on proteids resemble magnesium sulphate.

III. **Coagulants of Proteids.**—A coagulum differs from a precipitate in that it is no longer soluble in its original solvents. In other words, its physical or chemical nature has undergone some change.

Coagula may be produced by the following means :—

(1) **Heat.**

Experiment XI. Add to about 5 c.c. of diluted egg-white a drop of dilute acetic acid and boil—a white coagulum is formed.

The acetic acid is added to prevent the formation of alkali albumin, which is much more easily formed than is acid albumin, and which is not coagulated by heat.

Different proteids coagulate at different temperatures—globulin and albumin at 75° C., fibrinogen at 56° C.—and certain proteids, viz. caseinogen, do not coagulate at all when heated.

(2) **Mechanical Agitation.**—By shaking a solution of egg albumin with sand, strings of coagulated proteid like strings of fibrin are deposited.

(3) **Mineral Acids and Salts.**—Any strong mineral acid, (HCl, HNO_3, etc.), or mineral salt, ($HgCl_2$, $CuSO_4$, etc.), if added to a solution of proteid, will cause a coagulum to form. Certain other bodies, such as tannin and picric acid, yield a similar result.

(4) **Prolonged Action of Alcohol.**—The addition of alcohol to a proteid solution at first forms a precipitate, but if this be kept standing for some time under the alcohol it changes into a coagulum. Pepton and fibrin ferment (see blood) take longer to undergo this change than other proteids, and this property is sometimes taken advantage of to separate these from other proteids.

(5) **Ferments.**—When blood clots, the soluble proteid fibrinogen is changed by the action of a ferment (fibrin ferment) into the coagulated proteid fibrin. A similar change is produced in milk, the soluble proteid caseinogen being changed into an insoluble modification by the action of the ferment rennin.

CHAPTER IV.

PROTEIDS—Continued.

Classification of Proteids.

On account of the fragmentary nature of our knowledge regarding the chemical constitution of proteids, it is at present impossible to classify them with anything like the same precision as is the case with carbohydrates. As noted above, Kossel has suggested that all proteids contain as their basis of construction (or nucleus) the substance protamin and that to this are attached different side groups, such as mon-amido acids, aromatic bodies, sulphur, etc., the nature and the relative amounts of these side groups determining the kind of proteid.

It has been found, however, on putting this proposed classification to the test, that certain proteids (*e.g.* elastin) do not contain the three hexone bases necessary for the production of protamin, so that it will be necessary to adopt at present the older classification of Drechsel, adding to it a new group containing the protamines.

The following classification is modified after Drechsel and Kossel:

I. **Protamines** ($C_{30}H_{57}N_{17}O_6$) contain only the hexone bases, and exist, combined with nucleic acid, in the spermatozoa of certain fishes.

II. **Albuminoids** contain hexone bases plus mon-amido acids, such as leucin ($C_6H_{13}NO_2$), along with a variable amount of sulphur. Certain of the members of this group also contain traces of aromatic radicles, such as tyrosin ($C_9H_{11}NO_3$). The chief representative of this group is *collagen*, which, on boiling with water, is changed into *gelatine*.

Experiment XII. Take a piece of gelatine; dissolve it in a test-tube full of warm water; divide the resulting solution into three parts, *a*, *b*, and *c*; allow *a* to cool, when the gelatine solution will set into a jelly.

To *b* apply the biuret reaction; a violet colour is produced.

To *c* apply Millon's test; a very faint red is produced on boiling, as gelatine only contains a trace of an aromatic radicle on which this reaction depends. Some observers consider that the aromatic radicle is not really built up in the gelatine molecule, but is only attached to it as an impurity.

The other important albuminoids are *keratin* (the chief constituent of epidermis, hairs, nails, etc.) and *elastin* (composing elastic ligaments). (See Advanced Course.)

III. **True Proteids.**—These contain hexone bases, mon-amido acids, and aromatic radicles. They include all the commoner proteids, and may be further subdivided into four sub-groups, viz.:

(*a*) **Native Proteids.**—These give *all* the proteid reactions, and are coagulated by heat and by the prolonged action of *alcohol*. There are two varieties, albumins and globulins, which differ from one another in solubility.

Albumins are soluble in distilled water and in saturated solutions of all neutral salts except ammonium sulphate and anhydrous sodium sulphate, in which they are insoluble. They are, however, soluble in half-saturated solutions of these salts.

Globulins are insoluble in distilled water and in saturated solutions of all neutral salts. They are, moreover, insoluble in half-saturated solutions of ammonium sulphate and anhydrous sodium sulphate. They are soluble in weak saline solutions.

EXPERIMENT XIII.—Take some blood-serum. This consists of a weak saline solution containing albumin and globulin. Divide it into three parts, *a*, *b*, and *c*. Allow *a* to drop into a beaker filled with distilled water; a cloud forms round each drop as it mixes with the water. This is due to precipitation of the globulin, as there is now too little saline to keep it in solution. (See Advanced Course.)

Saturate *b* with crystals of $MgSO_4$—a precipitate of globulin is produced. Filter. The filtrate gives all the proteid reactions because it contains the albumin; the precipitate can be dissolved by mixing it with water, the weak saline solution thus produced dissolving the globulin, which can be recognised by applying the proteid tests.

To *c* add an equal bulk of a saturated solution of Am_2SO_4. This produces half saturation in the mixture, as a result of which globulin is precipitated. The precipitate and filtrate should be examined in the same way as the $MgSO_4$ precipitate.

The chief kinds of albumins are ovo- and serum-albumin.

The chief kinds of globulins are ovo- and serum-globulin, myosinogen (the chief proteid of muscle) and fibrinogen (an important proteid of living blood).

TABLE FOR SEPARATION OF ALBUMINS AND GLOBULINS.

Solvent.	*Albumin.*	*Globulin.*	*Practical method of separation.*
a. Distilled water,	soluble	insoluble	dialysis.
b. Weak saline,	soluble	soluble	—
c. Saturation with $MgSO_4$, NaCl, etc.,	soluble	insoluble	Saturation with crystals of either salt.
Half saturation with Am_2SO_4, Na_2SO_4,	soluble	insoluble	Half saturation by adding an equal volume of saturated solution of either salt.

(*b*) **Albuminates.**—These are compounds of native proteids with mineral salts, acids, alkalies, or the halogen elements.

EXPERIMENT XIV. To a few c.c. of diluted egg-white add a few drops of mercuric chloride solution; a white coagulum of albuminate of mercury is formed.

EXPERIMENT XV. To some diluted egg-white add two or three drops of 10 % HCl. Place this on the water-bath at body-temperature for five minutes. Divide into two parts *a* and *b*. Boil the portion *a*—no coagulum appears because the native proteid has been changed into acid albumin or 'syntonin' which does not coagulate on boiling.

To the portion *b* add sufficient litmus solution to stain it red, and then, drop by drop add 1 % solution of Na_2CO_3 till the solution is neutral; a precipitate of acid albumin comes down. Add a slight excess of the alkaline solution, and the precipitate redissolves. Acid albumin is insoluble in neutral saline solution, but soluble in weak acids and alkalies. It is also insoluble in distilled water.

Alkali albumin can be produced by using weak caustic potash instead of the acid in the above experiment. It gives the same reactions as acid albumin, but differs in containing less sulphur and nitrogen, for, as has been explained on p. 169, treatment with alkali separates the loosely combined portions of these elements from proteid. Acid albumin can, therefore, be changed into alkali albumin, but the reverse change of alkali into acid albumin is impossible. Both albuminates can be precipitated in weak acid or alkaline solution by neutral salts. They, therefore, behave like globulins (see below) in this respect, but differ from them in that they cannot be coagulated by heat. If, however, the neutralisation precipitate[1] of either albuminate be heated it changes into a coagulum insoluble in its original solvents.

Acid albumin, when prepared from myosin, is called **Syntonin**, and alkali albumin, when prepared by the action of strong caustic alkali on proteid, is called '**Lieberkühn's jelly.**' Acid albumin is the first product of peptic digestion of proteids, as alkali albumin is the first stage of pancreatic digestion.

(*c*) **Proteoses and Peptones.**—These are produced from native proteids by hydrolysis, either by means of mineral acids or superheated steam, or through certain ferments such as exist in the digestive juices secreted respectively by the stomach and pancreas. They will be studied under "Digestion."

(*d*) **Coagulated Proteids.**—As explained above, coagulation of proteids may be produced by various agencies, of which ferment action

[1] "Neutralisation precipitate" means the precipitate produced by neutralising a solution containing either albuminate.

is the most important, the resulting coagula including *fibrin* (in clotted blood), *casein* (in clotted milk), *myosin* (in 'rigor mortis' muscle). These bodies will be studied in their proper places.

IV. **Compound Proteids.**—These are combinations of native proteids with another organic substance.[1] This latter may be :—

(*a*) a carbohydrate—glucoproteids.
(*b*) nucleic acid—nucleins.
(*c*) an iron containing pigment—haemoglobin.

(*d*) Further compounds also exist where two different kinds of proteid are combined, such as nucleo-proteids (nuclein + native proteid) and **histones** (albumin + protamin). To the latter group belongs globin, the proteid which is separated from haemoglobin by decomposing it with acids or alkalies (see p. 193).

It will only be possible to merely indicate the chief properties of these bodies here, a more complete study of them being reserved for the advanced course.

(*a*) **Gluco-proteids.**—The most important member of this group is **Mucin.**

Experiment XVI. Collect some saliva in a test tube, add to it a drop of 10 per cent. acetic acid ; a stringy precipitate of mucin results. Add a few drops of weak sodium carbonate solution when the precipitate will redissolve.

Experiment XVII. Mucin has been prepared from connective tissue where it is very abundant, by extracting the latter with a weak alkali (lime water). The mucin has been precipitated by a weak acid. The resulting precipitate has then been boiled for about ten minutes with hydrochloric acid (1 part concentrated acid + 3 parts water), and the resulting solution cooled and neutralised. Examine portions of the resulting solutions. Divide the solution into two portions, *a* and *b*.

To (*a*) apply the biuret reaction—a violet or pink colour is produced, showing the presence of the proteid moiety.

To (*b*) add a drop of copper sulphate solution and, if necessary, some caustic alkali till a blue solution is obtained. Now boil, when reduction to cuprous oxide will occur, demonstrating the presence of the carbohydrate moiety.

Besides forming the ground substance of the connective tissues, mucin is also secreted on to the surface of all mucous membranes, where it acts as a lubricant.

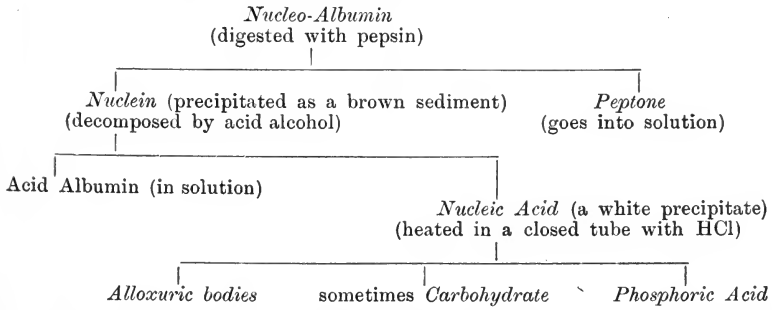
[1] In contrast to albuminates, which are compounds of native proteids with inorganic substances.

(*b*) **Nucleins, etc.**—**Nucleic acid** is a compound of alloxuric bodies (hypoxanthin $C_5H_4N_4O$, xanthin $C_5H_4N_4O_2$, guanin, and adenin) which are basic in nature (see Urine) with phosphoric acid. It does not give the proteid reactions, but seldom exists free, being usually united with albumin to form **nuclein**. This nuclein, again, is almost invariably combined with another molecule of albumin, the resulting compound being **nucleo-albumin**. Nuclein is best prepared by digesting nucleo-albumin with gastric juice, whereby the albumin changes into peptone; the peptone goes into solution, and the nuclein, since it is insoluble, is thrown down as a brown precipitate. If the caseinogen of milk be similarly treated, a sediment is also produced, and peptone goes into solution. This sediment is not, however, true nuclein, since on further decomposition, it only yields phosphoric acid and proteid, but no alloxuric bodies. It is hence called *Pseudo-nuclein.*

Experiment XVIII. Take a cellular organ (*e.g.* thymus gland or pancreas), mince it and then macerate it over-night with water made faintly alkaline by the addition of a drop or two of caustic alkali solution, or of ammonia. Strain the extract through muslin. Add litmus solution to it till it becomes distinctly blue, and then, drop by drop, add weak acetic acid. When the reaction is faintly acid a copious precipitate of nucleo-proteid results, which does not completely dissolve by adding excess of acid (distinguishing it from alkali albumin). Filter off the nucleo-proteid. Add weak alkali to the precipitate, and it re-dissolves.

Demonstration.—The precipitate of nucleo-proteid has been digested with pepsin hydrochloric acid for twenty-four hours; the albumin has been converted into peptone, and the liberated nuclein has fallen down as a brown sediment.

Schema of Relationships of Nuclein, etc.



This nuclein can be further decomposed into nucleic acid and albumin by dissolving it in alkali and then adding 0·3% hydrochloric

acid in alcohol, whereby the *nucleic acid* is precipitated. If this be collected and heated in a closed tube with hydrochloric acid, it splits up into alloxuric bodies and phosphoric acid. There are various forms of nucleic acid, and some of these, *e.g.* the nucleic acid obtainable from pancreas, contain a carbohydrate in their molecule.

C. Haemoglobin. (See blood.)

CHAPTER V.

FATS, FATTY ACIDS, LECITHIN AND CHOLESTERIN.

THESE bodies are classified together because they are all soluble in ether. After extracting any chopped-up organ or tissue with ether, and evaporating off the ether, a more or less syrupy mass is left behind consisting of a varying mixture of these substances. (See Advanced Course, p. 432.)

Fatty Acids.—These are the end-products of the oxidation of primary monatomic alcohols,[1] aldehydes being formed as an intermediate stage. Thus:—

$$CH_3CH_2OH + O = CH_3CHO + H_2O.$$

Ethylic alcohol = Acetic aldehyde.

and then

$$CH_3CHO + O = CH_3COOH.$$

Acetic acid.

The '$-COOH$' group is called *Carboxyl* and the $-OH$ of it is replaceable by a metal to form a salt.

$$CH_3COOH + NaOH = CH_3COONa + H_2O.$$

Sodium acetate.

If we take the monatomic alcohol containing sixteen carbon atoms and oxidise it, we obtain **Palmitic acid** ($C_{15}H_{31}COOH$), and if we take the eighteenth member we obtain **Stearic acid** ($C_{17}H_{35}COOH$). Both these fatty acids exist in the animal tissues, but they are not present in such large amount as a third one called **Oleic acid.** This differs from the other two in being derived from an unsaturated alcohol (*i.e.* an alcohol belonging to the olefine series and in which two of the neighbouring C. atoms are bound together by two valencies). The lowest member of this group of alcohols is allyl alcohol $CH_2 = CH - CH_2OH$. Moderate oxidation of this produces its alde-

[1] A monatomic alcohol is one containing one $-OH$ or hydroxyl group, *e.g.* C_2H_5OH (ethylic alcohol); if it contain three hydroxyl groups, it is called triatomic, *e.g.* $C_3H_5(OH_3)$ glycerine, and so on.

hyde acrolein, $CH_2 = CH - CHO$, and this again can be further oxidised to form acrylic acid, $CH_2 = CH - COOH$. The eighteenth alcohol of this series yields on oxidation oleic acid, which has therefore the formula $C_{17}H_{33}COOH$. Now all bodies in which some of the C atoms are bound together by two valencies are unstable and tend to change into bodies in which the C. atoms are bound together by only one band. To do this, they must have another element to satisfy the extra valency so that they will act as reducing bodies. It is on this account that oleic acid blackens osmic acid (tetroxide of osmium) by appropriating some of its oxygen, and reducing it to a lower oxide which is black. This reaction is given by all fats containing *oleic* acid.

Not only are the lower fatty acids, such as acetic, capable of forming salts with metals, but so are the higher members, such as palmitic, stearic, and oleic. The compound in this case is called a *Soap*.

Thus:—

$$\underset{\text{Palmitic acid.}}{C_{15}H_{31}COOH} + NaOH = \underset{\substack{\text{Sodium palmitate} \\ \text{(a soap).}}}{C_{15}H_{31}COONa} + H_2O.$$

If, instead of using an inorganic salt, we use an alcohol to combine with the carboxyl group, a body called an ester is formed, and if, for this purpose, we use the triatomic alcohol (*i.e.* containing three hydroxyl groups) glycerine, we obtain a **neutral fat**.

$$\left.\begin{matrix} CH_2 - OH \\ | \\ CH - OH \\ | \\ CH_2 - OH \end{matrix}\right\} + \left\{\begin{matrix} HOOC\,C_{15}H_{31} \\ HOOC\,C_{15}H_{31} \\ HOOC\,C_{15}H_{31} \end{matrix}\right. = \left\{\begin{matrix} CH_2 - OOC\,C_{15}H_{31} \\ | \\ CH - OOC\,C_{15}H_{31} \\ | \\ CH_2 - OOC\,C_{15}H_{31} \end{matrix}\right\} + 3H_2O$$

Glycerine. *Palmitic acid.* *Palmitin:* a neutral fat.

By boiling the neutral fat with caustic alkali it is split up into its constituents, the glycerine being set free and the fatty acid uniting with the alkali to form a soap.

This process of saponification is usually carried out by using an alcoholic solution of caustic potash.

EXPERIMENT I. **Saponification of neutral fat.**—Place about 50 c.c. of alcoholic potash in a small flask, and heat on a water bath to near boiling point. Melt some fat (about 10 grammes) in an evaporating dish and drop the melted fat into the heated alcoholic potash, shaking the latter every now and then. After all the fat has been added, continue heating until the spirit begins to boil, and then test to see if saponification be complete. This is done by dropping some of the solution in the flask into a test tube containing about 10 c.c. of distilled water, when,

if saponification be complete, a clear solution of soap will be obtained and no globules of oil will separate out on the surface of the liquid.

EXPERIMENT II. **Separation of fatty acid from soap.**—Place about 40 c.c. of 20% sulphuric acid in a small beaker, and heat it to near boiling point; drop into this the contents of the flask, (which should have been previously allowed to cool) stirring with a glass rod between each addition. The acid displaces the alkali from its combination with the fatty acid, and the latter separates out as an oily layer on the surface of the water.

EXPERIMENT III. **Reactions of fatty acids.**—(1) Remove some of the fatty acid with a clean glass rod, and place it on a piece of *glazed* (ordinary) paper, and a greasy stain will result.

(2) Allow the contents of the beaker to cool, when the fatty acid will solidify into a skin, which can be easily removed with a pen-knife and transferred to distilled water in a test tube. Shake up the fatty acid in the water so as to wash it free of sulphuric acid, pour the contents into a flat dish, again remove the fatty acid to more distilled water, and repeat until the wash-water no longer reacts acid. Now divide the fatty acid into two portions, *a* and *b*. Place *a* in a dry test tube, and dissolve in ether. The resulting solution reacts acid to phenolphthaleine, an indicator which reacts red with alkali, and is bleached by acid, being especially sensitive to fatty acids. To apply this test, two or three drops of the phenolphthaleine are dropped into a test tube containing a very dilute solution of alkali (one drop of 20% KHO in 5 c.c. water), the resulting red solution being then dropped into the solution of fatty acid, when the red colour at once disappears.

Add to *b* some half saturated solution of sodium carbonate, and warm; the fatty acid dissolves, carbonic acid gas is liberated and a solution of soap obtained.

Besides these reactions of the fatty acid produced from it, neutral fat gives an important reaction, *depending on the glycerine* which it contains.

EXPERIMENT IV. Place a small piece of fat in a thoroughly dried test-tube, add to it a crystal of acid potassium sulphate, and heat. A pungent vapour of acrolein[1] is given off, which blackens a piece of filter-paper which has been dipped in ammoniacal silver nitrate solution, thus demonstrating that the vapour acts as a powerful reducing agent.

Emulsification.—When oil is mixed with water it floats to the surface, but when a soap is present in solution in the water the oil

[1] Acrolein is the aldehyde of allyl alcohol and has the formula $CH_2=CH-CHO$.

globules remain suspended, and an emulsion results. This is more permanent if some suspending medium such as mucilage be added.

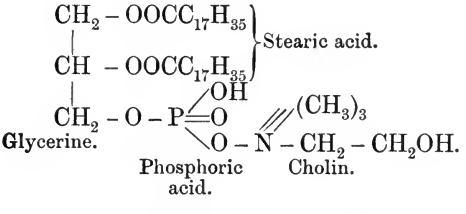
EXPERIMENT V. In one test tube (*a*) place some soap solution; in another (*b*) some water. To each add some neutral olive oil and shake. Allow to stand, and note that *a* remains emulsified, *b* does not.

EXPERIMENT VI. Place some rancid oil (*i.e.* containing free fatty acid) in a test tube—add some weak KOH solution and shake; an emulsion forms, soap being formed by the alkali combining with the fatty acid.

EXPERIMENT VII. Divide the emulsion produced in Experiment VI. into two; to one of these add a little mucilage or egg-albumin and shake, and note that the emulsion '*stands*' much longer than that to which no suspending medium has been added.

Lecithin.—This body is found chiefly in nervous matter, in the stroma of red blood corpuscles, and in bile. Chemically it consists of a molecule of glycerine, two of the hydroxyl groups of which are combined with fatty acid, and the third with phosphoric acid, which on the other hand has attached to it a nitrogen containing body called *cholin*.

Thus:—



This cholin is a poisonous alkaloid, and is broken off from lecithin during digestion, but is at once destroyed by the intestinal bacteria, the substances thus produced being methane, carbonic acid, and ammonia.

Lecithin has all the solubilities of fat, but is identified by its ash containing phosphorus, and by the presence of cholin (see Advanced Course, p. 434). Since it contains glycerine it will give the acrolein reaction.

Cholesterin.—This is found in most tissues, but especially in the bile and red blood-corpuscles. It is chemically of the nature of a monatomic alcohol containing twenty-six carbon atoms. Except that it is soluble in the same solvents as are fats it does not give any of the above reactions. It has, however, certain characteristic reactions of its own, and these are as follows:—

EXPERIMENT VIII. Some cholesterin crystals are given round—

place them on a microscopic slide and break them up with a glass rod, and examine under the microscope, or, better, dissolve some in absolute alcohol, place a drop of this on a slide, and allow it to evaporate. The crystals are colourless, glancing rhombic plates having usually a square piece removed from one corner (Fig. 141).

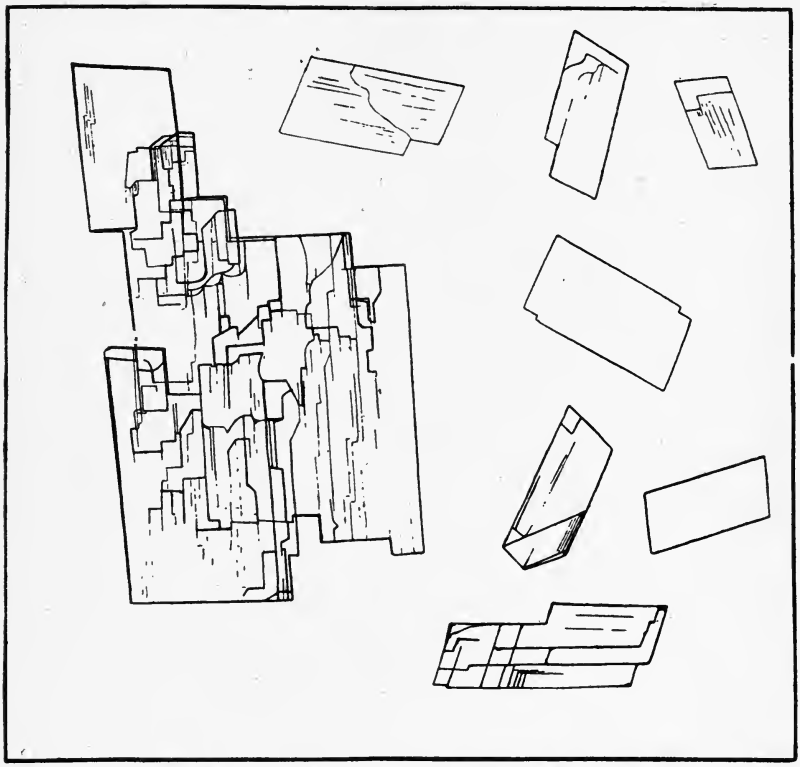


FIG. 141.—Crystals of cholesterin magnified 300 diameters.

EXPERIMENT IX. Place a drop of 20 per cent. sulphuric acid on the crystals and notice that the edges gradually become red.

EXPERIMENT X. To another specimen add some iodine solution and then a drop of concentrated sulphuric acid—a play of red, blue, and green colours results.

Although cholesterin is not a fat, it is studied with this group because it possesses the same solubilities, and is, therefore, sometimes present in ethereal extracts.

CHAPTER VI.

MILK.

MILK consists of a watery solution of various proteids, a carbohydrate and salts, containing in suspension emulsified fat.

The percentage composition varies somewhat in different animals. The more quickly the young animal grows the higher is the percentage of proteid and salts in the mother's milk. Thus a puppy doubles its weight in eight days, and bitches' milk contains 7·1 per cent. proteid and 1·3 per cent. ash. On the other hand, a child takes one hundred and eighty days to double its weight, and human milk contains only 1·6 per cent. proteid and 0·2 per cent. ash. It forms a perfect food for the young growing animal, but as time goes on it requires to be supplemented by other food stuffs in order that the tissues may be properly developed.

From a medical point of view the two kinds of greatest importance are cow's milk and human milk. These differ from one another only in their quantitative composition:

	Proteid.	Fat.	Carbohydrate.	Salts.
Cow's milk, -	3·5	3·7	4·9	·7
Human milk, -	1·7	3·4	6·2	·23

(*Bunge*).

The amount of fat and carbohydrate is nearly the same in both, there being, however, twice as much proteid and nearly three times as much salts in cow's as in human milk. To bring cow's milk to the same composition as human milk it is therefore necessary to dilute the former with an equal bulk of water, at the same time adding some fat and carbohydrate.

In order to study the chemistry of milk, we usually employ cow's milk because it is easily obtainable.

Cow's Milk.—This is an opalescent solution, possessing a characteristic taste, and of amphoteric reaction.

EXPERIMENT I. Place a drop of fresh milk on a piece of red litmus paper, and wash it off with distilled water; a blue stain is left: if the drop be placed on blue litmus, a red stain is left. This peculiar reaction is due to the fact that milk contains a mixture of acid and alkaline salts. By ascertaining how much decinormal acid or alkali[1] are

[1] A normal acid is one in which the amount of acid in 1000 c.c. corresponds to its molecular weight. A normal solution of HCl would therefore contain $1+35{\cdot}5=36{\cdot}5$ gr. HCl in 1000 c.c. water. A decinormal $\left(\frac{n}{10}\right)$ solution would contain 3·65 gr. HCl. A normal alkaline solution is prepared in a similar way.

required to produce neutralisation with the aid of different indicators the amounts of each of these kinds of salt can be determined. (See Titration Methods, p. 245.)

The *specific gravity* of fresh milk varies between 1·028 and 1·0345. The more fat (*i.e.* cream) the milk contains the lower is the specific gravity.

Experiment II. Estimate by a hydrometer (p. 240) the specific gravity (*a*) in skimmed milk and (*b*) in fresh milk. In the former it is about 1·0345, in the latter 1·028. By adding water to (*a*) the specific gravity obviously falls, and by removing the cream from (*b*) it rises. In dairy hygiene, a rough estimate of the richness of milk in cream is obtained by ascertaining its specific gravity, but it is clear from the above experiment that some of the cream can be removed and the consequent rise in specific gravity masked by the addition of water. This fraudulent trick of dairymen must, therefore, be borne in mind before giving an opinion of the quality of the milk.

Fresh milk does not coagulate on boiling, but a skin forms on its surface. A similar skin is produced when any emulsion containing proteid is boiled, and in the case of milk it is composed chiefly of caseinogen entangling some fat globules.[1] Its formation is due to drying of the proteid at the surface of the milk.

The Chemical Constituents of Milk.

I. Proteids.—The chief proteid of milk is a pseudo-nuclein called *Caseinogen*. This can be precipitated by adding to the diluted milk a weak acid, or by saturating it with a neutral salt.

Experiment III. Place about five cubic centimetres of skimmed milk in a test tube, and dilute with an equal bulk of water. To this diluted milk add, drop by drop, a weak solution of acetic acid; a precipitate of caseinogen, entangling fat, falls down. Filter off this precipitate and wash it with water. Now add to it a weak solution of Na_2CO_3; the precipitate dissolves, and an opalescent solution of caseinogen, still however containing some fat, passes through the filter. By repeated reprecipitation and filtration comparatively pure caseinogen can be obtained, from which the last traces of fat can be removed by treating with ether.

The chief property of caseinogen is its power to clot when treated with *rennin* (a ferment contained in gastric juice) in the presence of soluble calcium salts.

[1] An emulsion of cod-liver oil in diluted blood-serum is given round; warm it to about 50° C., and a skin will form on the surface. Be careful not to heat above 50° C., as then coagulation of the proteids will be produced.

Experiment IV. Take a pure solution of caseinogen. Divide it into two portions *a* and *b*. To both add about ten drops of rennin ferment. To *b* add also a few drops of a 5 per cent. solution of calcium chloride. Place both in the water bath at 40° C.; after about five minutes examine to see if clotting has occurred. It will be found that clotting has occurred in *b* where both rennin and soluble Ca salts were present.

Experiment V. Repeat the experiment with two samples of fresh milk *a* and *b*. To *b* add, instead of $CaCl_2$, some potassium oxalate solution. In this case only *a* will clot. The potassium oxalate has robbed the portion *b* of its soluble calcium salts. It can be made to clot by adding calcium chloride solution.

From these experiments we see, therefore, that the clotting of milk depends on the transformation of a soluble proteid, *caseinogen*, into an insoluble derivative, *casein*, by means of a ferment, *rennin*; and, further, that the presence of *soluble calcium salts* is necessary for this process.

It is probable that there are two stages in this transformation: the rennin first splits the caseinogen into two bodies, the more important being soluble casein, which then combines with Ca salts to form caseinate of calcium or casein, while the other passes into solution in the whey as whey-proteid (see Advanced Course, p. 436).

Caseinogen

Whey-proteid (goes into solution).

Soluble casein + soluble Ca salts = caseinate of calcium or *casein*.

The casein thus produced entangles the emulsified fat globules of the milk, and forms the *curd* of milk. This curd shrinks after a few hours, and an opalescent fluid is squeezed out of it called *whey*. The whey contains, besides whey-proteid, traces of other proteids and also the carbohydrate (lactose) and salts of milk.

Experiment VI. Apply the xanthoproteic reaction to some whey: a positive result is obtained. Acidify some of the whey with acetic acid and boil; the proteid is coagulated. The proteids are called lacto-albumin and lacto-globulin.

II. The Carbohydrate is Lactose.

Experiment VII. Boil some whey which has been weakly acidified with acetic acid. Filter off the coagulated proteids. To the filtrate apply Trommer's or Fehling's test; reduction is effected.

Lactose does not, like dextrose, readily ferment with yeast, but it is

capable of undergoing a special fermentation, which changes it into lactic acid. This is called the lactic acid fermentation. It depends on the presence of a microbe, the Bacterium lactis. It occurs in two stages as follows:—

$$\underset{\text{Lactose.}}{C_{12}H_{22}O_{11}} + H_2O = 4\underset{\text{Lactic acid.}}{CH_3 - CHOH - COOH}.$$

Some of the lactic acid is then further split up into butyric acid.

$$2CH_3 - CHOH - COOH = \underset{\text{Butyric acid.}}{CH_3 - CH_2 - CH_2 - COOH} + 2CO_2 + 2H_2.$$

The presence of these free acids in the milk leads to the *precipitation* of caseinogen, and this explains the production of the *curd* in sour milk. It is quite a different thing from the *curd* which is produced by rennin. Thus it can be dissolved by means of a weak alkali, and if rennin be added to the resulting solution true clotting will follow.

Experiment VIII. Take some sour whey. Add a few drops of it to Uffelmann's reagent,[1] when the dark purple colour of the latter will be changed to yellow. Test for lactic acid (see p. 207).

III. The **salts of milk** are chiefly phosphates and chlorides of the alkalies and alkaline earths. A trace—0·00035 per cent.—of iron is also present.

Experiment IX. The Detection of P_2O_5.—Add to five cubic centimetres of proteid-free whey half its bulk of nitric acid and about twice its bulk of a solution of molybdate of ammonia in nitric acid. Warm gently on the water bath, and a yellow precipitate of *phosphate* forms.

Experiment X. The Detection of Calcium Salts.—To some whey, freed from proteid by boiling, add a few drops of a solution of potassium oxalate—a white haze of calcium oxalate results.

IV. The Fats of Milk.—Examine a thin film of milk under the microscope, and note that the fat consists of small spherical bodies, which are transparent and do not adhere to one another.

The fat can be removed by shaking the milk with ether after the addition to it of a few drops of weak NaOH solution.

Experiment XI. To about five cubic centimetres of milk in a test tube add two drops of caustic potash (20 per cent.), and then about five cubic centimetres of ether. Cover the top of the tube with the thumb and shake the mixture, occasionally lifting the thumb slightly to allow the vapour of ether to escape. The ether will dissolve the fat, and the milk will become much less opaque. By adding alkali, a certain amount of the caseinogen is changed in its physical condition, so that the caseinogen molecules, which lie

[1] This reagent is made by adding a trace of ferric chloride to a 1 per cent. solution of carbolic acid.

between and thereby hold apart the fat globules, are diminished, and consequently the fat globules are dissolved by ether. So long as they are surrounded by caseinogen molecules they are not acted on by ether. Not only alkalies, but also acids can effect this change.

When the milk stands for some time, the fats, being specifically lighter, rise to the surface to form the *cream*, and if this be mechanically agitated it solidifies to form *butter*. Analysis of an ethereal extract of milk shows that the fats are olein 40 per cent., palmitin 33 per cent., stearin 16 per cent., and about 7 per cent. of lower fatty acids such as butyrin. There are minute traces of lecithin and cholesterin.

Colostrum.—The milk which first appears during lactation is yellower in colour and of higher specific gravity than that secreted later. On boiling, it yields a distinct coagulum of albumin and globulin, and if examined under the microscope it will be found to contain numerous cells—*colostrum corpuscles*—in the protoplasm of which fat globules are present. These cells are, in reality, secretory cells of the mammary glands which have been extruded in the first portions of milk.

CHAPTER VII.

BLOOD.

By means of the blood the food-stuffs, absorbed from the intestine, and the oxygen, absorbed from the lungs, are carried into the tissues, where a complex chemical process takes place, resulting in the production of tissue-energy, which may be in the shape of muscular movement, heat, glandular activity, etc. In this chemical process the complex food-stuffs are probably first of all decomposed, the resulting substances being then partially oxidised, so that the ultimate effete products pass into the venous blood, partly in a fully oxidised state (CO_2 and H_2O), partly unoxidised (lactic acid, precursors of urea).

Structurally, blood consists of minute solid discs called *corpuscles*, suspended in a clear fluid called the *plasma*.

When the blood leaves the blood-vessels it is a red opaque fluid of peculiar odour and alkaline in reaction. It soon undergoes a change however, in that it solidifies, or *clots*. If this clot be left standing some time a clear straw-coloured fluid—*the serum*—begins to separate out, which gradually increases in amount, so that, after some time, the clot floats free as a compact dark red solid mass.

Before proceeding with the chemistry of the blood we must, accordingly, study **the phenomenon of clotting**; and in order to do this the

process must be retarded, so that we may have time to observe the different stages. Moreover, it is inconvenient to study the process in blood as it contains solid elements in suspension, so that we usually separate the plasma for this purpose, and, in order to do this, we must retard clotting.

Since the blood does not clot so long as it is contained in the healthy blood-vessels, we may prevent the process by excising a blood-vessel (such as the jugular vein of the horse), after ligaturing it in two places, so as to retain the blood in it. The excised piece of vein is popularly called a "*living test-tube*," and, since the walls do not die for a considerable period, the blood remains fluid. If the *test-tube* be hung up, the solid elements of the blood (the corpuscles) sink to the bottom, and the fluid portion (the plasma) can be separated from them by means of a pipette. This is a very interesting method, but one which is, of course, impracticable for obtaining large quantities of plasma. For this purpose *certain neutral salts* (magnesium sulphate, sodium sulphate, sodium citrate, etc.) are mixed with the blood to prevent it clotting. The corpuscles and plasma are then separated by allowing the blood to stand, when the corpuscles sink to the bottom of the vessel, or this separation may be hastened by placing the salted blood in a centrifuge. The supernatant plasma—called *salted plasma*—is then removed by a pipette or syphoned off. There are many other methods for preventing clotting, but the description of these we will leave till after the process itself has been studied.

Experiment I. Fresh blood has been mixed with an equal volume of saturated sodium sulphate solution, or with one-quarter its volume of saturated magnesium sulphate solution.[1] The corpuscles have settled to the bottom of the vessel, and the straw-coloured or reddish plasma is pipetted off. Divide this into three portions, and label them *a*, *b*, *c*. To each add ten times its volume of water, so as to remove the action of the salt by dilution. Place *a* in water-bath at 37° C. To *b* add a piece of blood-clot or a few drops of serum. To *c* add a few drops of a 1 per cent. solution of potassium oxalate. Observe that first *b*, then *a*, pass into a jelly-like state—clotting—but that *c* remains fluid.

From this we learn that plasma itself can clot, but that the process is accelerated by adding blood which has already clotted, and is hindered by adding a soluble oxalate. The clotted blood must, therefore, contain an excess of some element necessary for clotting, and since the previous heating of it to about 60° C. robs it of the power of assisting clotting we assume that it contains some ferment—**fibrin**

[1] In order to do this the artery or vein is allowed to bleed into a vessel containing one of these solutions.

ferment (see ferments, p. 214, and for preparation see p. 476). On the other hand, the addition of a soluble oxalate must have removed from the plasma some necessary agency, namely, **soluble calcium salts**. It does this by forming calcium oxalate, which is insoluble. We can prove that this explanation is the correct one by the following experiments:—

Experiment II. To the test-tube *c* in the above experiment add about 5 drops of a 2 per cent. solution of calcium chloride; then replace in water-bath. Clotting now occurs, since soluble calcium salts are present in excess.

Experiment III. Clotting has been prevented in blood by mixing it with one-quarter its volume of a 1 per cent. solution of potassium oxalate in normal saline. Add to a few c.c. of this oxalate blood in a test-tube a few drops of a 2 per cent. solution of $CaCl_2$, and place in water-bath. Clotting occurs.

We see, then, that the plasma must contain some proteid in solution which is rendered insoluble by the action of a ferment acting in the presence of soluble calcium salts. This soluble proteid can be precipitated from the plasma by half saturating with sodium chloride, and it is called **Fibrinogen** (for preparation see p. 476).

Since no fibrin ferment can be prepared from absolutely fresh blood, it must exist there as a precursor, and since the greatest yield of it is obtained from that portion of the clot containing most leucocytes,[1] it must be in these that this precursor exists. The exact chemical nature of this substance is unknown, but there is much evidence to show that it is of the nature of nucleo-proteid. Fibrin ferment is sometimes called **thrombin**, and the precursor **pro-thrombin**, and it is supposed that, when the latter changes into the former, calcium salts are added to it.

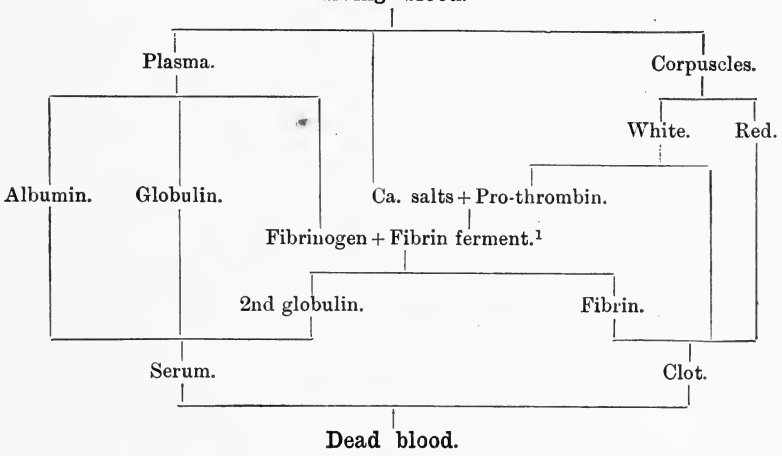
To sum up, therefore, the process of *clotting begins by the leucocytes disintegrating and liberating pro-thrombin, which immediately combines with soluble calcium salts to form thrombin. This thrombin then acts on fibrinogen and splits it into two bodies, the one, unimportant, remains in solution* (Hammarsten's secondary globulin), *and the other solidifies and forms fibrin.*[2] This fibrin is thrown down as fine threads which cross one another in all directions, forming a meshwork in which the corpuscles become entangled when the process occurs in unseparated blood. If fresh blood be whipped with a bunch of twigs, the strings of fibrin collect on these, and the fibrin can thus be removed from the serum and

[1] *I.e.* from the *buffy coat*, which is the paler upper portion of the clot of bloods, such as horses' blood, which clot slowly.

[2] It has been supposed that the thrombin transfers its calcium salts to this fraction of the fibrinogen molecule, and that fibrin is really *fibrinate of calcium.*

corpuscles. Such blood is called *defibrinated or whipped blood*, and cannot be made to clot by any means whatsoever, because it has already clotted in the process of whipping. Besides containing fibrinogen, the plasma also contains *albumin* and *globulin*, which remain unchanged during clotting and pass into the serum (see proteids).

The following schema exemplifies the process:—



Conditions which retard Clotting.—(1) *Cold*—receive the blood into a vessel placed in ice (*i.e.* keep it at a temperature a little above freezing point).

(2) *Contact with blood-vessel wall*—"Living test tube."

(3) Addition of *certain neutral* salts—"Salted plasma."

(4) Addition of *an oxalate*—"Oxalate plasma."

(5) Addition of *leech extract*—This is a secretion produced by the salivary glands of the leech, and which can be obtained by extracting the heads with water.

(6) *Contact with oil.*—Receive the blood into a smooth vessel smeared with oil. The oil forms a layer on the surface.

(7) *Intra-vitam methods.*—These consist in injecting certain substances into the blood-vessels of the animal before bleeding it. They seem to act by depriving the blood of some of the factors necessary for clotting, but their exact action is not understood. These substances are:—

(α) Commercial peptone, which consists mainly of proteoses.

(β) Soap solution.

(γ) A weak alkaline solution of nucleo-proteid injected slowly—"*negative phase*" of nucleo-proteid injection.

[1] The excess of fibrin ferment passes into the serum.

Conditions which hasten Clotting.—(1) *Body temperature.*

(2) *The addition of some clotted blood* (clot or serum).

(3) *Agitation*, *e.g.*, whipping the blood with a bunch of twigs.

(4) Contact with a *rough surface.*

(5) *Intra-vitam methods.* Certain conditions cause the blood to clot within the blood-vessels. These are:—

(α) Injury or death of the blood-vessel wall, *e.g.*, when an artery is crushed, as in a contused or lacerated wound, a clot forms which acts as a natural plug to prevent haemorrhage. When the arterial wall undergoes degeneration, a clot, or *thrombus* may form.

(β) Rapid injection into a vein of a strong alkaline solution of nucleo-proteid—"*positive phase*" of nucleo-proteid injection.

The Chemistry of the Leucocytes.

These are morphologically the same as other cells, and they contain the same chemical substances. The *protoplasm* consists mainly of water. The solids consist of various proteids, which chiefly belong to the group of compound proteids (gluco-proteids and nucleo-proteids), and there is also a small amount of albumin and globulin. The protoplasm may also contain such substances as glycogen, fat, mucin, etc., which have either been produced by the activity of the protoplasm, or which are simply deposited in the cell for storage purposes.

The *nucleus* seems to consist mainly of nucleo-proteids, nuclein and nucleic acid. The nucleo-proteid of the nucleus is said to contain a higher percentage of phosphorus than does that of the protoplasm.

The Haemocytes or Red Blood Corpuscles.

Structurally these are said to consist of a **stroma** containing in its meshes a peculiar proteid called **Haemoglobin.** It is, however, impossible to demonstrate this stroma histologically, and the whole question of the structure of haemocytes seems shrouded in mystery.

Chemically they contain about 60 per cent. of water and nearly 36 per cent. of haemoglobin, the remaining 4 per cent.—represented by the so-called stroma—consisting of lecithin, cholesterin and nucleo-proteid.

Haemoglobin.—This is a compound proteid containing 0·4 per cent. of iron. When decomposed by acids or alkalies it splits up into a proteid of the nature of a histon (see p. 178) called **globin** and into a pigment called **haematin**, which contains all the iron. A pure solution of haemoglobin can be obtained by centrifugalising defibrinated blood,[1]

[1] Horses' blood should be used for this purpose as the corpuscles sink more quickly than the corpuscles of any other blood do.

removing the serum with a pipette, shaking up the corpuscles with a 0·9 per cent. sodium chloride solution[1] (which is nearly *isotonic* for the blood of the ox, horse, or man), and again centrifugalising.

By this means the corpuscles are thoroughly washed free of serum, etc. They are then collected and treated with two or three times their bulk of distilled water, in which the haemoglobin dissolves, a deep red solution resulting.

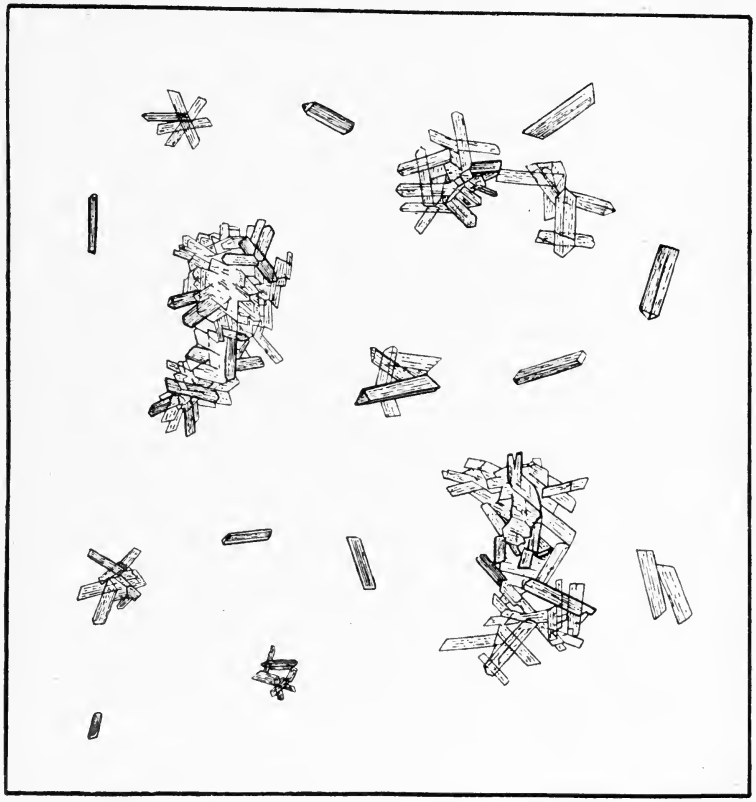


FIG. 142.—Haemoglobin crystals. × 800.

The solution gives several of the ordinary proteid reactions, but in each case a splitting into proteid and haematin simultaneously ensues.

EXPERIMENT IV. Heat carefully some haemoglobin solution. It decomposes at about 60° C., and the proteid coagulates on further heating.

[1] A salt solution of this strength has the same osmotic pressure as the contents of the red blood corpuscle, and consequently no swelling or crenation of the corpuscle is produced.

It exists in two conditions, viz., as haemoglobin and as oxyhaemoglobin, and these differ from one another in that oxyhaemoglobin contains loosely combined oxygen, whereas haemoglobin only contains the firmly combined oxygen of the proteid molecule. Unlike other proteids haemoglobin can easily be obtained as crystals.

EXPERIMENT V. Mix a drop of rat's blood with a drop of distilled water on a slide: place a cover slip over it: as evaporation slowly proceeds

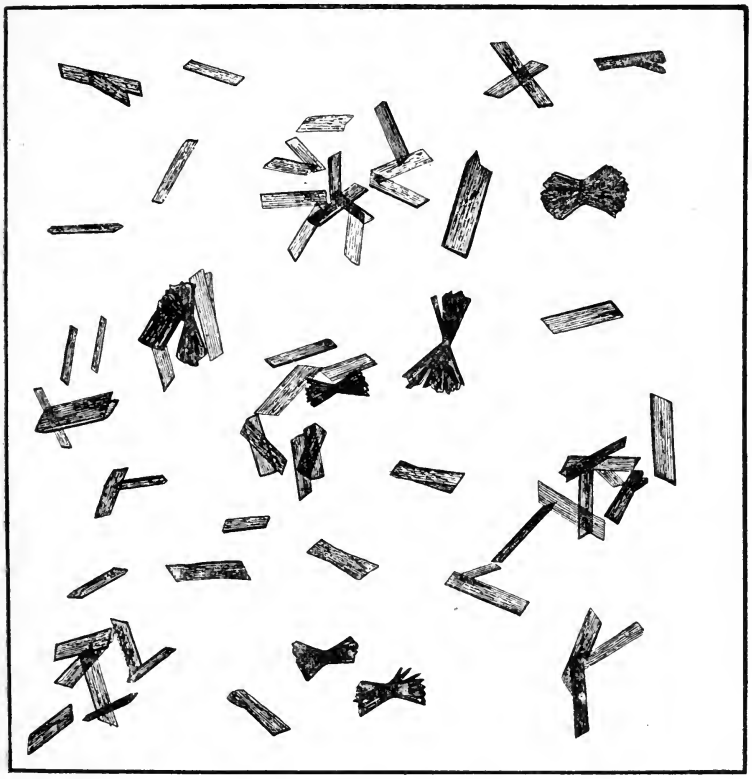


FIG. 143.—Haemin. × 1500.

the haemoglobin, which has been dissolved out of the corpuscles by the water, will crystallise out. These crystals will of course be first seen at the edge of the preparation. The crystals may be permanently mounted by allowing complete crystallisation to take place, then removing the cover slip and mounting in Canada Balsam. The crystals are orange-red in colour, and usually assume the form of rhombic prisms (see Fig. 142).

Of the compounds of haemoglobin the most important from a medico-

legal point of view is **Haemin**. Chemically it is hydrochloride of haematin, and is obtained by decomposing haemoglobin by means of an acid in the presence of free hydrochloric acid. It is important because it forms very characteristic crystals, and can be obtained with no great difficulty even from blood stains several months old. It is, therefore, a useful medico-legal test for blood.

EXPERIMENT VI. Smear a small drop of blood on a slide and allow to dry. Place a cover slip over it, and then run some glacial acetic acid underneath the cover slip. Warm till small bubbles begin to appear, cool, examine for haemin crystals. These are small dark-brown elongated rhombic crystals, usually collected into star-shaped or rosette-like groups (see Fig. 143). In this preparation the sodium chloride of the blood reacts with the acetic acid and hydrochloric acid is liberated, which then combines with the haematin simultaneously produced from the decomposition of haemoglobin. If it be desired to do the test with an old blood stain it is necessary to supply a crystal of sodium chloride.

EXPERIMENT VII. A piece of rag which has some time previously been soaked in blood is given round. Prepare haemin crystals from it by placing a piece of it the size of a split pea on a slide, adding two or three drops of glacial acetic acid and one crystal of common salt, covering with a cover slip, and then warming till bubbles of gas arise. On cooling, crystals of haemin will be seen.

The other compounds and derivatives of haemoglobin will be considered in connection with the spectroscope since it is by means of their spectra that they are identified.

CHAPTER VIII.

THE SPECTROSCOPIC EXAMINATION OF HAEMOGLOBIN AND ITS DERIVATIVES.

A SPECTROSCOPE consists essentially of a screen, in which there is a small *slit*, through which light from any desired source can pass, a *prism*, and a series of lenses forming the *telescope*, through which the observer looks.

For qualitative work the small direct vision spectroscope (Fig. 144 A) is serviceable. When the position of the bands, however, is required, one of the larger compound forms is necessary.

Adjustment of the Spectroscope.—It is necessary to have an exact focus of the image of the slit. In the *small direct vision spectroscope* this

may be obtained by directing the instrument towards a white cloud, and moving the eye-piece till the various Fraunhofer lines are clearly defined, or, in absence of daylight, obtaining a clear image of the upper and lower edges of the slit, *i.e.* of the upper and lower edges of the spectrum. The slit should not be too widely open. If the source of light include a sodium flame, a clear image of the D-line will be obtained when the slit is in focus.

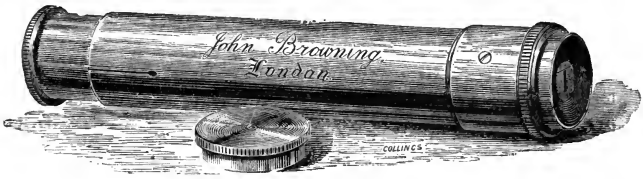


FIG. 144 A.

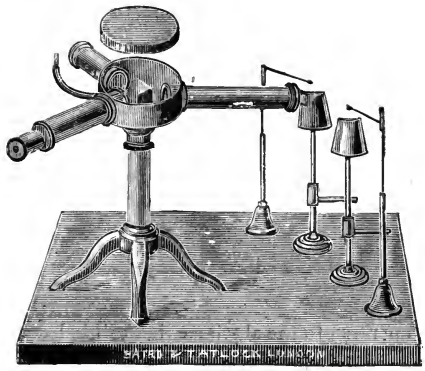


FIG. 144 B.

FIG. 144.—Spectroscopes. A, Small direct vision spectroscope. B, Compound spectroscope.

In the larger forms of spectroscope, three tubes are generally found radiating from the central prism or prisms[1] (Fig. 144B). One of these has its end blocked by a screen, in which there is a slit where width can be varied by a small screw. Attached near to the slit there is generally a small prism, which can be moved so as to cover half the slit, and affords the means of introducing a second source of light into the instrument. The tube, at the end of which is the slit, is called the *collimator* tube, and contains a lens so that the image of the slit can be brought to bear on one face of the prism. The distance of the slit from the lens is variable, and should be adjusted so that the rays issue from tube into the prism as parallel rays. After refraction through the prism they are

[1] For studying absorption spectra, the two-prism form with its greater dispersion of the spectrum is less well adapted.

collected by a second tube, *the telescope*, and the eye-piece of this should be arranged to receive parallel rays from the surface of the prism. The eye-piece is frequently fitted with cross wires, if so, the eye-piece should be first adjusted so that they are seen distinctly.

It will be found that the telescope has some lateral movement so that the vertical cross-wire can be made to coincide with any required part of the spectrum.

The third tube will contain a small scale which can be focussed on to the surface of the prism and will then become reflected along the axis of the telescope. It will be necessary first to adjust the movable end of the scale-tube so that the scale is in focus, and it may be necessary to move it laterally so that the scale is brought into the field of the telescope.

The scale must remain in a fixed position during any series of observations.

Construction of a chart for determining wave-lengths of bands on the spectrum. With the scale in fixed position, notice the position on the scale of the sodium line, and the lines observable when the sodium flame is replaced by one coloured with a salt of strontium, calcium, lithium, barium, caesium, and potassium. Take observations of the positions of about twelve of these lines, the wave-lengths of which are known. Obtain the values corresponding to these wave-lengths, and on a piece of paper, ruled in squares, plot out their position, regarding the abscissae as degrees of the scale and the ordinates as wave-lengths. Draw a curve through the several points. The wave-length of any part of the spectrum can now be ascertained. Observe where such a part intersects the scale, follow the ordinate corresponding to the degree of the scale to the point of intersection with the curve, and a line parallel to the abscissae line will indicate the wave-length.

Having arranged the spectroscope so that the scale is illuminated and visible through the eye-piece, and the slit is illuminated by a light (an argand or incandescent burner) placed about one foot off; notice the position of the D-line on the scale. If sodium chloride be sprinkled into the illuminating flame, the D-line will be manifest, but a better method is to arrange between the illuminating flame and the slit a Bunsen flame in which asbestos soaked with a strong solution of sodium chloride is placed.

A piece of glass tubing about two feet long may be taken, which has the lower six inches bent back to form an angle of 60° with the main stem. This is filled with 6 p.c. solution of sodium chloride. The short arm is then plugged with asbestos. At the end of the long arm is a short piece of rubber tubing clamped fairly tightly. This tube is held by a burette clamp, and the projecting asbestos can be allowed to just touch the Bunsen flame. In this manner a constant D-line is furnished.

1. **The visible Spectrum of Oxyhaemoglobin.**—Take some defibrinated blood which has been thoroughly shaken with air, and dilute

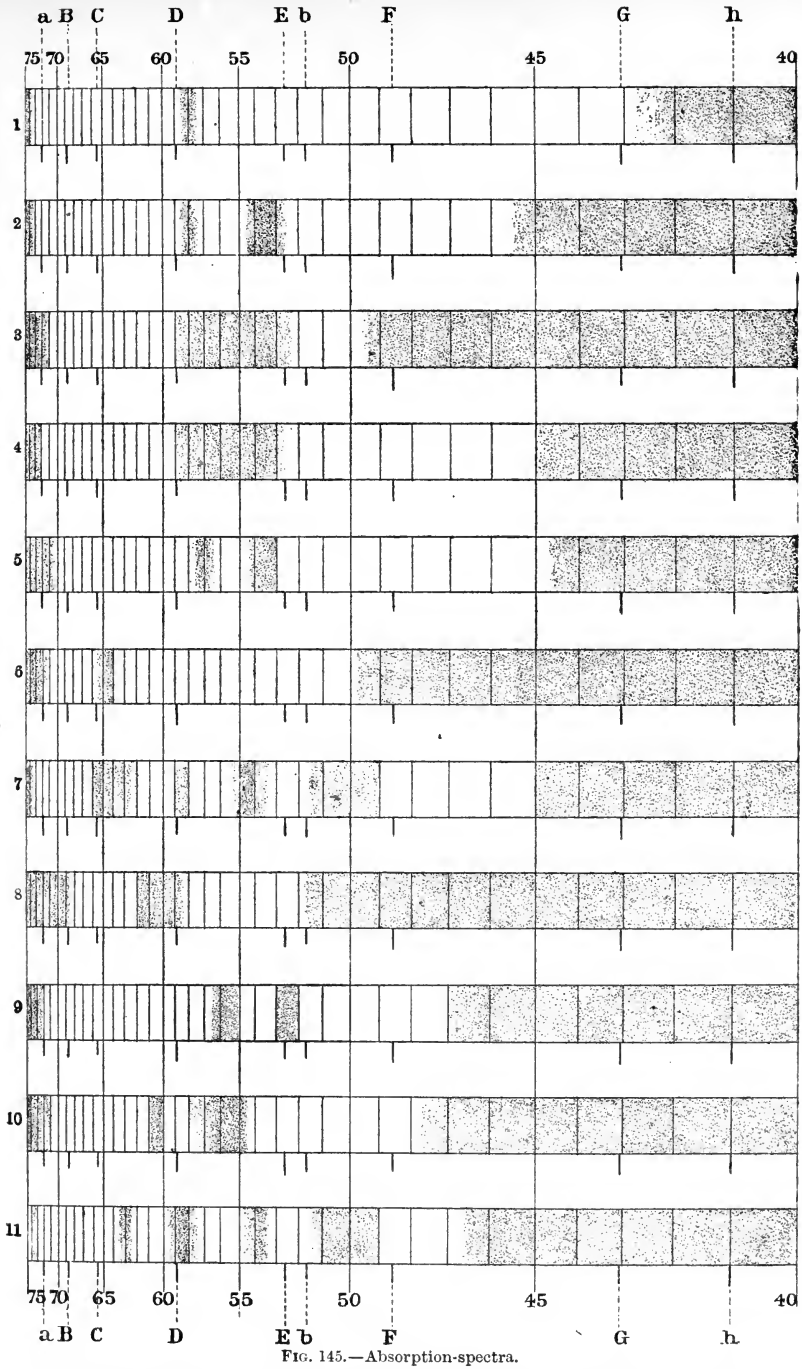


FIG. 145.—Absorption-spectra.

1, Oxyhaemoglobin (very weak solution); 2, Oxyhaemoglobin (weak solution); 3, Oxyhaemoglobin (strong solution); 4, Haemoglobin (reduced haemoglobin); 5, Carbonmonoxide haemoglobin; 6, Acid haematin; 7, Acid haematin (ethereal extract); 8, Alkaline haematin; 9, Haemochromogen (reduced haematin); 10, Haematoporphyrin (acid solution); 11, Haematoporphyrin (alkaline solution).

it with about ten times its volume of water. Place some of this behind the slit of the spectroscope, preferably in a flat-sided vessel about 1 cm. thick, but a test-tube will answer fairly well. It will be noticed that the whole of spectrum is blocked out except a portion of the red end.

Dilute this solution carefully. At a certain stage some of the green will be evident (see Spectrum 3 in Chart), there being a wide absorption band between the red and green. On diluting still further, this wide absorption band will resolve itself into two bands (Spectrum 2). These two bands are both on the blue side of the D-line, and their centres correspond to λ 579 and λ 543·8. Note carefully the position of these centres on the scale and the width of the bands. Observe also the limits of the visible spectrum at the red and blue ends.

On diluting still further it may be possible to cause the band on the blue side to disappear, whilst the band on red side is still just appreciable (Spectrum 1).

2. **The visible Spectrum of Haemoglobin (reduced Haemoglobin).**—If some diluted defibrinated blood be left standing undisturbed for 24 hours, the oxyhaemoglobin will lose its oxygen. This result may be arrived at more rapidly by treating some diluted defibrinated blood which shows fairly wide oxyhaemoglobin bands with a reducing reagent, such as ammonium sulphide or Stokes' reducing fluid.[1] If ammonium sulphide be used, the mixture should be warmed. It will now be noticed that the blood loses its bright scarlet appearance and becomes more purple in tint. Examine this by the spectroscope, and it will be found that the two bands of oxyhaemoglobin have disappeared, and are replaced by one band, the centre of which is between the two bands of oxyhaemoglobin. The band is a broad one, shading off more gradually on the red side, and the darkest part corresponds in wave-length to λ 550 (Spectrum 4 in Chart).

3. **The visible Spectrum of Carbon-Monoxide Haemoglobin.**—If a stream of carbon monoxide, or even of coal-gas, be passed through some diluted defibrinated blood, the scarlet tint is changed to a carmine or cherry colour. The oxygen is replaced by carbon monoxide. Examined spectroscopically the blood shows two bands differing from those of oxyhaemoglobin in being slightly shifted towards the blue end. The two bands have centres corresponding in wave-length to λ 575 and λ 540 approximately (Spectrum 5).

The proportion of red and blue unabsorbed at the ends of the spectrum is different in oxyhaemoglobin and CO-haemoglobin, there being more

[1] 2 gms. of ferrous sulphate are dissolved with 3 gms. tartaric acid in 100 cc. of water. Ammonia is added till the solution is alkaline. Stokes' fluid must be freshly prepared.

blue unabsorbed in CO-haemoglobin than in the former. Hence, comparing dilute solutions of similar strength in test tubes of the same diameter, the CO-haemoglobin has a distinct bluish tinge, contrasting markedly with the yellowish-red of the oxyhaemoglobin. This difference of end-absorption can be best shown as follows: Take a fairly dilute solution of oxyhaemoglobin showing the two characteristic bands clearly, but not strong enough to produce any intermediate shading. Note as carefully as possible where the red and blue are first visible. Pass a stream of coal gas or carbon monoxide through the solution by means of a fine nozzle for two or three minutes. Note the change on colour produced, and again examine the spectrum. It will now be found that rather more of the blue is visible, whilst the red is unaltered or slightly more absorbed.

An important difference between oxyhaemoglobin and CO-haemoglobin is seen in the effect of reducing reagents. If CO-haemoglobin be treated with Stokes' fluid or ammonium sulphide, it is unchanged.

4. **The visible Spectrum of Methaemoglobin.**—To a solution of oxyhaemoglobin, in which the two bands are so wide as to partially overlap, add a few drops of a strong solution of potassium ferricyanide. The colour changes to a chocolate tint. If this be spectroscopically examined, a distinct band is seen on the red side of the D-line, the wave-length of its centre being about λ 635 (Spectrum 12). On diluting the solution down, other bands may be seen—one just on the blue side of the D-line (λ 581), another still further towards the blue (λ 540), and a fourth may be made out on the bluish-green (λ 500) (Spectra 13 and 14). The two middle bands are probably not due to any traces of oxyhaemoglobin, but are characteristic of methaemoglobin.

If such a solution of methaemoglobin be treated with ammonium sulphide, a transient spectrum of oxyhaemoglobin may be seen, succeeded by a permanent spectrum of reduced haemoglobin.

If the solution of methaemoglobin be rendered alkaline with ammonia, the colour changes to a more distinct red, and the absorption band in the red disappears and is replaced by a band immediately on the red side of the D-line (Spectrum 15 in Chart).

By the action of nitric oxide on oxyhaemoglobin, a product is formed called **nitric oxide haemoglobin.** This is characterised by two bands, which are between the D and E-lines: the band on the red side is somewhat nearer the red end than the corresponding band of oxyhaemoglobin (Spectrum 16).

If oxyhaemoglobin be treated with a nitrite, as sodium nitrite or

amyl nitrite,[1] there is formed a certain amount of methaemoglobin and a certain amount of nitric oxide haemoglobin. The combination of the two gives a spectrum very similar to simple methaemoglobin (Spectrum 17 in Chart).

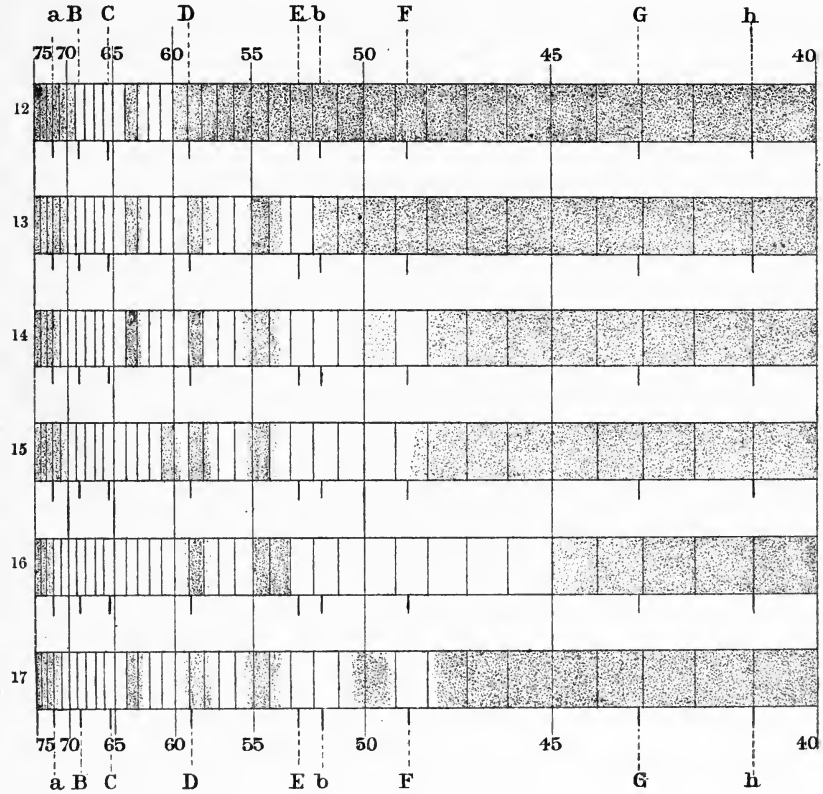


FIG. 146.—Absorption-spectra.

12, Methaemoglobin (strong solution); 13 and 14, Methaemoglobin (weak solutions); 15, Methaemoglobin (alkaline solution); 16, Nitric oxide haemoglobin; 17, Mixture of Methaemoglobin and nitric oxide haemoglobin.

If the product of the action of nitrites be treated with ammonium sulphide, the spectrum passes through a transient oxyhaemoglobin stage, succeeded by reduced haemoglobin, and later becomes nitric oxide haemoglobin.

5. **The visible Spectrum of Acid-haematin.**—If some diluted defibrinated blood be treated with a little glacial acetic acid and gently warmed, it will assume a dark brown colour. If it be diluted suffi-

[1] Care must be taken to avoid excess of amyl nitrite, or so-called photo-methaemoglobin, characterised by one broad band between D and E, will be formed.

ciently, and examined spectroscopically, it will present a spectrum characterised by one band on the red, the wave-length corresponding approximately to λ 645. The blue end of the spectrum will be very largely absorbed (Spectrum 6 in Chart).

There is frequently a considerable amount of general absorption in the acid-haematin prepared as above, and the band referred to may only be made clear by filtering the solution. More satisfactory results are obtained by extracting the colouring matter with ether, or treating with chloroform and excess of acetic acid, as follows :—

(*a*) Take defibrinated blood, and add about half its volume of glacial acetic acid and about an equal quantity of ether. Shake at once. The ether will extract the colouring matter, and, on examining the same spectroscopically, three distinct bands will be seen—one on the red similar to that already described, but apparently shifted nearer the D-line (λ 640), one on the green (λ 550), another on the green but nearer the blue (λ 515). A very indistinct band may be seen on the yellow (λ 590) (Spectrum 7 in Chart).

(*b*) Take defibrinated blood, warm and add half its volume of glacial acetic acid. Cool and add half the volume of chloroform, and more acetic acid if any precipitate appears. The solution will become clear and give a spectrum similar to that shown in the ethereal extract.

6. **The visible Spectrum of Alkaline Haematin.**—Take some diluted defibrinated blood, and add a few drops of strong caustic soda, and warm. The colour will change to a greenish-brown tint, and when the solution is examined spectroscopically, it will show a single band on the orange (wave-length, λ 600). A more satisfactory method of preparing the alkaline haematin is to form a paste of potassium carbonate and defibrinated blood; dry it over a water-bath and extract with alcohol, when a reddish-brown solution is obtained which shows the distinguishing absorption band clearly (Spectrum 8 in Chart).

7. **The visible Spectrum of Haemochromogen (reduced Haematin).**—If a watery solution of alkaline haematin be warmed with a few drops of ammonium sulphide, the brownish colour is replaced by a more marked red. If the solution be examined spectroscopically, the one band of alkaline haematin is found to be replaced by two bands on the green, the wave-lengths of their centres being approximately λ 557 and λ 525 (Spectrum 9).

8. **The visible Spectrum of Haematoporphyrin.**—Take some strong sulphuric acid (10 c.c.) in a test tube and add a few drops of blood, and shake up the mixture. A purple colour will result, due to the decomposition of the haemoglobin and the formation of the iron-free pigment, haematoporphyrin. This examined spectroscopically will, in the above

acid solution, show two bands, the centres being approximately λ 605 and λ 565 (Spectrum 10 in Chart).

If a large excess of water is added to the above a precipitate is thrown down. If this be dissolved in a little caustic soda, a solution of haematoporphyrin in an alkaline medium is obtained, which shows a four-banded spectrum when examined, the positions of the bands being λ 630, λ 580, λ 550, and λ 520 approximately (Spectrum 11 in Chart).

Haematoporphyrin may be regarded as iron-free haematin, and identical in composition with bilirubin. The following equation represents the change brought about by sulphuric acid :—

$$\underset{\text{Haematin.}}{C_{32}H_{30}N_4FeO_3} + 3H_2O = 2(\underset{\text{Haematoporphyrin.}}{C_{16}H_{18}N_2O_3} + Fe).$$

Solutions of haematoporphyrin exhibit a red fluorescence. This pigment must be regarded as normally present in small quantities in urine.

CHAPTER IX.

MUSCLE.

Muscle forms the most abundant tissue in the body. It is here that the food-stuffs undergo combustion, as a result of which energy is liberated and appears either as muscular movement or as heat. The food-stuffs, along with the oxygen necessary for their combustion, are carried to the muscle, and the effete products are removed by the blood going to and coming from the muscle.

Muscle also constitutes one of the commonest food-stuffs, meat being the form in which we take most of our proteid and a large amount of our fat.

We must accordingly study it from two standpoints, firstly as the "combustion" tissue of the body, and secondly as one of the most important food-stuffs.

Chemical Composition of Muscle.

Water,	75 per cent.
Proteids,	20–21 ,,
Organic Extractives,	0·3–·4 ,,
Fat,	2–3 ,,
Inorganic salts,	1·0–1·3 ,,

The Proteids.—These may be divided into two classes:—(1) Proteids peculiar to muscle; and (2) Proteids common to muscle and blood.

EXPERIMENT I. A rabbit is killed, and a cannula tied into its aorta, by which the blood-vessels are washed free of blood. The muscles are then removed and quickly passed through the mincing machine. The mince is then mixed with a 5 per cent. solution of magnesium sulphate, the mixture being placed on ice and left standing all night. The resulting extract, strained through muslin, should be prepared beforehand by the demonstrator.

Divide it into two parts, *a* and *b*.

(*a*) Dilute with four volumes of water, and place in the water-bath at body temperature. A clot forms.

(*b*) Add some acetic acid. A precipitate forms. Filter. Neutralise the filtrate with Na_2CO_3 solution, and dilute it with water. Place it in the water-bath, and note that no coagulum forms.

These two experiments show us that the extract contains in solution a substance which is precipitated by acetic acid, and which becomes transformed into an insoluble clot under suitable conditions. This body is proteid in nature, as can be proved by dissolving the clot in (*a*) a 10 per cent. sodium chloride and applying the proteid tests. The soluble body is called *myosinogen*, and the clot *myosin*.

Besides myosinogen the extract contains, however, other proteids.

EXPERIMENT II. Take some of the muscle serum in (*a*), or of the filtrate in (*b*), and half saturate with ammonium sulphate. A precipitate of *globulin* results. Filter off this globulin and test the filtrate for *albumin*.

Organic Extractives.—(For preparation of extract see Advanced Course, p. 439.) These are organic substances, which are soluble in water, and which are not proteid in nature.

These are divided into two classes:—(*α*) **Nitrogenous** and (*β*) **Non-Nitrogenous.**

(*α*) The former group include creatin and alloxuric bodies; **creatine** ($C_4H_9N_3O_2$) is the most abundant extractive in muscle[1] (0·2–0·3 per cent.). Chemically it is very closely related to *urea*, and can be changed into this by boiling with baryta water (see Advanced Course, p. 440). By boiling with dilute mineral acids it loses a molecule of water, and changes into **creatinine**, in which form it appears in the urine. The *total* amount of creatine in the muscles is over *seventy* grammes, but the amount of creatinine in the urine *per diem* is only about *one* gramme. On the other hand, the urine contains about thirty grammes of urea *per diem*, whereas the muscles contain only a trace. These facts, taken in conjunction with the close chemical relationship of creatine to urea,

[1] It crystallises out from the proteid-free muscle extract after this has been evaporated to a syrup (see Fig. 147).

would at once suggest that urea is derived from creatine, but all experiments to prove that such a relationship actually exists have proved entirely futile.

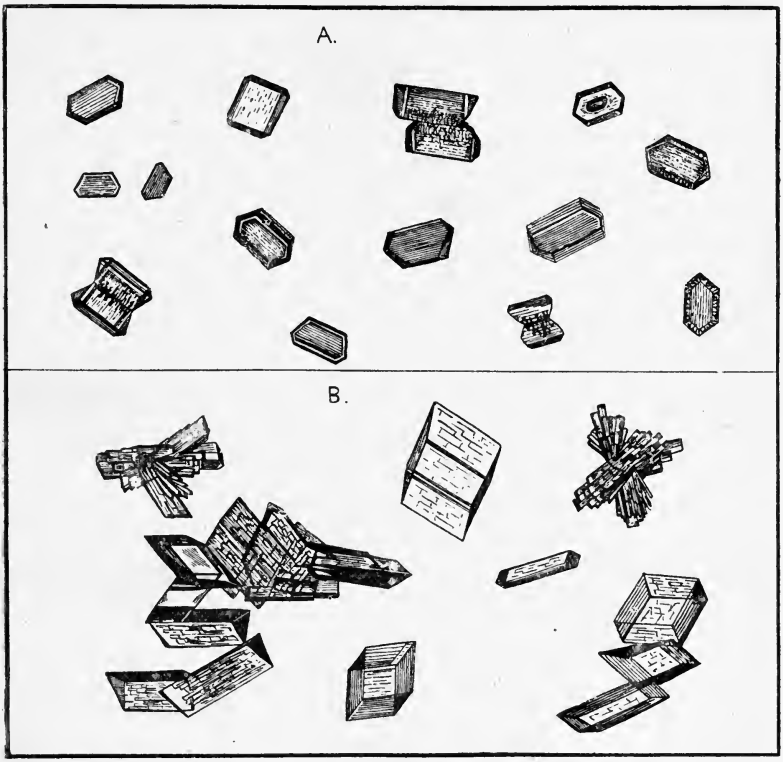
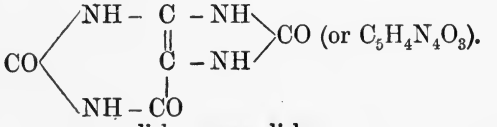


FIG. 147.—Crystals obtained from meat extract, mostly creatine, a few sodium chloride.

The other nitrogenous extractives, **alloxuric bodies**, are scarcely less interesting.[1] They are members of a large group of bodies containing as their basis of construction the so-called *purin ring*, which consists of two urea radicles linked together by a central chain of carbon atoms.

The most familiar member of this group is uric acid[2] which has the formula



[1] They are precipitated from the creatin-free extract by ammoniacal silver nitrate (see p. 441).

[2] Uric acid does not, however, occur in a muscle extract.

If there be only two oxygen atoms present we obtain **Xanthin**, if only one **Hypoxanthin**, and these two are the alloxuric bodies occurring in muscle extracts. They result from the breakdown of nucleins in the muscle. These nucleins contain alloxuric bodies in their molecule, and the latter are excreted in the urine mainly as the more highly oxidised body uric acid. A certain proportion is, however, split up probably into urea, at any rate it is impossible to recover the whole of hypoxanthin as uric acid in the urine when the former is given with the food.

The chief non-nitrogenous extractive is **Lactic acid** ($C_3H_6O_3$). It is extracted from the creatin and purin-free liquor by shaking with ether. After evaporating off the ether an acid syrup is obtained which is neutralised by adding zinc carbonate, this leading to the formation of zinc lactate. Chemically it consists of α-hydroxy-propionic acid, *i.e.* propionic acid, CH_3CH_2-COOH, in which one of the H atoms of the methyl radicle *next* the carboxyl group is replaced by hydroxyl (OH).[1] An acid of exactly the same constitutional formula is obtained by the fermentation of lactose (see carbohydrates, p. 165), and under special conditions of cane sugar. The acid obtained by the fermentation of milk sugar differs from *sarco*-lactic acid, however, in the fact that it does not rotate the plane of polarised light in either direction, whereas sarco-lactic acid rotates it to the right.[2] This different behaviour depends on the exact position of the various side groups in relation to a central carbon atom (see Advanced Course). The test for sarco-lactic acid is the same as for ordinary lactic acid, namely, Uffelmann's reaction (see Milk, p. 188).

Experiment III. Apply Uffelmann's test to the muscle extract.

Another important nitrogen-free extractive is **glycogen** $(C_6H_{10}O_5)n$. The relative amount of this is small (about ·04 per cent.), but it varies in different animals, and is much diminished after muscular activity. Although the percentage is small the *total* amount contained in all the muscles of the body has been found, in the case of the cat at least, to be nearly the same as that contained in the liver.

The less important extractives are: **Urea, carnic acid** ($C_{10}H_{15}N_3O_5$) (which is identical with antipeptone and exists in muscle combined with phosphoric acid as phospho-carnic acid), **dextrose** (trace) **inosinic acid** (also contains phosphorus, but its exact formula is unknown) and **lecithin.**

[1] If it had been in the other methyl radicle that the substitution of OH for H had occurred, the resulting body would have been β-hydroxy-propionic acid.

[2] The lactic acid obtained by the growth of certain bacteria in a solution containing cane sugar is levo-rotatory.

Inorganic Salts.—These consist of salts of the alkalies and alkaline earths. The chief acid radicle present is *phosphoric acid*, and this exists in several states—(*a*) Inorganic phosphates, (*b*) phosphorus of lecithin, (*c*) phosphorus of nuclein, (*d*) phosphorus of phospho-carnic acid, (*e*) phosphorus of inosinic acid. (*f*) Besides these the watery extract contains another phosphorus containing organic compound of unknown composition.

Phosphorus, therefore, seems to be a very important constituent of muscle, and its form of combination changes after muscular work, the organically combined phosphorus being split off as inorganic phosphates which are then washed out of the muscle by the blood, and appear in the urine. It is on this account that the phosphates in the urine are increased after muscular work.

Experiment IV. A watery extract of muscle has been freed of proteids by boiling it. Add to the clear filtrate a few drops of ammonia till faintly alkaline, and then a 10 per cent. solution of calcium chloride. A white precipitate of phosphates results.[1]

CHAPTER X.

FOODS AND THE PRINCIPLES OF DIETETICS.[2]

In the tissues, more especially in the muscles and glands, chemical processes are constantly going on which lead to the production of the energy—thermic and mechanical—necessary for life. These chemical processes consist of the decomposition and oxidation of complex molecules into simpler ones, during which a large amount of potential energy, which had been stored up in the complex molecules, is set free as actual or kinetic energy. The complex substances necessary for this process are supplied by the food-stuffs absorbed from the intestine. It is consequently necessary to supply these bodies to the organism from without, and besides, to supply water, which is required for the circulation of the blood and lymph.

The tissues themselves are also subjected to a considerable amount of tear and wear, and the repair of this is effected by the proteids and inorganic salts, and by these alone.

[1] That this precipitate consists of phosphates can be proved by dissolving in it nitric acid and testing with molybdate of ammonia.

[2] The advanced chapter on this subject is under the heading of Metabolism.

We may, therefore, divide the proximate principles of foods into two groups, viz.:

Tissue formers—Proteid, inorganic salts, water.

Combustion material—Proteid, fats, carbohydrates.

It will be observed that, of the organic food stuffs, proteid may functionate in either capacity, and hence, that a diet containing it alone can serve as an efficient food. That this is so, is proved by the fact that the Indians of the Pampas live entirely on flesh. Without proteid it is impossible to maintain life, but if more than is necessary for the repair of the broken-down tissues be supplied, the excess may serve as fuel.

The most serviceable combustion material seems, however, to be carbohydrate; next comes proteid, and the least available is fat, this latter being pre-eminently the form in which the excess of food over present requirements is laid by for future use. Thus, during summer, hibernating animals store up a large quantity of fat, and this is called upon during the winter sleep to furnish the energy necessary for life.

In judging whether any diet be efficient the first thing we must see to, therefore, is that it contains a sufficient amount and a suitable mixture of the nutritive constituents[1] of food. In practice it is found that these facts can be determined by estimating the amount of *carbon* and of *nitrogen* which the diet contains. We can determine how much of these two elements is necessary, by estimating the amount of them contained in the excreta.

An ordinary man under ordinary circumstances eliminates about 300 grammes of carbon per diem (chiefly as CO_2 in expired air), and about 15 grammes of nitrogen (chiefly as urea, etc., in the urine). Now, the only food-stuff which contains both these elements is proteid, and to supply the required amount of nitrogen it would be necessary to give only about 100 grammes of this. Such an amount would, however, only furnish about one-sixth of the necessary amount of carbon. This difficulty could be surmounted by giving about 600 grammes of proteid, but then the tissues would be supplied with six times more nitrogen than they required. It is advantageous, therefore, to mix with the proteid some food stuff containing an excess of carbon but no nitrogen. Such a food stuff is fat or carbohydrate. Experience teaches us that of these two the more serviceable is carbohydrate, and for two reasons: firstly, because it is more easily digested, and secondly, because it is cheaper.

[1] These nutritive constituents are sometimes called the *proximate principles* of food, because, consisting as they do of carbon, hydrogen, oxygen, and nitrogen combined more or less into highly complex bodies, they are really elementary constituents of the organism.

When muscular work is performed the excretion of carbon rises, whereas that of nitrogen is scarcely affected at all, so that in such cases the diet should contain an excess of carbon.

Another method of determining how much food will be required, is to estimate *how much energy* must be liberated in order to meet the needs of the organism. We can do this by placing the person in a respiration-calorimeter in which all the actual heat which leaves the body is collected and measured. By adding this result to the thermal equivalent of the muscular work which he meanwhile performs, we obtain the total amount of energy eliminated. This result is expressed in calories, a kilo-calorie being the amount of heat necessary to raise the temperature of one kilo of water through one degree centigrade. In this way it is found that about 3,500 kilo-calories are necessary per diem for an adult doing ordinary work.[1]

Having determined how much energy will be required, we must now find out how much food must be supplied to yield it. We can determine the caloric value of the various food-stuffs by burning them in a chemical calorimeter. Since the end products of the combustion of fats and carbohydrates (viz., CO_2 and H_2O) are the same in the body as *in vitro*, their physical caloric values are the same as their physiological, viz., 4·1 for carbohydrates and 9·3 for fats. A very important end product of the metabolism of proteid is, however, urea which still contains some potential energy, so that it has a physical heat-value of its own. In order to find the physiological heat value of proteid, therefore, we must deduct from its physical value the physical value of the amount of urea arising from it. By this means it has been shown that the physiological heat value of proteid is practically the same as that of carbohydrate, viz., 4·1.

By an examination of the diets taken by various classes of people, averages of the relative amounts of the various classes of food stuffs have been obtained. Such a table for a man doing an ordinary amount of work is the following:

Proteid, - - - - - - - - - - -	125 grammes.[2]
Carbohydrate, - - - - - - - - - -	500 grammes.
Fat, - - - - - - - - - - -	50 grammes.

[1] In physical chemistry the unit of heat chosen is one thousand times smaller than the physiological Calorie, it being in this case the amount of heat necessary to raise the temperature of one gramme of water through one degree centigrade. The small calorie is written with a small "c," the large one with a capital "C." *The heat unit can be transformed into units of work* by multiplying by 425·5, a unit of work being expressed as the amount of force necessary to raise a weight of one gramme to a height of one metre—a gramme metre—or of one kilogramme to the same height, a kilogramme metre.

[2] Dryweight.

This diet yields:

	Carbon.	Nitrogen.	Kilo-Calories.
Proteid, - - -	62 gr.	20 gr.	$125 \times 4{\cdot}1 = 512{\cdot}5$ C.
Carbo., - - - -	200 gr.	—	$500 \times 4{\cdot}1 = 2050$ C.
Fat, - - - -	38 gr.	—	$50 \times 9{\cdot}3 = 465$ C.
Total, - - -	300 gr.	20 gr.	3027·5 C.

These facts show us how important it is to know the exact composition of the various food-stuffs, so that we may be in a position to draw up an adequate diet sheet.

The organic food stuffs may conveniently be divided into two classes, the animal and the vegetable.

The Animal Food Stuffs.—The two most important of these, viz. *milk* and *meat*, have been discussed in separate chapters.

Eggs form another important class of animal foods.

Eggs.—A hen's egg weighs about two ounces or fifty grammes. The *shell* forms about 12 per cent. of the total weight, and consists mainly of carbonate of lime. The *white* consists of a multitude of very fine fibrous envelopes filled with a solution of proteid (chiefly egg-albumin, but also traces of egg globulin and egg mucoid), containing traces of sugar (0·5 per cent.), fatty substances, and inorganic salts. About 85 per cent. of the white is water. The *yolk* contains about 51 per cent. of water, the solids being mainly fats (31·75 per cent.), the chief of which is the phosphorised fat lecithin. It also contains about 16 per cent. of proteid, and this is mainly of the nature of a nucleo-proteid called vitellin. The proteids and fats are intimately united with one another in the yolk, the exact nature of the resulting compounds being very little understood.

The yolk contains about 1 per cent. of inorganic salts, and it is important to note that the *phosphorus exists mainly in organic combination* (partly as lecithin and partly as nuclein). The same is true of the iron, which exists in organic combination with nuclein. Both these inorganic bodies are much more easily assimilated when presented to the tissues in organic combination.

The Vegetable Food Stuffs.—One of the most important groups of these is

Cereals.—These are obtained from the seeds of various artificially cultivated grasses, and they all contain representatives of the various

nutritive constituents of foods. The following table gives their general average composition :—

Water,	10-12 per cent.
Proteid,	10-12 per cent.
Carbohydrate,	65-75 per cent.
Fat,	0·5-8 per cent.
Mineral matter,	about 2 per cent.

The more important varieties have the following composition :—

	Water.	Proteid.	Fat.	Carbo-hydrate.	Cellulose.	Ash.
Wheat,	12·0	12·11	1·7	71·2	2·2	1·9
Oats,	10	10·9	4·5	59·1	12·0	3·5
Barley,	12·3	10·1	1·9	69·5	3·8	2·4
Rice,	12	7·2	2	76·8	1·0	1·0

Taken from Hutchison's *Food and the Principles of Dietetics.*

The most important of the cereal foods is wheat, of which it is estimated that 6 bushels per head of population are consumed every year. It is in the form of flour and bread that wheat is mainly consumed.

FLOUR.

Ordinary white flour is obtained from the endosperm of wheat grains and contains from 70 to 75 per cent. of starch, about 8 per cent. of proteid, and about 1 per cent. of fat. The proteid is mainly of the nature of a globulin, and it has the property of becoming viscid when mixed with water. This viscid body is called *gluten*, and it is in virtue of this body that dough is formed when water is added to flour, as in the manufacture of bread.

Experiment I. Roll up some flour loosely in a piece of muslin; place the bag thus formed in a small beaker containing some water and knead it. The starch grains pass through the muslin into the water, so that this soon becomes opaque and a sample placed in a test-tube gives a blue colour with iodine. Apply Trommer's test to another sample and note that no sugar is present. After kneading for some time remove the bag and examine the contents, when it will be found that a sticky mass has been produced—gluten. Remove a piece of this and suspend it in water in a test-tube. Apply Millon's and the xanthoproteic tests to it, and note that the suspended pieces of gluten react positively to both tests.

Grains poor in gluten-forming globulins do not form dough when mixed with water, *e.g.* rice, oats, etc.

Good flour does not contain sugar, and if that be present it shows that a certain amount of germination has occurred.

Whole flour is obtained by crushing the entire grain minus the husk and outer portion of the bran. It contains somewhat more proteid and fat than does white flour and is accordingly more nutritious, but on account of the admixture of bran which it contains, it is less digestible and acts as a mild laxative on the intestine.

BREAD AND BREAD MAKING.

The gluten which is formed when water is added to flour cannot be directly used as a food, for, on account of its soddenness, it is impervious to the digestive juices, and cannot therefore be digested. Before it can be digested it must be aerated, *i.e.* rendered porous, and in this state it forms *bread*. The agency employed to aerate the bread is carbon dioxide gas, which is generated in the mass of gluten or 'dough' by the action of the yeast plant on sugar.

The following is a brief *résumé* of the process:—The first stage in the process consists in preparing an active culture of the yeast plant. This was originally done by allowing dough to stand exposed to the air, when some of the yeast cells, which appear to be omnipresent in the atmosphere, settled on it, and grew and multiplied there until a fermenting mass or '*leaven*' was obtained. Unfortunately for this process, however, the atmosphere contains other bacteria which also settle on the dough, and by their growth lead to the production of organic acids, in consequence of which the mass became very sour. To make bread a little of the leaven was added to fresh dough, in which it grew and multiplied until the whole was leavened, the aerated mass being then heated so as to stop the fermentation. Such bread is very sour, and although the process is still carried out in some parts of Germany, it is almost obsolete.

In *the modern process* the leaven gives way to the so-called *ferment*, which is produced by adding some pure yeast obtained from the brewery to a culture medium consisting of a mixture of mashed potatoes and flour, the culture being kept in a warm place for about five hours. By this time the mass is swarming with young actively-growing yeast cells, and, provided that contamination with bacteria has been prevented, none of the sour acids which develop in leaven are present. Besides the yeast, an unorganised ferment called *diastase*, originally present in the flour, becomes active and

hydrolyses some of the starch of the flour into sugar. The yeast cells then act on this to produce alcohol and carbonic acid gas, so that a fermenting mass is obtained. More flour is now added to this, and the process allowed to proceed five or six hours longer, until the developed gas causes the top to burst, after which the remainder of the flour is added. The mass is now called *dough*. It is thoroughly mixed by machinery, and allowed to ferment for another hour, when it is weighed out into *loaves*, which are then placed in pans and heated to about 232° C. in an oven for an hour and a half. The heat kills the yeast, but at the same time causes the enclosed bubbles of gas to expand, so that the dough becomes filled with little cavities. The heat also causes the outer part of the dough to become hardened by coagulating the proteid, and at the same time it converts the starch into dextrin and soluble starch, and so forms the *crust*. The crust is glazed because of the dextrin, and it is coloured and its taste different from the rest of the bread, because of the *caramel* produced by the action of the heat on the sugar which is developed.

Experiment II. Shake up some bread with water and filter off the extract. Test the residue for starch by adding iodine, and for proteid by the xanthoproteic, or Millon's tests. Test the filtrate for sugar by Trommer's test. All the reactions are positive. If a similar extract be made of the crust it will be found to give a purplish colour with iodine, due to the soluble starch and dextrin which it contains.

CHAPTER XI.

DIGESTION. FERMENTS.

The organic food-stuffs—proteids, fats, and carbohydrates—exist mainly as very large molecules which are incapable of diffusing through animal membranes. Before the food can be absorbed by the gastro-intestinal mucosa it is necessary that these large molecules be resolved into smaller ones. Digestion is the process by which this resolution is effected in the animal body, and although absorption is not a mere physical process of osmosis it can nevertheless only proceed *efficiently* with simple molecules.

The agencies which act on the food-stuffs during digestion, and which produce this resolution, are called **ferments**.

These ferments are of two kinds—*organised* and *unorganised*. An

example of the former is yeast. When this grows in a solution of dextrose it splits the dextrose into alcohol and carbonic acid:

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

An example of the latter is the ferment present in saliva, namely, ptyalin. This resolves polysaccharides into monosaccharides:

$$(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6.$$

In the case of yeast, the chemical process goes on *in the body of the cell*, and the energy which is liberated by the splitting up of the complex molecule into simpler ones is used up in the life of the plant. The agent which causes this process is not, however, the vital activity of the yeast cell, but it is an unorganised ferment, called a *zymase*, manufactured by its protoplasm. If yeast-cells be killed by grinding them in a mortar with sand, and then be subjected to a pressure of 500 atmospheres, a clear golden fluid is expressed from them, which, if added to a solution of sugar, produces alcoholic fermentation. We see, therefore, that there is no actual difference between the two kinds of ferments, the organised or living cell ferment owing its properties to an internal secretion in the meshes of its protoplasm. In connection with their *conditions of activity* there are, however, certain points of difference between unorganised and organised ferments, and especially with regard to the temperature at which they are destroyed. All ferments, organised and unorganised, are destroyed at a temperature of 70° C. in the presence of water. If, however, unorganised ferments be dried, they stand a very much higher temperature.[1] Certain chemical substances—*e.g.* chloroform, alcohol, ether, etc.—abolish the action of organised, whereas they do not affect unorganised, ferments. These differences are, however, more apparent than real, for it is evident that when the cell, in the case of organised ferments, has been killed by any of these agencies, it will no longer be capable of absorbing into its meshes the substance to be fermented; and although there may be zymase present in these meshes, it is incapable of acting, because it is locked up in the dead protoplasm of the cell body.

Certain bacteria, such as B. diphtheriae and B. tetani, behave like gland cells, in that they excrete an unorganised ferment, which, when it gains entry into the blood, produces the symptoms of the disease. Thus, if the fluid in which either of these organisms has been grown be filtered free of bacilli and injected into an animal, the characteristic symptoms are produced. There are bacteria, such as B. coli

[1] The spores of certain bacteria can, however, stand a very high temperature without being destroyed. For example, the spores of the tetanus bacillus can usually withstand boiling for five minutes.

and B. typhosus, on the other hand, which do not excrete their ferment, but retain it in their cell bodies as a zymase. The bacilli-free culture medium, when injected into an animal, does not in this case produce any symptoms.

The unorganised ferments which exist in the animal body are divided into certain groups depending on their action. Some convert polysaccharides into monosaccharides. These are called **amylolytic ferments,** examples being ptyalin in saliva and amylopsin in pancreatic juice. Others convert native proteids into peptones. These are called **proteolytic,** and are found in the gastric (pepsin) and pancreatic juices (trypsin). Others invert disaccharides, and are called **inversive**—*e.g.* invertin of intestinal juice. Others split neutral fat into fatty acid and glycerine, and are called **steatolytic**—*e.g.* steapsin of pancreatic juice; whilst others convert soluble into insoluble proteids—*e.g.* the rennet of the gastric juice converts caseinogen into casein, and are called **coagulative.** Fibrin and myosin ferments have a similar action on certain of the soluble proteids of blood and muscle.

These ferments all act best at the temperature of the body. They become inactive at low temperatures, but this does not destroy them, as they again reassume activity on raising the temperature. As stated above, a temperature of 70° C. destroys them if water be present. If they are dried, however, they can stand much higher temperatures. Some of them act best in an alkaline reaction (ptyalin); others in an acid reaction (pepsin).

The substances produced by their activity tend to stop their action, *e.g.* the alcohol produced by the action of yeast on cane sugar will, if allowed to accumulate, ultimately put a stop to the fermentation. In the case of organised ferments these products may actually kill the cell, and completely stop any further fermentation. In the case of unorganised ferments, on the other hand, if these substances be removed, the ferment resumes activity.

Most ferments, *aërobic,* require free oxygen for their activity, others, *anaërobic,* can act in the absence of the free gas.

The ferments cannot be isolated as tangible substances, but they can be precipitated along with proteids by saturation with ammonia sulphate, or by the addition of alcohol. They are soluble in glycerine, and active solutions of them are usually prepared by extracting the gland with this substance. There are no chemical tests by which we can identify them, their presence in any fluid being detected by allowing them to act on suitable substances.

Nearly all ferments act by producing hydrolysis, *e.g.* the inversion of cane sugar, the peptic or tryptic digestion of proteids, etc.

Digestion in the Mouth.

The salivary glands—parotid, sublingual, and submaxillary—along with the numerous isolated gland acini scattered over the mucosa, pour into the mouth a secretion known as saliva. The composition of this mixed saliva is as follows :—

Water, - - - - - - - - - - - 99·42 per cent.
Organic Matter, - - - - - - - - - - 0·36 ,,
Mucus and epithelial cells. Ptyalin and soluble proteid. KCNS
Inorganic Matter, - - - - - - - - - ·22 per cent.
Chlorides, phosphates, and carbonate of alkalies and alkaline earth.

It is, therefore, a very dilute secretion, its specific gravity being only about 1005, ordinary water being taken as 1000.

The total secretion during 24 hours amounts to about the same as that of the urine, *i.e.* 1500 c.c.

The saliva excreted by the different glands differs somewhat in composition ; that from the parotid contains no mucus, and is consequently a thinner fluid than that of the submaxillary, which contains much mucus, or than the sublingual saliva, which also contains a certain amount of that substance.

Collect some saliva in a test tube,[1] and perform the following reactions with it :—

I. To Identify the Various Constituents.

Experiment I. A drop placed on red litmus paper produces a blue stain. The reaction may, however, become acid where decomposition is taking place in the mouth, as is the case in decaying teeth.

Experiment II. If a drop of saliva be placed on a slide, covered and examined under the microscope, two kinds of cells will be seen, viz. : (1) *large, flat, squamous cells*, which have been desquamated from the surface of the stratified epithelium of the mouth, (2) *small round cells* like leucocytes, which come either from the glands themselves or from the tonsils.

Experiment III. Place some saliva in a test tube and dilute it with an equal quantity of water ; now add a few drops of 10 per cent. acetic acid, when a stringy precipitate of **mucus** will occur. Filter off this precipitate, and note that the filtrate is watery, the stringy character of saliva being due to the mucus which it contains. To the filtrate add a few drops of Millon's reagent and boil. The result shows the presence of **proteid.**

[1] The secretion of saliva may be stimulated by inhaling, through the mouth, some acetic acid.

Experiment IV. Add to some saliva in a test-tube a drop of a weak solution of ferric chloride (Liq. Ferri. Perchlor. B.P.). A red colour is produced due to the production of ferric sulphocyanide, the ferric chloride reacting with a **sulphocyanide** (viz. KCNS) which is contained in saliva. The red colour is discharged by adding a few drops of a solution of mercuric chloride (1-1000).

Experiment V. If some saliva be allowed to stand for an hour or so it becomes milky or a thin surface film forms on it. This is due to the precipitation of calcium carbonate which exists in fresh saliva as calcium, bi-carbonate, which is soluble. On standing exposed to the air, however, carbonic acid gas is given off, in consequence of which the bi-carbonate changes into the carbonate.[1] A similar precipitation of calcium carbonate carrying with it a certain amount of calcium phosphate ($Ca_3(PO_4)_2$) sometimes occurs in the ducts of the glands and leads to the formation of calculi, or a similar precipitate may form on the teeth, where it leads to the formation of tartar.

II. To Study the Action of the Ferment Ptyalin.

Experiment VI. Place a few cubic centimetres of a 0·5 per cent. solution of starch in two test tubes, *a* and *b*. To *b* add about an equal amount of saliva and place both in the water-bath heated to body temperature. By means of a glass rod transfer drops from each solution about once a minute to a white slab and add to each drop a little iodine solution. In the case of the test tube *b* the blue colour becomes at first purplish and then reddish brown, and ultimately disappears. When this stage has been reached apply Trommer's or Fehling's test to the contents of the test tube, and note that reduction occurs. In the case of *a* the blue colour persists throughout and reduction of cupric salts does not occur.

What has occurred in *b* is that the ptyalin has hydrolysed the polysaccharide starch (blue with iodine and no reducing power), first into a simpler polysaccharide dextrin (red with iodine, no reducing power), and then into the disaccharide maltose (no colour with iodine, reduces cupric salts). If left in contact with the maltose for some time the ptyalin can invert this, yielding dextrose. There are really two forms of dextrin formed, the one which gives the iodine reaction described above is called **erythro-dextrin**, but there is another **achroo-dextrin** which gives no reaction with iodine. The latter exists as an intermediate stage between erythro-dextrin and maltose.

Experiment VII. Place some of 0·5 per cent. solution of starch in

[1] $2CaH_2(CO_3)_2 = 2CaCO_3 + 2CO_2 + 2H_2O$
(soluble) (insoluble)

the mouth, and after about two minutes transfer this to a test tube. Ascertain if reduction of cupric salts occurs. Repeat this experiment with a grain of unboiled starch and note the difference in both cases (see Carbohydrates, p. 167).

The ptyalin will only act in neutral or alkaline reaction, but not in the presence of free acid.

Experiment VIII. If experiment VI. be repeated with the addition of a few drops of 0·2 per cent. hydrochloric acid, so that the fluid reacts acid to litmus, it will be noticed that no dextrine is produced. If the mixture be heated for a considerable time a trace of a reducing sugar may appear because of the hydrolysing action of the acid.

From the results of this last experiment we see, therefore, that it will be impossible for the action of the ptyalin to proceed in the acid gastric contents. If the stomach be empty at the beginning of the meal the process goes on for about half an hour, as the first portion of acid which is secreted is bound to proteid, so that it does not exercise its inhibiting influence on the ptyalin which has been swallowed.

Only a small percentage of the starch taken with the food, however, is changed by the time it leaves the stomach, and it would appear that ptyalin, the only ferment in saliva, is of very little importance for the digestion of starchy foods, the main seat of this being in the duodenum by means of the *amylopsin* of the pancreatic juice.

The actual function of the saliva is undoubtedly a mechanical one, acting as a solvent for certain foods and assisting in the mastication and swallowing of others. A body must be in solution before it can be tasted, so that the saliva assists in the appreciation of taste. It is also necessary for articulation and for preserving the sensitiveness of the nerve endings of taste and common sensation. This explains why a fever patient cannot taste things so well as during health. It is interesting to note that in some animals the saliva contains little or no amylolytic ferment.

CHAPTER XII.

DIGESTION IN THE STOMACH.

The food, after being masticated in the mouth, is passed down the oesophagus into the stomach, where it is collected, and remains for two or three hours, meanwhile being acted on by the gastric juice. In order that each particle of food may be efficiently digested, there is a

constant movement of the muscular wall of the stomach, whereby its contents are kept in motion; and when these have been sufficiently digested, they are collected into the funnel-shaped pyloric portion of the stomach, and passed through the pyloric sphincter, which meanwhile relaxes to allow of their passage. On entering the stomach the food is very little changed, except that it has been masticated. On leaving it, however, its appearance is quite altered, being now a thick, more or less coloured fluid called **chyme.**

Various methods have been adopted for studying gastric digestion—*e.g.* observing the process through a gastric fistula, removing samples of the gastric contents by means of a stomach tube, etc.

In order to obtain pure gastric juice, the most reliable method is that of Pawlow, which consists in resecting a portion of the fundus of the stomach, and sewing it up so as to form a bag, which is then sutured to an abdominal wound. This isolated sac of stomach secretes pure gastric juice along with the main stomach, and the juice may be collected and analysed.

The Composition of Gastric Juice.—Pure gastric juice obtained from the sac in Pawlow's experiment is a clear, colourless fluid, with a specific gravity of 1003-1006, and of an acid reaction.

Its percentage composition varies in different animals, that of the dog and of man being as follows:

	Man.	Dog.
Water,	99·44	97·3
Organic matter, chiefly pepsin,	0·32	1·71
Inorganic matter—		
(*a*) free hydrochloric acid,	0·2-0·3	0·3[1]
(*b*) chlorides and phosphates of alkalies and alkaline earths,	0·1-0·2	0·66

The most important features to be considered in connection with this table are: (1) the presence of free hydrochloric acid and (2) the nature of the organic matter.

The Acidity of the Gastric Juice.—This might be caused either by a free acid or by an acid salt, such as acid sodium phosphate (NaH_2PO_4). We can decide which it is by testing with Congo red, a red pigment which is turned blue by a free acid, but is unaffected by an acid salt.

EXPERIMENT I. To a 0·2 per cent. solution of HCl add a few drops of a solution of Congo red. The red colour is at once changed to blue. Repeat with a solution of acid potassium phosphate. This turns blue litmus red, but a solution of Congo red is unaffected.

The acidity is therefore due to a free acid. We must now find out if this acid be organic or inorganic.

[1] Pawlow always found more than 0·2 per cent.—namely 0·5-0·6 per cent.

Experiment II. Place a drop of Günzberg's reagent (a solution of phloroglucin and vanillin in absolute alcohol) on an evaporating basin, and mix with it a drop of 0·2 per cent. hydrochloric acid. Slowly evaporate to dryness. A deep red stain is left. Repeat this experiment with a 0·8 per cent. solution of lactic acid. No red stain is obtained.

This reagent reacts to mineral acids, but not to organic acids, even when these are present in considerable amount. The mineral acid with which it reacts most sensitively is hydrochloric acid, and since the reaction is always very distinct in gastric juice, we are led to expect that the acidity is due to this acid. That this is actually the case has been proved by estimating on the one hand the total amount of bases, and on the other the total amount of acids in gastric juice. It was found that the latter were much in excess of the former, the acid radicle present being chlorine, which must exist in the gastric juice as hydrochloric acid.

How this Free Acid is Secreted.—There is perhaps nothing more surprising in the whole of physiological chemistry than that a strong mineral acid should be secreted from a distinctly alkaline fluid such as the blood is. The salts from which it is produced are, of course, the chlorides (especially of sodium), and these are very abundant in the blood, which also contains sodium carbonate, to which is mainly due its alkaline reaction. How then is the hydrochloric acid liberated from this alkaline fluid? Although alkaline in reaction the blood nevertheless contains weak acids, either as acid salts (NaH_2PO_4) or as carbonic acid. Now it is a well known fact in physical chemistry that any acid, however weak its acidity may be, displaces a certain amount of any other acid from its combinations, and this displacement becomes very distinct if the weaker acid be in large excess of the stronger. This property is known as **mass-influence,** and in virtue of it even the weakest acid—*e.g.* ordinary water—can displace a certain amount of the strongest mineral acids from their combinations.[1]

Since very little carbonic acid is in simple solution in the blood it is probable that it is the acid phosphates which furnish the weak acid. They do this by giving off a portion of their hydrogen, the place of this being then taken by the free alkali liberated from the chloride.

$$NaH_2PO_4 + NaCl = Na_2HPO_4 + HCl.$$

So far, then, the reaction is a merely chemical one, but now we must discover what agency it is which causes this free acid to be secreted into the stomach. If a pouch be made of the cardiac end of the

[1] Thus if a solution of bismuth nitrate be diluted with a large excess of water a white precipitate of bismuth oxynitrate falls out.

stomach, and another of the pyloric end, by Pawlow's method, it will be found that the secretion of the former alone contains the free acid. If now the mucous membrane from these two regions of the stomach be examined microscopically it will be found that the glands of the cardiac portion contain—besides the ordinary secreting cells, which are similar to those of the pyloric portion—large round cells. To these cells, then, probably belongs this acid secreting power, *i.e.* they can take up the acid and transfer it to the lumen of the gland.[1]

If an animal be fed on bromides instead of chlorides, hydrobromic takes the place of hydrochloric acid.

The Uses of the Free Acid are Two: (1) It is necessary for the efficient action of pepsin, and (2) it acts as an antiseptic, preventing fermentation in the stomach, which would otherwise certainly occur, since a considerable number of micro-organisms are present in our food-stuffs, and nothing could be more favourable for their growth than the semi-fluid contents of half-digested food kept at body temperature. A certain form of dyspepsia is due to deficiency of hydrochloric acid in the gastric secretion. If the reaction of the gastric contents in such a case be tested, however, it is found to be strongly acid, and if the amount of this acidity be determined (see Advanced Course, p. 445) it may be found to be greater than that of healthy gastric juice. By applying appropriate tests the cause of the acidity is found to be chiefly due to lactic acid. This is developed by the growth of certain micro-organisms (bacillus lactis) on carbohydrates (see Milk, p. 188). This lactic acid may be further decomposed, giving rise to butyric acid.

$$(1)\ \underset{\text{Lactose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Lactic acid.}}{4C_3H_6O_3}$$

$$(2)\ \underset{\text{Lactic acid.}}{4C_3H_6O_3} = \underset{\text{Butyric acid.}}{2C_4H_8O_2} + 4CO_2 + 4H_2$$

By examining the equations it will be noticed that gas (CO_2) is evolved during the transformation of lactic into butyric acid. The accumulation of this in the stomach leads to '*flatulence.*'

The presence of lactic acid in these cases can be detected by employing Uffelmann's reaction (see Milk, p. 188).

The Organic Matter.—If pure gastric juice, obtained by Pawlow's method, be cooled to 0° C. a precipitate falls down. On analysis this is found to have nearly the same percentage composition as proteid, and on testing its action on a solution of proteid it is found to be '*pepsin.*'

[1] As to the exact *rôle* which these 'oxyntic' cells play in this mechanism there is considerable difference of opinion. Some state that the above-mentioned chemical reaction takes place in their bodies, others that they simply transfer 'H' ions, which then react with the chlorides in the stomach itself.

Nearly pure '*pepsin*' can also be prepared by saturating gastric juice with ammonium sulphate, which precipitates it. Whether the actual ferment pepsin is what we obtain by these methods, or whether it is simply proteid with the ferment adherent to it, cannot as yet be decided.

The Action of the Gastric Juice.—To study this we employ an artificial gastric juice prepared by macerating the mucosa of the stomach for several days with twenty times its weight of glycerine containing 0·1-0·2 per cent. hydrochloric acid. The acid changes the pepsinogen (the mother substance or *zymogen* of pepsin) present in the gland cells into pepsin, and this then dissolves in the glycerine.

We may employ any proteid for the investigation, the most convenient one being blood fibrin, which has been very thoroughly washed with boiling acidulated water so as to remove all impurities.

Experiment III. Label six test tubes *A*, *B*, *C*, *D*, *E*, *F*, and place a piece of fibrin in each. Half fill *A* with water, *B* with 0·2 per cent. HCl, *C* with water and a few drops of the peptic extract, *D* with 0·2 per cent. HCl and a few drops of peptic extract, *E* same as *D* but boil the mixture, and *F* with 1 per cent. sodium carbonate and a few drops of the peptic extract.

Place all these in a water-bath kept constantly at body temperature (40° C.).[1] Observe (1) that in *A* the piece of fibrin remains unchanged, whereas in *B*, *D* and *E*, which all contain 0·2 per cent. HCl, it becomes swollen and transparent. In *F*, which contains alkali, it does not swell.

Experiment IV. After about half an hour, remove a sample of the contents of any of the tubes containing acid, colour it with some litmus, and then carefully neutralise with weak sodium carbonate solution (1 part 1 per cent. Na_2CO_3 + 2 parts water). A precipitate of **acid albumen** or syntonin is produced (for reactions see proteids, p. 177).

The first stage in gastric digestion of proteids consists, therefore, in the production of acid albumen by the ·2 per cent. HCl. As we shall see later, this preliminary change is necessary before pepsin can further hydrolyse the proteid.

Experiment V. (2) Remove a sample of the contents of *D* and apply the following tests:—(*a*) The Biuret reaction—rose-pink colour, (*b*) Add HNO_3 (con.)—white precipitate, which clears up on heating and returns on cooling. These two results show us that **proteoses** have been produced.

[1] This can be approximately determined by dipping a finger into the water; if the water be at the same temperature as the skin it will feel neither cold nor hot.

This constitutes the *second stage* of peptic digestion, and it is the pepsin which produces the change. If samples of any of the other test tubes be examined no proteose will be found, either because no pepsin has been present (as in *A* and *B*), or because, though present, its action has been destroyed by heat (as in *E*), or there has been no acid present to produce syntonin and help its action (as in *C* and *F*).

There are two principal varieties of proteoses developed, namely '**primary**' and '**secondary**'; the former are precipitated by saturation with sodium chloride, the latter are not (see Advanced Course).

EXPERIMENT VI. (3) Take a sample of a digest of two days' duration. Heat this to near boiling point, and add ammonium sulphate crystals till no more will dissolve. Now change the reaction of the fluid to alkaline and allow to cool.[1] Filter and test the filtrate for **Peptone.**

1. By Biuret reaction—(remember to add a large excess of caustic alkali, so that more than is sufficient to decompose the Am_2SO_4 may be present in the fluid)—rose-pink.

2. By nitric acid test—no precipitate. This constitutes the *final stage* in the peptic digestion of proteids. There are two varieties of peptone developed, anti- and hemi-peptone, which differ from one another in that the proteolytic enzyme of pancreatic juice can further decompose the *hemi-*, but not the *anti-*peptone.[2] These are the products of peptic digestion only when the process is of short duration. By prolonged action digestion proceeds much further and yields the same crystalline products as result when a strong mineral acid is allowed to act on proteid.

The various stages can therefore be tabulated as follows:

Precipitated by saturation with Ammonium Sulphate.	(1) NATIVE PROTEID,	coagulated by heat,	Biuret test = violet.
	(2) ACID ALBUMINATE,	precipitated by neutralisation,	Alcohol = coagulates.
	(3) PROTEOSE, or pro-peptone, Primary,	precipitated by HNO_3, clearing up on heating, etc., precipitated by saturation with NaCl,	Biuret test = pink. Alcohol = precipitate.
	Secondary,		
	(4) PEPTONE—ANTI—unacted on by trypsin.[2] HEMI—acted on by trypsin.		

Besides pepsin, the gastric juice also contains the milk curdling

[1] It is only by thus saturating in the heat, both in acid and alkaline reaction, that all traces of secondary albumoses are precipitated.

[2] Much doubt has recently been cast on the existence of anti-peptone (see p. 449).

ferment **rennin.** It will be remembered that caseinogen is precipitated by weak acids; it might be naturally supposed, therefore, that the curd which forms when milk enters the stomach was due to precipitation by acid, and not to coagulation by the ferment. That it is the rennin which acts, however, is proved by neutralising the gastric juice before adding the milk, when curdling will occur as usual, or by treating some of the curd with weak alkali in which it will not dissolve, whereas a precipitate of caseinogen would dissolve with ease.

The gastric juice scarcely affects other food-stuffs. In the case of fat, however, it dissolves the proteid envelope of the fat cell, and liberates the contents, which now float in the chyme as oil globules. It inverts disaccharides, but has no action on polysaccharides.

The conditions which influence the activity of gastric digestion are discussed in the Advanced Course.

CHAPTER XIII.

DIGESTION IN THE INTESTINE.

In about twenty minutes to half an hour after the food enters the stomach, small portions of it begin to pass through the pyloric sphincter into the duodenum. These have undergone gastric digestion and constitute *chyme.* This leakage goes on till the stomach has completely emptied itself, the length of time necessary for this (3-10 hours) varying with the quantity and quality of the food, and with the activity of the gastric juice.

The chyme, as it leaves the stomach, is strongly acid in reaction, of a dirty yellow colour, with no characteristic smell, and has floating in it unemulsified globules of oil. In the duodenum it becomes mixed with the secretions of the pancreas and liver, which are poured into that portion of the intestine by one common duct, and as it travels on to the jejunum it also becomes gradually mixed with the intestinal juice, secreted from Lieberkühn's follicles. These three secretions are alkaline in reaction, in consequence of which the acid of the chyme is neutralised, so that the contents of the lower portion of the duodenum and of the upper portion of the jejunum become alkaline in reaction. Now, although the acidity of the gastric juice *prevents* the growth of organisms in it, it does not *kill* their spores, and these are carried into the intestine along with the chyme. When this latter becomes alkaline, however, the conditions are very favourable for bacterial growth, and

the spores become transformed into the active organisms which multiply quickly, meanwhile receiving their nourishment from the half-digested food-stuffs, which become partially decomposed as a consequence. Among the products of this bacterial growth are several organic acids, so that the food, before it has gone far along the intestine, again becomes acid in reaction. The mucosa of the large intestine does not secrete any digestive juices, its sole function being one of absorption. In its passage along it the fluid of the intestinal contents becomes gradually absorbed, and the unabsorbed residue forms the faeces.

It will be seen, therefore, that there are *four distinct digestive agencies* at work in the intestine, and we will now study the action of each of these separately.

The Pancreatic Juice.—*Composition*—This can be collected by producing a fistula of the pancreatic duct. The juice is strongly alkaline in reaction, gives a coagulum of proteid on boiling, and contains, besides this, a considerable amount of organic matter.

Its percentage composition varies very much with the method adopted for collecting it, that obtained immediately after the establishment of the fistula being very much richer in solids than that secreted a few days later.

	Directly after operation.	Permanent fistula.
Water	90·08	97·68
Total solids	9·92	2·32
Organic	9·04	1·64
Inorganic	0·88	0·68

In studying its digestive action we employ, as in the case of gastric digestion, an extract of the gland. This extract may be made with glycerine, after treating the minced gland with weak acid, or allowing it to stand some time, so as to convert the zymogens into the active ferments. Glycerine does not extract all the ferments, however, so that it is more usual to employ the minced gland itself, or a watery extract of it.

The result of the investigations has shown that there are four active ferments, one proteolytic—**trypsin** ; one amylolytic—**amylopsin** ; one steatolytic—**steapsin** ; and one coagulative—a milk curdling ferment.

I. **Trypsin.**—Like pepsin this hydrolyses proteid, and leads to the production of proteoses and peptones. In this case, however, digestion does not stop here, but the hemipeptone is further hydrolysed, amido acids and hexone bases resulting ; the ultimate decomposition products are, in fact, almost the same as when a strong acid is used as the hydrolysing agency (see Proteids, p. 170).

Experiment I. A solution of pancreatic extract in 1 per cent. sodium carbonate solution is prepared (Liq. Pancreaticus, Benger, diluted 30 times with 1 per cent. sodium carbonate solution). In order to study the action of this on proteids, add to it a piece of fibrin which has been soaked over night in 1 per cent. sodium carbonate solution, and place on a water-bath at body temperature.

The following points of difference may be noted between this and the peptic digestion of fibrin: (1) The reaction is alkaline; (2) There is no preliminary swelling of the fibrin, it is gradually eaten away (erosion); (3) When the piece of fibrin has nearly disappeared remove a sample of the digest, and neutralise with weak acetic acid. A precipitate of **alkali albumin** results (for Reactions, see p. 177).

Apply to another sample the tests for **proteoses** and **peptones**, and note that they are positive.[1]

Experiment II. If the pancreatic extract in Experiment I. be boiled before the fibrin is added, no digestion will result. The digestive agency is, therefore, a ferment which is destroyed by heat.

Experiment III. Repeat Experiment I., making the reaction acid by hydrochloric acid. Note that, although the fibrin becomes swollen up—as this depends on the acid, not on the ferment—no formation of proteoses or peptone occurs. The trypsin cannot act in acid medium.

(4) Trypsin can carry digestion a stage further than can pepsin.

Experiment IV. A minced pancreas has been macerated with 1 per cent. sodium carbonate solution, and to the resulting preparation were added the whites of several eggs. The mixture was then divided into two parts, to one of which a few crystals of the antiseptic thymol were added. Both were placed in the incubator for several days.[2] After this time it will be noticed that the portion to which no thymol was added has a very faeculent odour, and that this is absent from the other portion. The odour is due to bacterial growth, the effete products of this on proteids belonging to the aromatic series of organic compounds (**phenol**, **kresol**, **indol** and **skatol**, see p. 238).

The portion containing thymol is boiled and filtered, and the filtrate then evaporated to small bulk. A sample of the resulting syrup is placed in a watch-glass, and allowed to stand exposed to the air for a day or so. Then examine a drop on a microscopic slide, and note the crystals of Leucin and Tyrosin.

Leucin and Tyrosin.—During digestion of hemi-peptone by trypsin a number of **amido acids** are produced, of which leucin and tyrosin are

[1] There is, however, no *primary* proteose formed by tryptic digestion; there is, however, a considerable amount of *secondary* proteose (see p. 449).

[2] These digests are prepared beforehand by the demonstrator.

examples. An amido acid is derived from an *organic* acid (containing therefore the – COOH group) by the substitution of one of the hydrogen atoms of a methyl (CH_3) group by the amido group (NH_2).

Thus acetic acid has the formula CH_3COOH.

If now we displace one of the 'H's' of the CH_3 group by NH_2 we obtain

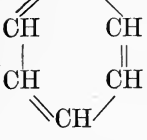
$$CH_2\begin{matrix}NH_2\\COOH\end{matrix}$$

which is amido acetic acid, also called *glycin* or *glycocoll.*[1] It is formed during the digestion of gelatine, but not of native proteids. It also exists in the bile, where it enters into the formation of one of the bile salts (*e.g.* glycocholate of soda is glycin + cholalic acid). It likewise occurs in combination with benzoic acid as hippuric acid in the urine of herbivorous animals, and in traces in the urine of man.

If we take the next acid of the acetic acid series, viz., propionic acid CH_3CH_2COOH, we obtain amido propionic acid, or *alanin*,

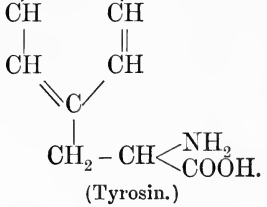
$$CH_3CH\begin{matrix}NH_2\\COOH.\end{matrix}$$

In the free state it is only produced from a few proteids,[2] and is unimportant, but it is frequently combined with oxyphenyl, the resulting compound being **tyrosin.** If in the formula of oxyphenyl



(Oxyphenyl.)

the H atom opposite to the OH group be replaced by amido propionic acid, we obtain para-oxyphenylamido propionic acid, which is tyrosin. It therefore belongs to the aromatic group of organic bodies, and it will



(Tyrosin.)

be remembered that it is on account of its containing oxyphenyl that it reacts red with Millon's reagent (see Proteids, p. 172).

Experiment VI. Add Millon's reagent to some pancreatic digest; a white coagulum of proteids results. Filter. Boil the filtrate. It turns red, because it contains tyrosin.

[1] This relationship to the fatty acids is demonstrated by the following reaction. If acetic acid be treated with bromine gas, brom-acetic acid is formed,

$$CH_2\begin{matrix}Br\\COOH.\end{matrix}$$

If now we treat this with ammonia we get :—

$$\underset{\text{Brom-acetic acid.}}{CH_2\begin{matrix}Br\\COOH\end{matrix}} + \begin{matrix}NH_3\\NH_3\end{matrix} = \underset{\text{Glycin.}}{CH_2\begin{matrix}NH_2\\COOH\end{matrix}} + NH_4Br.$$

[2] By the hydrolysis of haemoglobin, however, alanin is a very abundant decomposition product.

Experiment VII. Examine the crystals of tyrosin under the microscope, and note that they consist of fine needles grouped into star-shaped masses (Fig. 148).[1]

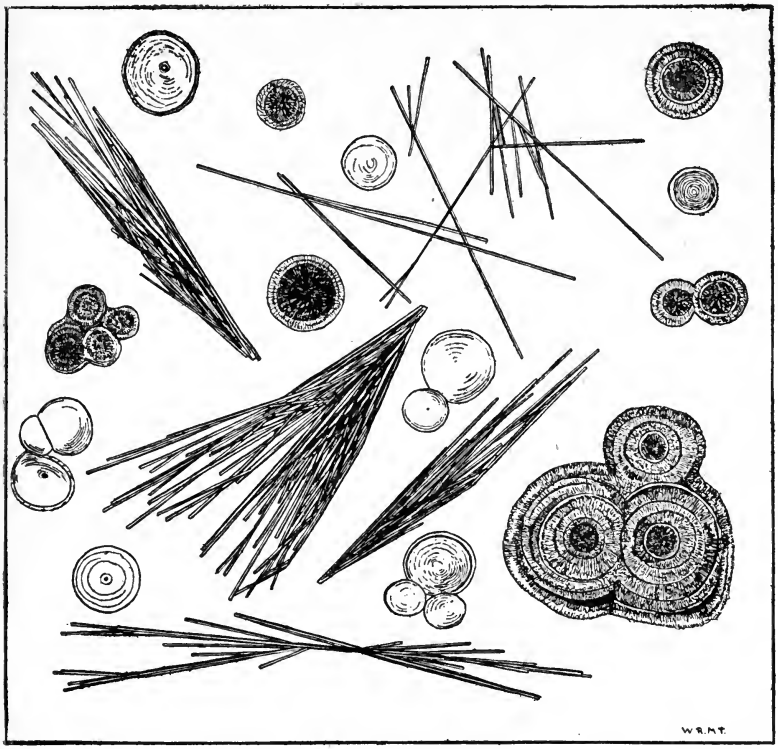


Fig. 148.—Crystals of leucin and tyrosin.

There are no other important amido acids till we come to the member of the series which contains six carbon atoms. This is amido caproic acid or **leucin.**[2]

$$CH_3 - CH_2 - CH_2 - CH_2 - CH{<}^{NH_2}_{COOH}.$$

Experiment VIII. Examine the crystals of leucin under the micro-

[1] A body very closely related to tyrosin in its chemical structure has recently been described amongst the products of hydrolysis of proteids by acid. This is phenylalanin, differing from tyrosin only in that it does not contain an – OH group. It has also been discovered in the products of the prolonged action of pepsin on proteids.

[2] It has recently been shown, however, that the true constitution of leucin is rather α amido isobutylacetic acid

$$CH_3{>}_{CH_3}CH - CH_2 - CH{<}^{NH_2}_{COOH}.$$

scope, and note that they consist of round balls not unlike oil globules, but having concentric markings (Fig. 148).

Leucin and tyrosin were among the first-discovered decomposition products of proteids, and, on account of the ease with which they are isolated, they have been detected in nearly every organ and tissue of the body, being probably produced, however, by the chemical agencies employed in examining these, and not existing as such in the living tissue. They also occur, along with free ammonia, in the urine of patients suffering from severe disease of the liver.

Not only are amido derivatives of mono-basic acids produced during proteid decomposition, but we may also have *similar derivatives of dibasic acids.*[1] One of the simplest of these latter is succinic acid.

$CH_2 - COOH$
|
$CH_2 - COOH$
(Succinic acid.)

If now we replace an 'H' of a methyl radicle by the amido group (NH_2) we obtain **aspartic acid.**

$CH{<}^{NH_2}_{COOH}$
|
$CH_2 - COOH$
(Aspartic acid.)

Besides being produced in the intestine by the action of trypsin on proteid, it also occurs plentifully in plants.[2]

If one of the 'H' atoms of the other methyl group of this be replaced by CH_3, we obtain another important di-basic amido acid, viz., **glutaminic acid,** and this is also a common decomposition product.

All these amido acids retain to a certain extent their acid properties. Thus they can combine with bases to form salts. On the other hand, on account of the NH_2 group which they contain, they also show faint basic properties, in that they can unite with acids, forming weak salts. Most of them can also unite with metallic salts, forming double compounds, which are very useful in preparing the pure amido acid.

Besides these mon-amido acids, there are also produced bodies in which more than one amido group exists. These have a distinctly basic reaction, and combine with weak acids, such as phosphotungstic.[3] They also form double salts with silver nitrate. These two reactions are taken advantage of in separating these bases from the mon-amido acids. Since all these bases contain six carbon atoms, they are called **hexone bases,** and the most important are lysin (diamido caproic acid, $C_5H_9(NH)_2COOH$), arginin ($C_6H_{14}N_4O_2$), lysatin ($C_6H_{13}N_3O_2$), and histidin ($C_6H_9N_3O_2$).

By the action of trypsin on fibrin a body is produced belonging to none of the three groups described above. This is *cystin.* It is

[1] Acids are mono- or di-basic according to whether they contain one, or two, replaceable OH (hydroxyl) groups belonging to a COOH (carboxyl) radicle.

[2] If the OH group of the COOH radicle be further replaced by NH_2 we have asparagin.

[3] This complex acid has the formula $P_2O_5xWO_3yH_2O$.

especially interesting because it contains, besides an amido group, a sulphur group. It is probably the source of the taurin of the bile (see p. 456) and sometimes appears in the urine (see. p. 279). Cystin, it will be seen, is related to lactic acid.

$$\begin{array}{l} CH_2 - SH \\ | \\ CH - NH_2 \\ | \\ COOH. \end{array}$$

(Cystin.)

The hexone bases are the only decomposition products obtainable from protamines (Kossel's first group); if amido acids be also produced we have the albuminoids (second group); if aromatic bodies—tyrosin—be also produced, we have true proteids (third group); and if either of these three groups be in combination with other bodies, such as nuclein or carbohydrate, we have the compound proteids (fourth group). (See also Proteids.)

II. **Amylopsin.**—This acts on starch in exactly the same way as ptyalin does—*i.e.* it converts it into maltose. It can, however, also digest cellulose,[1] so that it is capable of acting on unboiled starch.

EXPERIMENT IX. Add some glycerine extract of pancreas to some powdered starch. Shake, and place in the water-bath at 37°. Remove drops every half minute, and mix on a slab with a drop of iodine solution. Note the appearance of the dextrine reaction. When this disappears apply Trommer's test to a sample of the digest; note the reduction due to maltose.

III. **Steapsin.**—This decomposes neutral fat into fatty acid and glycerin. (See Fats, p. 180.)

EXPERIMENT X. Some minced pancreas is shaken with water[2] and the resulting extract divided into two parts. One of these is boiled to destroy the ferment and is then cooled. To each portion (about 10 c.c.) are added five drops of melted and filtered butter fat, a few drops of an alcoholic solution of phenolphtalein and then $n/_{10}$ NaOH until a deep red colour is obtained. After vigorous shaking so as to obtain a partial emulsion the test-tubes are placed in the incubator, and, after about half-an-hour, examined. The steapsin-containing fluid will be decolourised (the fatty acid having bleached the phenolphtalein), and, to regain the original red colour, a certain number of c.c. $n/_{10}$ NaOH must be added to it. In this way an approximate estimate can be obtained of the fat-splitting power of the extract.

The liberated fatty acid combines with the free alkali of the pancreatic juice and bile to form a soap, which is absorbed into the epithelial cells of the villi, wherein it is again set free, and recombines with glycerin to form neutral fat.

IV. **Milk Curdling Ferment.**—Extracts of pancreas cause milk to clot; but, in normal digestion, since the rennin of the stomach has already produced this, the ferment can hardly ever come into play.

[1] This digestion of cellulose is, however, probably carried out chiefly by bacteria.

[2] Glycerin does not dissolve steapsin, so that a glycerin extract of pancreas is not suitable for this experiment.

CHAPTER XIV.

THE BILE. BACTERIAL DIGESTION.

THIS is perhaps the most puzzling secretion in the whole of physiological chemistry. Its digestive action is very slight, so that it would almost appear, at first sight, to be an *excretion* of effete products rather than a useful secretion. Against such an idea, however, stands the fact that it is poured into the beginning of the intestinal tract, and not into the end of it, as we would expect were it an excretion. Further, many of its constituents are reabsorbed into the portal blood and carried back to the liver, to be re-excreted in the bile. In other words, there exists a circulation of certain biliary constituents from the liver to intestine by the bile, and from intestine back to liver by the portal blood. It is obvious, therefore, that bile collected from a biliary fistula (produced by attaching the central end of the bile duct to a wound in the abdominal wall) will contain less solids than the bile obtained after death from the gall bladder.

Composition of Human Bile.—In *I.* the bile was obtained from the gall bladder of persons who had been accidentally killed, but were otherwise healthy; in *II.* the bile was obtained from a fistula.

100 parts contain—	I.	II.
Water,	86	97
Solids,	14	3
Viz. organic salts,	9	0·9–1·8
Mucin and bile pigment,	3	0·5
Cholesterin,	0·2	0·06–0·16
Lecithin and fat,	0·5–1·0	0·02–0·09
Inorganic salts,	0·8	0·7–0·8

The daily secretion amounts to about 750 c.c. To study the chemistry of bile we employ that of the ox since this is easily procurable.

EXPERIMENT I. Examine some ox bile. Note that it has a green colour, a peculiar musk-like odour, a bitter-sweet taste, a faint alkaline reaction to litmus paper, and that it is of a slimy consistence.

EXPERIMENT II. If a few drops of a weak acetic acid be added to a few cubic centimetres of bile, a stringy precipitate is produced. This consists in some animals (ox) of nucleo-proteid, in others (man) of mucin. Filter off this precipitate, and note that the filtrate has lost its slimy character. Boil the filtrate: no coagulum is produced, therefore bile contains *no* native proteid.

Experiment III. Test another portion of the bile for bile salts by **Pettenkofer's reaction.** To do this place a drop of bile in a capsule and move this about so that a thin film is produced. Now add to the film a drop of a concentrated watery solution of cane sugar, and then a drop of concentrated sulphuric acid. A purple colour is produced. This pigment shows absorption bands in the spectrum. The chemistry of this reaction is that the sulphuric acid acts on the cane sugar to produce a body called furfuraldehyde, which then reacts with the cholalic acid of the bile salts to produce the pigment. Where only traces of bile salts are present, the test may be made more delicate by using a solution of furfuraldehyde (1 in 1000) instead of cane sugar.

Experiment IV. **Matthew Hay's Sulphur Test.**—If some flour of sulphur be sprinkled on the surface of bile, or of a solution containing bile salts, it will sink to the bottom of the vessel, whereas with most other fluids it remains floating on the surface. This reaction depends on the fact that bile salts lower the surface tension of fluids in which they are dissolved.

The Bile Salts are two in number, glycocholate and taurocholate of sodium. The two acids (glycocholic $C_{26}H_{43}NO_6$ and taurocholic $C_{26}H_{45}NSO_7$) are very closely related to one another, for they both yield on boiling with stronger acids a common non-nitrogenous body called *cholalic acid*, and a nitrogenous body of the nature of an amido acid. The amido acid, which is obtained from glycoholic acid, is *glycin*. The other amido acid is *taurin*, and is peculiar in that it contains sulphur (for Chemical Constitution, see Advanced Course). Scarcely anything is known of the chemical constitution of cholalic acid with which these two bodies are combined. Its empirical formula is $C_{24}H_{40}O_5$. The relative amount of these two acids in the bile varies in different animals. In the bile of the herbivora, glycocholic acid is much in excess, whereas in that of carnivora the *only* acid is taurocholic. In omnivora (*e.g.* man, etc.) a variable mixture of the two is present. These bile salts are decomposed into their constituents by the action of the bacteria in the intestine. If we examine the faeces, however, no glycin and only a trace of cholalic acid can be detected. The fate of taurin has not been accurately determined.

It is evident, therefore, that most of the bile salts are reabsorbed from the intestine into the portal blood, and perhaps into the thoracic duct, to be ultimately re-excreted in the bile. This explains why fistula bile should contain so very much lower a percentage of these salts than does gall bladder bile. It also shows that these salts must subserve some very important function, and that they are too valuable

to be lost in the faeces. In the blood they seem to act as solvents for cholesterin, and in the intestine they assist in the absorption of fat.

A small amount (one eighth), however, escapes in the faeces, and, to make good the loss, more must be produced.[1] The substance which yields them is proteid, both glycin and taurin being undoubtedly derived from this. The evidence that glycin results in the decomposition of proteid we have already obtained in studying tryptic digestion, and the presence of sulphur, as well as of nitrogen, in taurin, betrays its derivation from the same source. Nothing certain is known of the derivation of cholalic acid.

The Bile Pigments.—These are **bili-rubin** and **bili-verdin**. The former occurs most plentifully in the bile of carnivorous, the latter in that of herbivorous animals. Their presence can be detected by oxidising a mixture containing them with nitrous acid, when a play of colours—green, blue, purple, and then yellow—is produced. This is called **Gmelin's test.**[2]

Experiment V. Dilute some ox bile with an equal amount of water. Hold the test tube as nearly horizontal as possible, and allow some fuming nitric acid to run down it, so that this forms a layer under the bile. Where the two fluids are in contact, a play of colours is produced.

Bili-rubin is the least oxidised pigment, and its empirical formula is $C_{32}H_{36}N_4O_6$. If we compare this with the formula of haematin—$C_{32}H_{32}N_4O_4Fe$—we see that it must be from this body that it is derived, the change being the abstraction of iron and the addition of two molecules of water. This is also the formula of iron-free haematin or haematoporphyrin, and of haematoidin, a pigment which crystallises out in old blood clots in the tissues. Although the same empirically, these bodies vary somewhat in their physical behaviour, so that we may assume that they have different constitutional formulae.

When it reaches the intestine, the bile pigment is reduced by the nascent hydrogen generated by bacterial growth, to another pigment called **stercobilin**. Most of this is absorbed into the portal blood along with the bile salt. This reabsorbed stercobilin is partially re-excreted in the bile, and partially excreted in the urine, where it goes by the name of **urobilin** (see Urine). The stercobilin which is not reabsorbed forms the colouring matter of the faeces.

[1] Increased proteid metabolism in the tissues is *not* accompanied by an increase in the nitrogen and sulphur excreted in the bile. This shows that the bile salts cannot be *excretory* products, but that they must be useful *secretory* bodies.

[2] This test depends on the various colours of the oxidation products of bili-rubin. The first oxidation product is *bili-verdin*, which is green; the next is *bili-cyanin*, which is blue; the next is *bili-purpurin*, which is purple; and the last is *choletelin*, which is yellow.

Lecithin ($C_{44}H_{90}NPO_9$) and **Cholesterin** ($C_{27}H_{45}OH$) (see Lesson III.) —These two bodies are kept in solution in the bile by means of the bile salts.

Experiment VI. Place some bile in a test-tube, and add one or two crystals of cholesterin to it and gently warm. The cholesterin dissolves.

Repeat this experiment with water, when the crystals will not dissolve.[1]

Both lecithin and cholesterin are excretory products. The tissues which contain the highest percentage of them are the nervous, so that the bile functionates as the channel by which the products of nervous metabolism are removed.

Inorganic Salts.—These are chiefly sodium carbonate (Na_2Co_3) and disodium hydrogen phosphate (Na_2HPO_4).

The Uses of the Bile in Intestinal Digestion.—(1) It is an alkaline fluid, containing a viscid substance (mucin, etc.), consequently it assists in the emulsification of fats.

Experiment VII. Shake up some rancid oil with bile in a test tube. Notice that a very stable emulsion is formed. (See Fats).

(2) In virtue of the bile salts which it contains, (*a*) it precipitates syntonin, and (*b*) to a certain extent also, proteoses and pepton.

Experiment VIII. Add to a sample of a 24 hours' peptic digestion of egg-white some bile, from which the mucin has been removed by alcohol. A precipitate of syntonin, etc., is produced.

Thus the fluid chyme becomes much thicker on mixing with the bile, and its condition, therefore, more favourable for being further digested in the intestine, since it will adhere to the intestinal wall.

(*c*) It dissolves the free fatty acid produced in the intestine.

On account of this latter action, and, to a certain extent, on account of its emulsifying powers, it assists materially in the absorption of fat. Where bile is not excreted into the intestine (as in Jaundice), the faeces become rich in fat, in consequence of which they appear greasy and pale in colour. The presence of excess of fat in the intestinal contents also hinders, to a certain extent, proteid digestion by coating the particles of food and preventing the juices getting at them. In consequence of this, bacterial growth becomes excessive. It is by this means that bile diminishes putrefaction in the intestine, and not on account of any antiseptic properties it possesses, for bile itself quickly becomes putrid on standing. Many other digestive properties have been ascribed to bile, *e.g.* that it assists

[1] These two bodies are also kept in solution in the blood because of the presence of bile salts.

the absorption of oil globules and that it acts as a laxative, but these are not of much importance. It may be mentioned that in some animals bile contains an amylolytic ferment.

To sum up, we may state that although bile contains no ferment by which a chemical change can be produced on any of the food-stuffs, it is nevertheless of great value as a digestive fluid, in that it assists the pancreatic juice (1) by neutralising the chyme; (2) by dissolving the fatty acid produced by the action of steapsin, and which has not united with alkali to form soap; (3) by assisting in the emulsification of neutral fat; (4) by assisting the absorption of fat, and consequently (5) of allowing proteid to be attacked by trypsin, thereby diminishing bacterial growth and consequent putrefaction; (6) and lastly, by precipitating the half-digested products of chyme, so that the trypsin may the better act on them.

Intestinal Juice.—This is secreted by Lieberkuhn's follicles. It may be obtained pure by isolating a piece of intestine and collecting the juice secreted by it. This may be accomplished by cutting out a piece of intestine and stitching both ends to abdominal fistulae (Vella's method), the severed ends of the intestine being sutured together. Or one end of the isolated piece may be sutured, the other being attached to a fistula (Thiry's method). In both these cases the mesentery of the isolated portion is left intact, and the juice can be removed from the loop and its action studied *in vitro*, or food may be placed in the loop and afterwards removed and examined.

Succus entericus seems to contain three ferments or ferment-like bodies. One of these has been known for long and is called inverting ferment because it 'inverts' (see p. 216) disaccharides. There are several varieties of *inverting ferment* depending on the exact nature of the disaccharide on which they act: *e.g.* one acting on maltose (maltase), one on lactose (lactase), and one on cane sugar (invertine). Lactase is present only when the food contains lactose.

The other two ferments act on proteids. One of them, *erepsin* by name, hydrolyses casein, proteoses and peptones into simple nitrogenous crystalline products, similar to those obtained when trypsin or strong acids act on native proteids. It, itself, cannot however act on native proteids. It is more plentiful in extracts of intestinal mucous membrane than in succus entericus. It is probably, therefore, an intracellular ferment, some of it leaking out of the cells into the succus entericus. Since the proteids (*i.e.* peptones) have to pass through these cells during absorption they will come under the influence of erepsin.

Another ferment-like body in succus entericus is *entero-kinase*. Alone, it has no action on any food stuff, but when mixed with trypsinogen it

TABLE SHOWING CHANGES WHICH TAKE PLACE IN FOOD DURING ITS PASSAGE ALONG THE INTESTINE.

	Proteid.			Carbohydrates.			Fats.
State of food-stuffs in the chyme.	Acid albumin proteoses.	Hemipeptone.	Antipeptone.	Cellulose.	Starches.	Dissaccharides.	Neutral fat.
Action of trypsin.	Antipeptone. Decomposition products of hemipeptone.	Hexone bases. Amido acids. Aromatic bodies (not phenol, etc.).	Nil.	Nil.	Nil.	Nil.	Nil.
Action of amylopsin.	Nil.			Nil.	Dissaccharides (Maltose).	Nil.	Nil.
Action of steapsin.	Nil.			Nil.	Nil.	Nil.	Glycerine. Fatty acid.
Succus entericus.	*Entero-kinase*—activates trypsinogen. *Erepsin*—hydrolyses proteoses and peptones.			Nil.	Monosaccharides.	Monosaccharides.	Nil.
Bacteria. Can do all that the ferments do as well as produce :—	Bodies of phenol group.			Butyric and acetic acids. Methane.	Alcohol, acetic and butyric acids. Carbon dioxide and hydrogen.		Lower fatty acids.
Action of bile	Precipitates products of gastric digestion in chyme.			Nil.			Dissolves unsaponified fatty acid.

converts it into trypsin. On a flesh-free diet, the pancreatic juice, as secreted from the duct of Wirsung, contains very little trypsin, and digests coagulated egg white scarcely perceptibly even after several hours. If to this inactive pancreatic juice a few drops of succus entericus be added digestion of the egg white proceeds actively. Trypsinogen, which is the form in which the proteolytic ferment is secreted on a flesh-free diet, remains inactive until it gets to the intestine where it is converted into trypsin by the entero-kinase. Entero-kinase is not secreted unless it is required, *i.e.* if the intestinal mucosa be mechanically stimulated a juice will be secreted containing, however, no entero-kinase.

Bacterial Digestion.—As has been explained above, the conditions necessary for bacterial growth are very favourable in the upper reaches of the intestine. As a result of their growth they decompose the food-stuffs and lead to the production of products in many cases the same as those of the digestive juices, in other cases of a different nature.

Their action on **proteids** leads to the production of proteoses, peptones, and amido acids. So far their action corresponds to that of pepsin, but they produce other substances belonging to the aromatic bodies.

These are arranged in two groups of bodies. The one contains phenol C_6H_5OH and its methyl derivative kresol $C_6H_4<^{CH_3}_{OH}$. These are produced from tyrosin, which, it will be remembered, has the formula $C_6H_4<^{OH}_{CH_2CH(NH)_2COOH}$, and when it changes into these bodies, the amido propionic acid side chain loses first its amido group as ammonia, and then its carboxyl and methyl groups are oxidised and given off as carbonic acid and water.

The other group is more complex, and contains indol $C_6H_4<^{NH}_{CH}>CH$ and its methyl derivative skatol

$$C_6H_4<^{NH}_{C}>CH$$
$$\qquad\qquad\quad\backslash CH_3.$$

These are derived from tryptophan, a product of tryptic digestion of true proteids. Its chemical reactions are described on p. 452. Its chemical constitution is skatol amido acetic acid

$$C_6H_4<^{C\ /CH_3}_{NH}>C-CH(NH_2)COOH.$$

It is anaerobic bacteria which first of all act on the tyrosin and tryptophan, and split off from them the amido (NH_2) groups as NH_3.

After this has been accomplished, aerobic organisms act on the remaining side chains yielding carbonic anhydride and water.

These aromatic bodies have a strong faeculent odour which they impart to the faeces. Certain proportions of them are, however, absorbed into the blood and reappear in the urine in combination with alkalies as salts (see Urine, p. 263).

These products also result when proteids undergo putrefaction in the air, but in this latter case other bodies called ptomaines are also produced which are powerful poisons. It is on account of the presence of these that it is dangerous to eat putrid flesh. The action of bacteria on **carbohydrates** is even more energetic than it is on proteids. They can do all that ptyalin and amylopsin can do, but besides this they can decompose the monosaccharides into simpler bodies such as ethylic alcohol, lactic and butyric acids. They have also the power of digesting cellulose whereby methane (CH_4) is produced as one of the products.

On **fats** they act like steapsin, but here also they can carry the process a stage further in that they transform the fatty acid into members lower in the series. They decompose lecithin, and prevent the poisonous action of the liberated cholin by further breaking it up into carbon dioxide, methane and ammonia. We see, therefore, that bacterial action is more beneficial than otherwise, and since they can accomplish so much it has been suggested that the intestinal bacteria are the active agents in digestion, and that the various digestive juices serve mainly as nutritive pabula for them to grow on. Such a view is, however, erroneous, for it has been shown that if guinea pigs be excised from the uterus just before full term under antiseptic precautions, and kept in a chamber aspirated with sterile air, and fed on sterile milk, they thrive, and if after some time the intestinal contents be examined, the latter will be found free of bacteria.

Further, the intestinal contents of animals in the arctic regions have been shown to be nearly sterile, so that there can be no doubt that bacterial growth is not essential for efficient digestion.

The Faeces.—These are composed of the following substances:

1. Substances which have escaped digestion, *e.g.* pieces of vegetables, muscle fibres, elastic tissue, casein, fat, nuclein, haematin, etc.

2. Remains of juices secreted into the intestine, *e.g.* mucin, traces of bile salts and pigments, inorganic salts (alkaline earths), epithelial cells, etc.

3. Products of digestion, *e.g.* aromatic bodies, fatty acids, methane, ammonia, etc.

CHAPTER XV.

CHEMISTRY OF URINE.

THERE is probably no other portion of Chemical Physiology of greater importance from a clinical standpoint than the chemistry of the urine. It is in the urine that the chief end-products of proteid metabolism (*e.g.* urea, uric acid, etc.) are excreted, so that a determination of the amount of these will afford us information regarding the activity of chemical interchange in the tissues, of which proteid matter forms the chief part. Again, it is by means of the kidneys that the blood is kept of constant composition, any excess of the normal constituents (*e.g.* sugar) or any foreign matter (*e.g.* drugs, unusual proteids) being removed by them and excreted in the urine. In disease of the urinary tract the pathological products (*e.g.* albumin, blood, cells, etc.) are admixed with the urine and can be detected in it in a more or less changed state, according to the exact site of the lesion. From a purely physiological point of view it is of peculiar interest, since it is in the urine that the incompletely oxidised products of metabolism are excreted, the carbonic acid and water excreted by the lungs representing the completely oxidised.

In studying its chemistry, therefore, we must ascertain firstly, the nature of the various constituents and their precursors in the blood and tissues; secondly, the total amount of those excretory products which contain the nitrogen of the decomposed tissue proteid; and thirdly, we must look for unusual products indicating improper composition of the blood or organic disease of the urinary tract.

We must remember that the quantity and composition of the urine vary considerably within the limits of health, and in order to form reliable conclusions we must collect the total urine for a period of twenty-four hours. Even with a fair sample thus obtained we must consider the intake and loss of water; copious drinking will increase the quantity and lower the specific gravity of the urine; on the other hand, profuse sweating will have the opposite effect. The nature of the diet in relation to the reaction of the urine and the quantity of urea must also be considered.

GENERAL CHARACTERS OF URINE.

Quantity.—A healthy man of average weight (65-70 kg.) and height, and living on an ordinary mixed diet, excretes about 1500 c.c. per 24 hours. A knowledge of the total daily excretion of urine is in-

dispensable if we wish to ascertain whether any one of its constituents is being excreted in normal amount, a mere determination of the percentage in an isolated sample being of *very slight* value. For accurate work (*e.g.* in making observations in metabolism) the method employed is to collect the total urine for the 24 hours in a graduated urine jar fitted with a glass lid, and then to remove a measured sample for analysis. The amount of urine is increased by the imbibition of large quantities of liquid and by certain drugs called diuretics which act on the renal circulation; it is diminished by excessive sweating or diarrhœa, and by failure of the heart's action.

FIG. 149.—The urinometer.

Specific Gravity.—This is determined by a special form of hydrometer—a urinometer—graduated so that the zero mark—1000—corresponds to distilled water (Fig. 149).

EXPERIMENT I. Fill a urine testing glass with urine and place the urinometer in it, and read off the graduation which is on a level with the surface of the urine.

The average density varies between 1015 and 1025, but a highly concentrated urine—*e.g.* after severe sweating—may reach 1035, or a very dilute one—*e.g.* after huge potations—1002, and still be healthy. A specific gravity over 1030, however, usually indicates the presence of sugar or the existence of high fever, and one much below 1010 is suspicious of some renal trouble.

It is important to remember that it is only dissolved substances which affect the density.

Reaction.—Healthy urine usually reacts acid to litmus.

EXPERIMENT II. Test the reaction of urine with litmus.

This acidity is not due to free acids but to acid salts.

EXPERIMENT III. Add some urine to about 5 c.c. of a solution of congo red in a test-tube, and the colour of the reagent remains unchanged. Congo red is turned blue by free acids, but not by acid salts (see Digestion, p. 220). The salt which produces the acid reaction in urine is acid phosphate of sodium NaH_2PO_4. There is also a certain amount of the alkaline phosphate Na_2HPO_4 present, and under certain conditions, this becomes increased

in amount till it may equal that of the acid salt when the reaction is amphoteric, or it may even overstep this and cause the urine to react alkaline. This increase of the alkaline salt is established during digestion—alkaline tide—because of the liberation of chlorine from the chlorides of the blood to produce the hydrochloric acid secreted by the stomach, and, as a consequence of which, bases are liberated and form the alkaline salt of phosphoric acid.

An alkaline reaction may also be caused by the presence of alkaline carbonates, which cause the urine to effervesce on the addition of a mineral acid. The excretion of these is increased by the administration of the salts of certain organic acids (*e.g.* citric, tartaric, etc.), the carboxyl radicles of which become oxidised into carbonates in the blood. It is on this account that the urine of herbivorous animals and of vegetarians reacts alkaline. Lastly, an alkaline reaction may be due to ammonia, which is produced by microbal hydration of urea (see Urea, p. 249). On account of this, stale urine always reacts alkaline.

Experiment IV. Some urine has been allowed to stand for two days; note that it smells of ammonia, and that the blue stain on litmus disappears if the paper be gently warmed.

If the newly excreted urine contains a detectable amount of ammonia[1] it points to decomposition of urine in the bladder, or to extensive disease of the liver cells.

Colour.—The pale straw colour of healthy urine is due to *Urochrome*, a pigment which is derived, probably by a process of oxidation, from another pigment called *Urobilin*, which, however, is present only in traces in healthy urine. In febrile urines, and in the urine of cases in which extravasation of blood into the tissues exists, the urobilin becomes much increased in amount, this suggesting its derivation from haemoglobin, a fact which is borne out by other evidence. Urobilin also exists in the urine as a colourless precursor, called a *chromogen*, and this may be changed into the pigment by the addition of strong acids.

A third pigment, called *uroerythin*, is also present in small amount. It is the colouring matter of pink urate deposits. Its exact chemical relationships are not known.

Another pigment, which occurs only in the minutest traces in normal urine, but which may be present in considerable amount in pathological urine, is *haematoporphyrin*. Chemically it is iron-free haematin and can be prepared artificially from haemoglobin (see p. 203).

Quantitative Composition of Urine.—Although it is from the chemical interchange or metabolism in the tissues that most of the

[1] *i.e.* detectable by the above test.

urinary substances are derived, the amounts of these vary enormously according to the nature of the diet. The reason of this is that the diet is the chief controlling factor of tissue metabolism. A proteid-rich food leads to more active tissue metabolism than does a proteid-poor one, as a consequence of which there are more solids excreted in the urine during the former than during the latter diet. In studying the composition of urine, therefore, we must bear this in mind before deciding whether any constituent is increased or diminished in amount. The average composition of the 24 hours urine during health in a young man is approximately as follows:

		I. Mixed diet.	II. Flesh diet.	III. Bread diet.
Total Amount,		1500 c.c.	1672 c.c.	1920 c.c.
Organic	Urea,	33·18 gr.	67·2 gr.	20·6 gr.
	Uric acid, etc.,	0·55 gr.	1·398 gr.	0·253 gr.
	Kreatinine,	0·91 gr.	2·163 gr.	0·961 gr.
	Ammonia,	0·77 gr.	0·9 gr.	0·4 gr.
	Hippuric acid,	0·40 gr.	—	—
Inorganic	K_2O,	*2·50 gr.	3·308 gr.	1·314 gr.
	Na_2O,	*11·09 gr.	3·991 gr.	3·923 gr.
	CaO,	*0·26 gr.	0·328 gr.	0·339 gr.
	MgO,	*0·21 gr.	0·294 gr.	0·139 gr.
	Cl,	7·50 gr.	3·817 gr.	4·996 gr.
	SO_3,	2·01 gr.	4·674 gr.	1·265 gr.
	P_2O_5,	3·16 gr.	3·437 gr.	1·658 gr.

*Calculated here as the metal and not as the oxide.

The Organic Constituents.—With the exception of some of the aromatic bodies absorbed from the intestine, the organic substances in the urine all contain nitrogen, and if we add to these the small amount excreted as ammonia, we can then account for over 90 per cent. of all the nitrogen resulting from tissue metabolism, the remaining fraction being lost mainly in the faeces (1 gramme per diem), and slightly in the sweat as urea. It is most important, therefore, first of all to become acquainted with some method by which the **total nitrogen** excreted in urine can be estimated. This is furnished by **Kjeldahl's method**, the principle of which is the following.

DEMONSTRATION.—1*st Stage—Incineration.*—A measured quantity of urine—5 c.c.—is placed in a combustion flask,[1] and to it about twice that amount of concentrated pure sulphuric acid is added. The mixture is then gradually heated till just below the boiling point of the acid (the flask being placed in a slanting position), and is kept at this temperature till it becomes clear. The chemical reaction which takes place is that the H_2SO_4 decomposes the organic matter, the carbon being oxidised to carbon dioxide, and the nitrogen changed into ammonia, which immediately on its formation combines with the

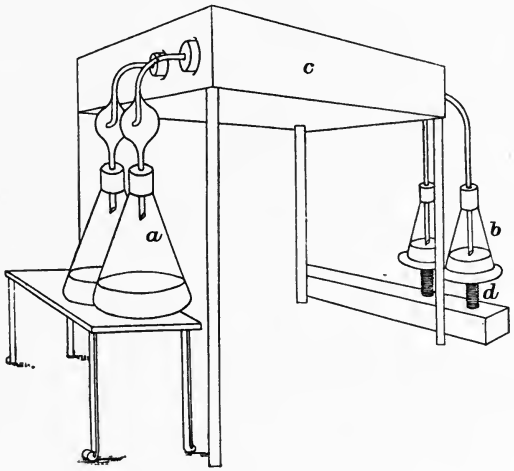


FIG. 150.—Distilling portion of Kjeldahl's apparatus.

excess of sulphuric acid present to form ammonium sulphate. The first effect of adding the acid is to produce charring (*i.e.* the mixture becomes black), and the reaction is complete whenever all this liberated carbon has been oxidised and the mixture has become colourless. This latter transformation can be considerably hastened by adding a few crystals of potassium permanganate which oxidise the carbon. (For other methods see Advanced Course.)

2nd Stage—Distillation.—The contents of the flask are allowed to cool, and are then dissolved in distilled water, the resulting solution being carefully transferred to a large Erlenmeyer's flask (*a*)—*i.e.* a conical flask made of combustion glass and holding 600 c.c. To this solution of ammonium sulphate (containing an excess of sulphuric acid) a few

[1] This flask should have a capacity of 300 c.c. ; it is made of hardened glass, has a long neck, and a round bottom.

pieces of granulated zinc are now added to prevent bumbing, and it is then mixed with a concentrated solution of pure caustic soda till faintly alkaline. The amount of the caustic alkali solution necessary to exactly neutralise the quantity of acid used in the incineration should have been previously determined by titration, and a little more than the necessary amount then carefully poured down the side of the distilling flask, so as to form a layer under the acid ammonium sulphate solution. By this means any ammonia gas which might be produced where the two fluids are in contact, is at once absorbed by the overlying layer of acid. The flask is now connected with the distilling apparatus (see Fig. 150), the other end of this being attached to a tube which touches the surface of a measured quantity of decinormal $\left(\frac{n}{10}\right)$ sulphuric acid contained in another Erlenmeyer flask (*b*).

When all is ready the contents of the distilling flask are shaken up so as to mix them, the burner is lighted underneath it, and distillation allowed to proceed till no more ammonia gas is given off. The chemical reaction which now ensues is that the excess of alkali in the distilling flask reacts with the ammon. sulph. liberating free ammonia, which distills over and is at once absorbed by the $\frac{n}{10}$ acid placed to receive it.

3*rd Stage—Titration.*—Before describing this it will be necessary to explain what is meant by a *normal or decinormal solution.*

A normal solution is one which contains the molecular weight of the substance in grammes dissolved in 1000 c.c. of water. Sodium hydrate has the molecular weight: $Na_{23}O_{16}H_1 = 40$, therefore a normal solution (*n*) would contain 40 grammes per 1000 c.c., and a decinormal solution $\left(\frac{n}{10}\right)$ 4·0 grammes. Hydrochloric acid has the molecular weight: $H_1Cl_{35\cdot5} = 36\cdot5$, therefore a normal solution would contain 36·5 grammes per 1000 c.c., and a decinormal 3·65 grammes.

In other words, a measured quantity (say 50 c.c.) of $\frac{n}{10}$ acid would exactly neutralise the same amount of $\frac{n}{10}$ alkali. In the case of an acid which forms two kinds of salts—*i.e.* a dibasic acid—such as sulphuric, which forms an acid—$KHSO_4$—and a neutral salt—K_2SO_4—the molecular weight must be halved in order to form a normal solution. The reason of this is quite evident when we consider that instead of only forming one salt, as does a monobasic acid, it forms two. The molecular weight of H_2SO_4 is 98, therefore a normal solution equals 49 grammes per 1000 c.c. and $\frac{n}{10} = 4\cdot9$ grammes per 1000 c.c.

In the case of a tribasic acid such as phosphoric a third of the molecular weight would be taken.

To Titrate a Solution.—An accurately measured quantity—say 50 c.c.—is measured into an Erlenmeyer's flask, and mixed with about two drops of a solution of methyl orange. This gives a deep red colour with acids and a faint yellow with alkalies. Suppose 50 c.c. $\frac{n}{10}$ acid had been taken, the red of the indicator would change to yellow when exactly 50 c.c. of $\frac{n}{10}$ alkali had been added. Litmus solution might also be employed, but it is not so delicate. (For standardising the decinormal solutions, see Advanced Course.)

The application of the above to the determination of the liberated ammonia in Kjeldahl's method will now be readily understood. A measured quantity—say 50 c.c.—of $\frac{n}{10}$ acid is placed in an Erlenmeyer's flask, and in contact with the surface of this is the distillation tube. As the NH_3 comes over, it at once combines with the acid to form ammonium sulphate. After distilling for half-an-hour, test to see if all NH_3 has come over. This may be done by raising the distilling tube a little and removing a drop of the distillate with a clean glass rod and placing it on sensitive red litmus paper; if no blue stain results all NH_3 is over; if a blue stain results wash the glass rod and litmus paper with a jet of distilled water, allowing the washings to run into the receiving flask, and continue the distillation.

When all the NH_3 is distilled remove the receiving flask and titrate with $\frac{n}{10}$ alkali. The number of c.c. of this which it is necessary to employ in order to produce neutralisation corresponds to the number of c.c. of $\frac{n}{10}$ acid which has not combined with NH_3, and if we deduct this from the quantity of $\frac{n}{10}$ acid originally employed, we obtain the number of c.c. of $\frac{n}{10}$ acid which has been neutralised by NH_3. This result multiplied by 0·0014[1] gives the amount of nitrogen in grammes contained in the urine.

Experiment V. Determine by the above method the amount of nitrogen contained in an acid solution of ammonium sulphate.[2]

[1] Because the molecular weight of nitrogen is 14. Each c.c. of an N solution equals $\frac{14}{1000} = \cdot014$; or of a $\frac{n}{10}$ solution ·0014.

[2] A suitable solution for the purpose is made by dissolving 1·32 grammes of ammonium sulphate crystals in 100 c.c. of 1 per cent. sulphuric acid; 5 c.c. of this solution contains 0·014 grammes N.

Measure out 5 c.c. of the solution with a pipette, place it in the distilling flask *A*, dilute to 200 c.c. with water. Now measure accurately 20 c.c. $\frac{n}{10}$ acid and place in receiving flask *B*, adjust distilling tube *C*, neutralise contents with NaOH, and distil as above.

The total amount of nitrogen excreted by the urine per diem varies between 15 grammes and 20 grammes, and if the total amount taken as food be ascertained it will be found to nearly correspond to this. By special precautions it can be made to accurately correspond when the person is said to be in *nitrogenous equilibrium.* The excretion during starvation will be discussed under Urea.

The Nitrogenous Compounds.—The nitrogen excreted in the urine is distributed in the various compounds in the following proportions:

Urea, - - - - - - -	86 per cent.
Ammonia, - - - - - -	3 ,,
Creatinin, - - - - - -	3 ,,
Uric acid and purin bodies, - - -	2 ,,
Pigments, nucleo-albumin, hippuric acid, etc.,	6 ,,

As will be pointed out later, the relationship of ammonia to urea is of great importance, since, in certain diseases of the liver, it becomes changed, relatively more ammonia being excreted.

CHAPTER XVI.

UREA: CHEMICAL RELATIONSHIPS.

Urea is a di-amide of carbonic acid. When studying the decomposition products of proteids (see Intestinal Digestion) we came across amido acids, in which an *H* atom of the methyl radicle was substituted by an amido group—NH_2. If, now, the hydroxyl—OH—radicle of the carboxyl—COOH—be replaced by NH_2, a body called an *acid amide* is produced. Take acetic acid, CH_3COOH, and its amido acid has the formula, CH_2NH_2COOH, and its acid amide, CH_3CONH_2. *Urea* is closely related to an acid amide. Carbonic acid has the formula $CO<^{OH}_{OH}$; if, now, we substitute both hydroxyl radicles by an amido group we obtain $CO<^{NH_2}_{NH_2}$, which is urea. Since this contains two ammonia residues it manifests weak basic properties, and forms loose salts with nitric and oxalic acids.

Experiment I. To some urine, which has been evaporated to small bulk on a water-bath, add some pure (not fuming) HNO_3, and cool the mixture by holding the test tube under the tap. Crystals of urea nitrate separate out. Examine these with the microscope, and note that they are either rhombic tables or six-sided plates, which overlap each other like the tiles of a roof (see Fig. 151).

Experiment II. Repeat Experiment I. with oxalic acid, and note that the crystals are not unlike those of the nitrate, being elongated plates with bevelled pointed ends (Fig. 152).

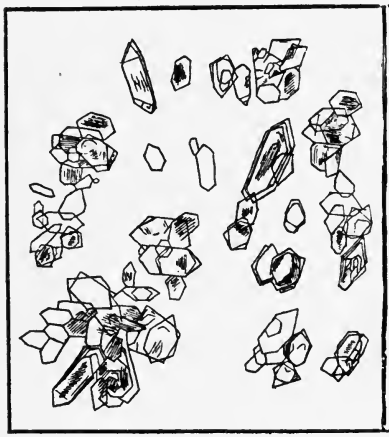


Fig. 151.—Urea nitrate.

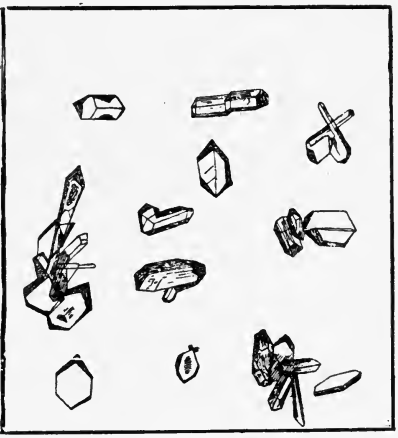


Fig. 152.—Urea oxalate.

Like all other substituted ammonias urea is decomposed by nitrous acid—HNO_2—carbonic acid gas and nitrogen being evolved:

$$CO\begin{matrix}<\\ \end{matrix}\begin{matrix}NH_2\\ NH_2\end{matrix} + \begin{matrix}O=N-OH\\ O=N-OH\end{matrix} = CO^2 + 2N_2 + 3H_2O.$$

Experiment III. Add some *fuming* nitric acid (*i.e.* containing HNO_2) to urine and note the effervescence which results. That one of the gases evolved is CO_2 can be proved by holding the mouth of the test-tube over another one containing lime water, when, on shaking, the latter will turn milky. A very similar reaction is obtained by adding a hypobromite or hypochlorite:

$$CO\begin{matrix}<\\ \end{matrix}\begin{matrix}NH_2\\ NH_2\end{matrix} + 3NaBrO = CO_2 + N_2 + 2H_2O + 3NaBr.$$

This reaction is taken advantage of in its quantitative estimation (see below, p. 250).

There are several reactions which are peculiarly interesting, since they demonstrate the chemical relationships of urea to its probable pre-

cursors in the tissues (see below). Thus if urea be hydrolysed (*i.e.* be caused to take up a molecule of water) it forms ammonia carbonate:

$$CO\begin{matrix}NH_2\\NH_2\end{matrix} + \begin{matrix}HOH\\HOH\end{matrix} = CO\begin{matrix}ONH_4\\ONH_4\end{matrix}$$

Urea. *Water.* *Ammonia carbonate.*

This process occurs in urine which has stood for some time, the hydrolysis being effected by several microbes. It may also be produced by boiling urea with weak acids or alkalies, in both of which cases the ammonium carbonate is further decomposed, liberating in the case of alkalies ammonia gas (the CO_2 being absorbed by the alkali present), and in the case of acids carbonic acid gas (the NH_3 being absorbed by the acid present).

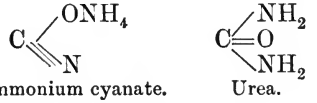
EXPERIMENT IV. Prepare a solution of pure urea, and divide it into two portions *A* and *B*. To *A* add about 10 drops 10 per cent. HCl and boil, meanwhile collecting the vapour which comes off in a second test-tube containing lime water. By this becoming milky, the presence of CO_2 gas is demonstrated. To *B* add about 5 drops saturated KOH and boil. NH_3 gas is evolved so that a moistened strip of red litmus paper is turned blue if held in the fumes, which also smell strongly of ammonia.

A substance intermediate between urea and ammonium carbonate, and having therefore the formula $CO\begin{matrix}ONH_4\\NH_2\end{matrix}$, can be formed by allowing dry CO_2 gas to act on dry NH_3. This is called *ammonium carbamate*, and if heated to 135° C. it splits up into urea and water.

Dry heat splits urea into ammonia gas and a body called *biuret*, which, by further heating, changes into *cyanuric acid* $(HCNO)_3$, which is isomeric with cyanic acid, HCNO.

EXPERIMENT V. Heat some urea crystals in a dry test-tube. Note that they melt and give off NH_3. Continue heating for a few minutes then cool the test-tube and dissolve the residue in H_2O, and to this solution apply the biuret test. A rose pink colour results (see Peptone, p. 224).

Conversely, we can change cyanic acid into urea by evaporating an aqueous solution of ammonium cyanate (NH_4CNO) to dryness. This has the same empirical formula as urea, but its structural formula is different:



Ammonium cyanate. Urea.

It was by this means that Wöhler first showed that inorganic could be changed into organic bodies.

For **quantitatively estimating urea** two methods may be employed :— I. Precipitate from the urine all nitrogen-containing bodies except urea, filter, then determine the amount of nitrogen in the filtrate, and from this calculate the urea (see Advanced Course). II. By decomposing urea with Sodium Hypobromite in the presence of free caustic alkali.

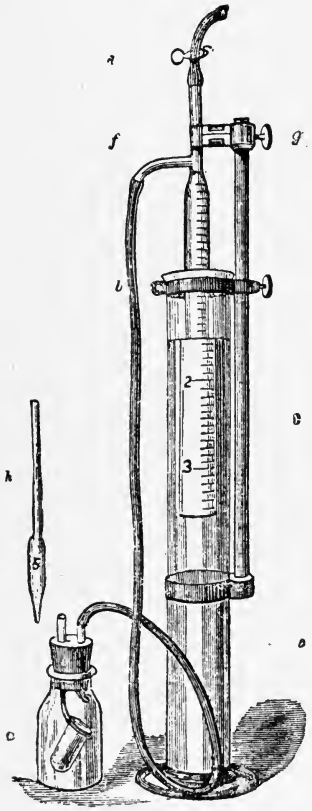


FIG. 153.—Dupré's urea apparatus.

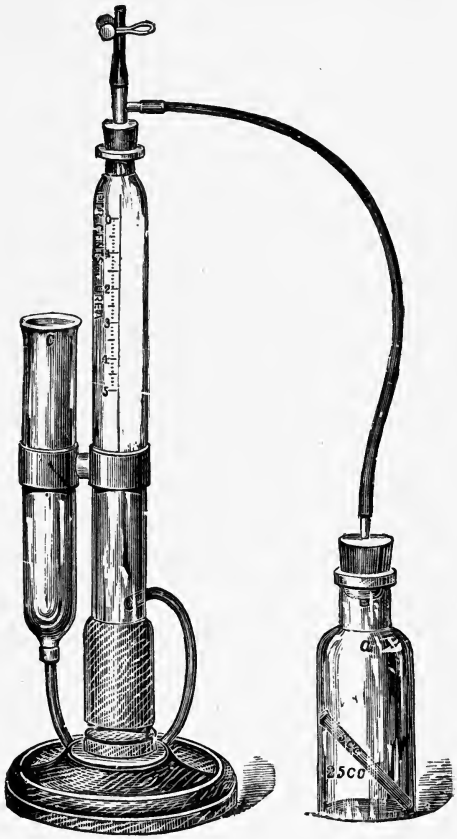


FIG. 154.—Gerrard's urea apparatus.

The alkali absorbs the liberated carbonic acid and the nitrogen is collected in a graduated tube. From the amount of nitrogen evolved the urea can be calculated by remembering that 0·1 gramme urea contained in urine, liberates 37·1 c.c. nitrogen.[1] There are various forms of apparatus used for collecting the liberated nitrogen. The most

[1] 0·1 gr. of pure urea really liberates 37·27 c.c. of N. In urine only about 92 per cent. of the urea nitrogen is liberated by the hypobromide. This deficit is, however, partly compensated for by a certain amount of nitrogen being simultaneously split off from the other nitrogenous bodies present.

accurate is that of *Dupré* (Fig. 153), which consists of an inverted burette (*a*) placed in a cylinder of water, and to the neck of which is connected a T piece (*f*). With the side tube of this the generating bottle is connected by indiarubber tubing, and the other tube is closed with a piece of tubing and a clip. To make the estimation 25 c.c. of the alkaline solution of NaBrO are placed in the generating bottle (*o*) and 5 c.c. urine in a small tube, which is then carefully placed in the generating bottle without allowing the two fluids to mix. The cork of the generating bottle is then inserted, and the water meniscus inside and outside the burette brought to the same level at the zero mark, the clip on the T piece being meanwhile open, and water being added to, or removed from, the outer vessel as necessary. The clip is now applied, and the burette raised to ascertain that no leakage exists. Now readjust the two menisci, and mix the contents in the generating bottle. The evolved N displaces the water in the burette. After the reaction is complete immerse the generating flask in a basin of water, so as to bring the temperature of the gas contained in it to the same as that of the gas in the burette. After two minutes bring the two menisci to the same level, and read off the number of c.c. of N. Another form of apparatus is that of Gerrard (Fig. 154).

The determination may also be made by means of the following improvised apparatus (Fig. 155).

The neck of a burette *C* is connected by means of a long piece of indiarubber tubing with a glass tube *D*, dipping under the surface of water contained in a bottle *B*. Through the cork of this a second tube *E* connects it with the generating bottle *A*, and a third tube *F*, closed by a piece of tubing and a clip, is also inserted in the cork of *B*. To make the estimation the burette is raised, and water poured into it till the tube *D* is full. It is then lowered till the water stands at the lowest graduation (50 or 100) of the burette, and is on the same level as the water in *B*. The urine and hypobromite solution are then placed in the generating bottle, the cork of this inserted, the tube *F* closed, and the solutions in *A* mixed. The gas pushes the water into the burette, and, after the generating bottle has been cooled, the meniscus of water in the burette is brought on a level with that in *B*, either by lowering the burette or raising *B*. The amount of displaced water is then read off, and from it the urea is calculated.[1]

The Metabolic Changes which give rise to Urea.—Urea is in man the chief end product of proteid metabolism, and consequently varies enormously with the nature of the diet.

Experiment VI. This can be very simply demonstrated by making a determination of the percentage contained in a sample of urine passed

[1] The corks must be of indiarubber.

about four hours after breakfast, the latter consisting, one morning, mainly of porridge or some similar proteid-poor diet, and on the next morning mainly of some form of flesh or eggs.

During starvation the excretion rapidly falls for the first few days, and then remains constant for a week or two—*the starvation level*—after which it again rises somewhat for a few days, this being followed by a sudden fall accompanied by the death of the animal. The reason of the primary fall is that no proteid food is being supplied to the vital tissues, and, as a consequence of this, their metabolism becomes less

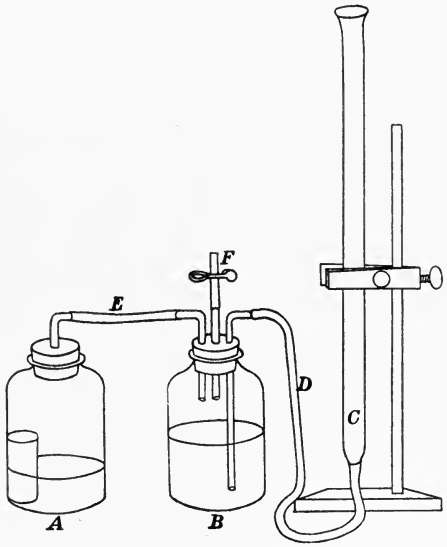


Fig. 155.—Improvised apparatus for estimation of urea.

active, the energy necessary for life being meanwhile supplied by the deposited fat. This latter ultimately becomes used up, however, and then the proteids are called upon to supply the necessary energy, and the urea excretion rises again. This disintegration of the vital tissues cannot last for long, however, as the proteid tissues are indispensable for life, and the animal dies.

As regards the *intermediate bodies* between serum albumin and globulin (as which the bulk of the proteids are absorbed into the portal blood), and urea, very little is known. The majority of the intermediate bodies have been already described in connection with the tissues in which they are found, and all that remains is to string them together in their proper sequence.

By the hydrolysis of proteid in the intestine, *amido acids and hexone*

bases are liberated. These are chemically closely related to urea, and if they be given in the food they at once lead to an increase in the urea excretion. It is almost certain, however, that no such process occurs physiologically since these bodies cannot be detected in the blood, so that, although large amounts of them can certainly be obtained in an artificial tryptic digest, it is probable that, in the intestine, the bulk of the proteid is at once absorbed when it reaches the stage of peptone, and that only a trace is further hydrolysed into these bodies.

The absorbed serum albumin and globulin are carried to the active tissues (*i.e.* muscles, glands, and nervous tissues) where they undergo complicated changes leading to the production of disintegrative products, among which is *creatin*. It will be remembered that this body can be very easily changed into urea in the laboratory (see p. 205), and that it exists in the tissues in very large amount, whereas the urine only contains a small quantity (as creatinine) (see p. 261). Many experiments have accordingly been made to study how the urea excretion behaves on administration of creatin as food, or after its injection into the blood. It has invariably been found that the urea is not increased in the slightest, but that all the administered creatin reappears as urinary creatinin. It is possible, however, that this foreign or exogenous creatin is not quite the same thing as the creatine naturally produced by the tissues, *i.e.* endogenous, and that, whereas the latter becomes changed into urea, the former does not undergo this change.

A third probable precursor of urea is *ammonium salts*. If ammonium carbonate or citrate be given by the mouth, the urea excretion at once rises. These salts have also been found in small quantities in the blood, more especially in that of the portal vein. The chemical transformation is a very simple one (see p. 248), and since they are found most abundantly in the portal blood, it is probable that before the amido acids or hexone bases, absorbed from the intestine, are transformed into urea, they are split up into ammonium carbonate and a residue containing most of their carbon, and which is further made use of by the tissues to produce energy, or to form fat.

From one of the hexone bases, viz. arginine, urea can be very easily obtained by boiling with baryta. Now arginine is chemically very like creatin, one point of difference being that the former, when administered as food or injected into the blood, causes an increase in urea, the latter does not.

There can be no doubt, then, that amido acids and hexone bases, and

for the reasons given above, probably also endogenous creatin are transformed by the tissues into urea. If we compare their formulae with that of urea, however, we find that they contain too many carbon atoms

Leucin,	$C_6H_{13}NO_2$	Creatin,	$C_4H_9N_3O_2$
Arginine,	$C_6H_{14}N_4O_2$	Urea,	CH_4N_2O

in relation to their nitrogen, so that before such a transformation can take place they must break off some of their carbon as other bodies—fat or carbohydrate—the nitrogenous moiety being then liberated probably as a salt of ammonia.

Other probable precursors of urea are the *alloxuric bodies* (see p. 206).

Regarding the Site of Formation of Urea, there can be no doubt that it is the *liver*. The products of tissue metabolism are conveyed here, and changed into urea which is then excreted by the kidneys. The proofs of this are the following:

(1) If the liver be excised and perfused with defibrinated blood, urea is formed if ammonium salts, or amido acids be added to the blood. To do this experiment an animal is bled, and its blood defibrinated. The liver is then excised, and cannulae inserted into the portal and hepatic veins. The former of these is connected with a pressure bottle containing the defibrinated blood to which some precursor of urea is added, and the latter with a dish to collect the perfused blood. The urea is estimated in a sample of blood from the pressure bottle—*i.e.* before perfusion—and another one from the collecting dish—*i.e.* after perfusion. It is found that the latter contains much more urea than the former.

If this experiment be repeated with any other organ, or with the muscles, no urea formation results.

(2) If the liver be removed from the circulation the urinary excretion of urea is diminished, this being accompanied by a corresponding increase in the ammonia. In mammals, the liver extirpation is soon followed by the death of the animal, but it can be removed to a large extent from the circulation by attaching the portal vein to the inferior vena cava, and thus diverting the blood stream so that no blood gets to the liver by this path—*Eck's fistula*. After such an operation it has usually been found that the diminution of urea is very slight, the liver being still supplied by blood from the hepatic arteries, and by blood eddying back through the hepatic veins. In certain cases, however, the diminution has been quite marked.

In birds and reptiles, uric acid takes the place of urea as the end product of proteid metabolism. In these animals, extirpation of the liver is practicable on account of a natural anastomosis between the portal vein and the *vena advehens* going to the kidneys. If, now, the portal

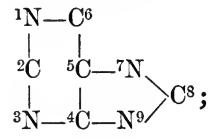
vein be ligatured above this branch, the portal blood is diverted into the kidneys. The liver can now be extirpated, the animal surviving the operation several hours, and during this time the uric acid almost entirely disappears from the urine, ammonia lactate taking its place. Reasoning from analogy, then, we may suppose that urea is also formed —from ammonia salts—in the liver of mammals.

(3) In certain diseases of the liver—*e.g.* acute yellow atrophy, phosphorus poisoning, marked cirrhosis—the hepatic cells die and lose their power of forming urea, and as a consequence of this, the urea diminishes in the urine, ammonia salts, and even amido acids (leucin and tyrosin) taking its place.

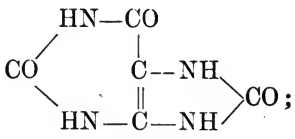
CHAPTER XVII.

URIC ACID AND THE OTHER PURIN BODIES.

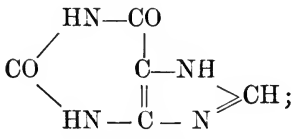
It will be remembered, from the description of the chemical structure of nuclein (p. 179), that there exist among its decomposition products several bodies belonging to the so-called purin group of chemical substances. Uric acid is also a member of this group. The group receives its name, because all the members of it contain as their nucleus of construction a body called purin, which exists as a double ring of carbon and nitrogen atoms. The various members of the group differ from one another according to the nature and position of the various side chains, which are tacked on to this purin ring. In order to make the relationship clear the structural formulae of the various members should be studied side by side, thus:



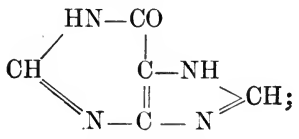
Purin.



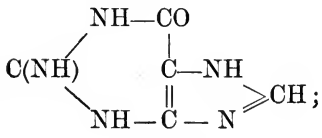
Uric acid.



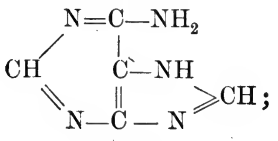
Xanthin.



Hypoxanthin.

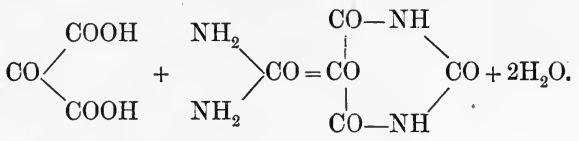


Guanin.



Adenin.

From a study of these formulae, it will be seen that purin really consists of two urea radicles joined together by a central chain of three carbon atoms. It would, however, be more accurate to describe it as consisting of a radicle of urea united with one of **alloxan,** which is produced by condensation of mes-oxalic acid with urea.



Mes-oxalic acid. *Urea.* *Alloxan.*

These substances are, therefore, sometimes named the alloxuric bodies. (For further details of chemistry, see p. 206.) For convenience of description the various atoms in the purin ring are numbered.

The lowest oxidation product of purin is **Hypoxanthin** (6 oxypurin). It occurs abundantly in muscle extract (p. 207) and in the extracts of other tissues, and also in the urine. It always exists along with **Xanthin** which is 2, 6 di-oxypurin.

If the oxygen in hypoxanthin be replaced by an amido group 6 amino-purin results. This is **adenin,** and is the chief purine body found in nuclein prepared from the thymus gland. It only exists in traces in the urine.

A similar derivative of xanthin (2—amino 6—oxypurin) is called **guanin.** It is the chief purin found in nuclein prepared from the pancreas, and exists in certain pigments of insects and fishes. It occurs abundantly in *guano,* but only exists in traces in urine.

If three oxygen atoms be present we have **uric acid** (2, 6, 8 trioxypurin), and this is the form in which nearly all the "tissue purins" are excreted in the urine.

The empirical formulae for these bodies are therefore:

	Purin,	$C_5H_4N_4$.
Purin Bases	Hypoxanthin,	$C_5H_4N_4O$.
	Xanthin,	$C_5H_4N_4O_2$.
	Adenin,	$C_5H_4N_4NH$.
	Guanin,	$C_5H_4N_4ONH$.
	Uric acid,	$C_5H_4N_4O_3$.

Of these the uric acid is by far the most abundant in urine, whereas the purin bases are most abundant in the tissues. In metabolism, therefore, the latter form the precursors of the former.

Preparation and Properties of Uric Acid.—Experiment I. To 100 c.c. urine are added 5 c.c. HCl (con.), and allow the mixture

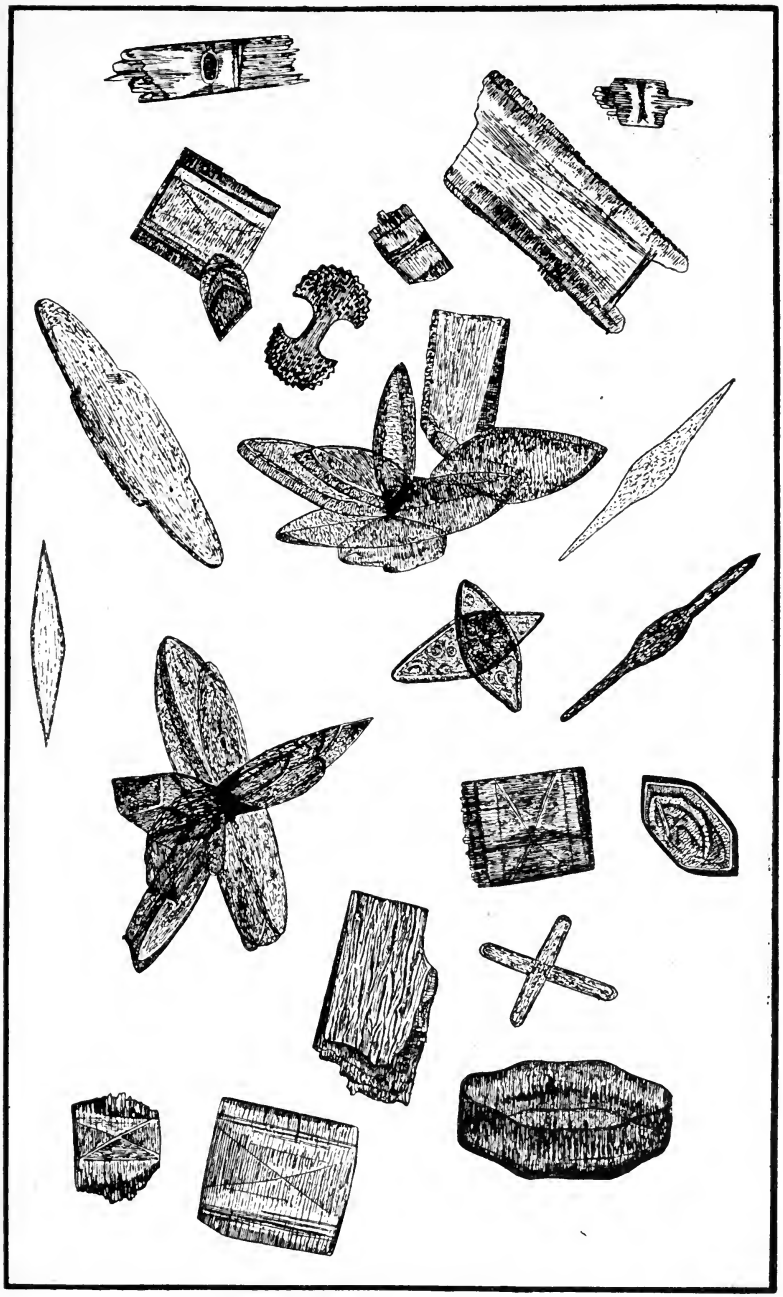


FIG. 156.—Crystals of uric acid.

to stand overnight. It will then be found that a dark brown sediment, like cayenne pepper, has settled down, and probably also that a brown scum has formed on the surface. Filter and examine the sediment under the microscope. It consists of large dark-brown clumps of crystals, whetstone or barrel-shaped (Fig. 156). These are crystals of uric acid admixed with pigment. They can be purified by solution in 5 per cent. KOH and reprecipitation by HCl.

EXPERIMENT II. Pure crystals can be obtained from the solid urine of a snake or bird. This urine, which consists of sodium urate, is dissolved in caustic potash and acidified with HCl. Pure uric acid separates out.

From these two experiments we learn that uric acid exists in urine as a salt. If this salt be decomposed by a mineral acid the liberated uric acid, being very insoluble, is precipitated.

Since uric acid contains two replaceable hydrogen atoms, it is a *dibasic* acid, and may be represented by the formula H_2U. Consequently it may form an acid salt—MHU—in which only one *H* atom is replaced by a monad,[1] or a natural salt—M_2U—in which they are both replaced.

The following are the most important *reactions of uric acid.*

EXPERIMENT III. **The Murexide Test.**—Place some uric acid or bird's urine in a capsule, add a few drops of dilute nitric acid, evaporate *slowly* to dryness on a water-bath. A yellow residue is obtained (consisting partly of *alloxan*, see p. 256). Add a drop of ammonia, a deep purple colour results, which is changed to blue by adding caustic soda. This reaction is due to the alloxan changing into ammonium purpurate.

Another way of doing the test is to leave the dry yellow residue on the water bath till it turns red, and to dissolve it in distilled water, when a purple solution will be produced without adding ammonia.

EXPERIMENT IV. The last test should be repeated with some urine.

EXPERIMENT V. Uric acid has the power of reducing metallic oxides in alkaline solution. This may be demonstrated by the following tests. Some urate is dissolved in weak sodium carbonate solution, which is then poured on to a piece of filter paper moistened with a solution of $AgNO_3$. A black stain of reduced silver results. This is called Schiff's reaction. In the presence of neutral salts, and more especially of magnesium mixture ($MgCl_2$, NH_4Cl, NH_2HO), the uric acid and other purin bodies unite with the silver to form a double salt. This salt separates out as a gelatinous precipitate, and is much employed for quantitatively estimating the purin bodies (Salkowski's method). Uric acid can also exercise its reducing powers on cupric salts in alkaline solution.

[1] In these equations M stands for the monad base.

Experiment VI. By applying Trommer's test to an alkaline solution of uric acid, it will be noticed that reduction ensues. The reduction precipitate is, however, of a dull brown colour instead of being yellowish red as it usually is. This is because a certain amount of the cuprous oxide unites with some of the uric acid to form a brown compound.

Quantitative Estimation of Uric Acid in Urine.—In this country the usual method adopted is that of Hopkins, the principle of which is as follows. The uric acid is precipitated as ammonium urate by saturating the urine with ammonium chloride crystals. The urate is then collected on a filter paper, washed with ammonium chloride or sulphate solution, and dissolved in warm water. The dissolved urate is then decomposed by adding a strong mineral acid, and the acid thereby liberated either collected and weighed, or titrated with a standard solution of permanganate of potassium, which it decolourises (see p. 462).

Metabolism of Purin Bodies.—The urinary purin bodies are derived from two sources, the food and the tissues. That portion which comes from food is called the *exogenous* moiety; the portion which comes from the tissues the *endogenous* moiety.

The Exogenous Moiety arises from food stuffs containing purin bodies. These may exist in the food in three states: (1) in combination with albumin and phosphoric acid, as nuclein (*e.g.* in sweetbreads, or other cellular tissues, p. 179), (2) in a free state, as in muscle extract, (p. 442); (3) as a methyl derivative of purin, such as thein, theobromin and caffein.

The purins of the first two groups (*i.e.* guanin, adenin, hypoxanthin and xanthin) are oxidised in their passage through the organism into uric acid, and appear in the urine as such, whereas those of the third group lose their methyl radicle, and are excreted as xanthin and hypoxanthin in the urine.

The whole of the administered purin does not, however, reappear in the urine, a certain fraction being further broken down by a splitting of the purin ring, the decomposition products being probably excreted as urea. In the case of hypoxanthin and xanthin (which are the purins existing in muscle, liver and spleen) about one half reappears in the urine, whereas in the case of adenin, and probably quanin (administered as nuclein), only one fourth can be recovered. In the case of the methyl derivatives, about one third can be recovered as purin bases.

The Endogenous Moiety.—This can be estimated by placing the person on a purin-free diet such as bread, eggs, milk, cheese, and butter. It usually amounts to 0·6 grammes[1] in the twenty-four hours,

[1] Calculated as uric acid.

and has been found to remain constant in the same individual under similar conditions, although it may vary in different individuals. It is not affected by varying the amount of the diet provided always that this be purin free. This can be seen from the following table:

Diet.	Alloxuric bodies excreted in urine.	Total nitrogen excreted in urine.
I. Containing purin bodies, - - -	1·017 gr.	16·8 gr.
II. Containing same amount of nitrogen, but no purin bodies, - - -	0·606 gr.	16·2 gr.
III. Containing half as much nitrogen as II., but no purin bodies, - - -	0·609 gr.	9·0 gr.

(Burian and Schur).

This endogenous moiety comes from the nuclein of the tissues, but just as in the case of the exogenous moiety, the whole of the liberated purin does not reappear in the urine, a certain amount undergoing rupture of its purin ring, and being excreted probably as urea. The fraction, both of endogenous and exogenous purins, which is thus further decomposed varies in different *species* of animals, man excreting as urinary purins one half, carnivora only one twentieth; and herbivora (rabbit) one sixth. In different individuals of the same species, however, this factor remains constant, so that we can tell accurately how much purin has been set free in the organism by multiplying the endogenous moiety by 2 for man, or by 20 for carnivora, or by 6 for herbivora. The chief organ in which this destruction, both of exogenous and endogenous purins, ensues is the liver in dogs and the kidneys in oxen.

If the endogenous moiety be calculated out for each kilo body weight, it will be found that it is greater in infants than in adults. This is probably due to the fact that the tissue metabolism is more active in the former, and consequently more nuclein is broken down. In certain blood diseases also (*e.g.* where the number of the polymorphous nucleated leucocytes is increased) an increase is noticed. In this latter case, however, it is not the leucocytosis which causes the purin increase, but rather, the leucocytosis and purin increase are both due to some metabolic changes in the blood glands.[1]

Hippuric Acid.—In herbivorous animals a large amount of nitrogen is excreted as hippuric acid, but in man and the carnivora only traces.

[1] In a case of *leucopenia* (where the leucocysts were reduced to one fifth the normal amount), Hutchison and I found the excretion of endogenous purins of normal amount.

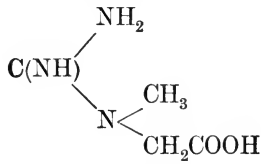
Chemically, hippuric acid consists of a combination of benzoic acid and glycocine, and can be prepared artificially by heating these two bodies together in a closed tube.

$$\begin{matrix} CH_2—NH_2 \\ | \\ COOH \end{matrix} + C_6H_5COOH = \begin{matrix} CH_2—NH—CO—C_6H_5 \\ | \\ COOH \end{matrix} + H_2O.$$

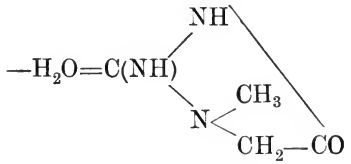
Glycin. *Benzoic acid.* *Hippuric acid.*

The explanation of its presence in the urine of herbivora is, that they take aromatic bodies in their food; these are oxidised to benzoic acid in the tissues, and this then combines with glycin to form hippuric acid. In man, etc., there is very little aromatic body in the food, and consequently, most of the glycin combines with cholalic acid to form one of the bile salts (see p. 233). The synthesis of hippuric acid takes place in the kidney, as has been shown by perfusing the excised kidney with defibrinated blood containing the two precursors. A similar experiment with any other organ yields no hippuric acid.

Creatinin.—It will be remembered in connection with creatin (see p. 205) that one of its reactions is that, by standing in acid solution, it loses a molecule of water, and changes into creatinin.



Creatin
(methyl guanidin acetic acid).



Creatinin
(anhydride of creatin).

When, from any cause, the urine reacts alkaline, creatin is excreted instead of creatinin. It is, therefore, the reaction of the urine which determines in what form this base will be excreted. It can be detected by the following test:

Experiment VII. **Weyl's Reaction.**—To five c.c. of urine are added four or five drops of a very dilute solution of sodium nitroprusside, so that the original colour of the urine remains unchanged. If a weak solution of caustic potash be now added drop by drop a ruby-red colour results, quickly changing to yellow. If an excess of acetic acid be added and the solution boiled, a greenish to blue colour results, and after standing some time a blue sediment (Prussian blue) settles to the bottom of the tube.

Aceton gives a similar colour with the nitroprusside and alkali, which, however, does not become yellow on standing, or even after adding acetic acid.

Creatinin possesses, to a certain extent, the power of reducing metallic oxides in alkaline solution, and this must be remembered as a possible source of fallacy in testing for dextrose.

Its significance in metabolism has been described on p. 252, where it was pointed out that it comes from two sources, and that it appears probable, that the *exogenous* portion undergoes a different metabolism from the *endogenous*, the former having certainly nothing to do with the metabolism of urea, the latter probably being an intermediate body, which afterwards becomes broken down into two moieties, one containing most of the carbon and the other an ammonia derivative. The latter then becomes transformed into urea.

The Free Ammonia.—This is determined by placing a measured quantity of urine, made alkaline with lime-water, under a bell-jar in which is a flat dish containing a measured quantity of $\frac{n}{10}H_2SO_4$. The alkali slowly expels the free ammonia, which is absorbed by the acid, and can be determined by titration. As mentioned on p. 255, the urinary ammonia is increased in certain liver diseases. It is also increased after the administration of certain ammonia salts (see Advanced Course).

CHAPTER XVIII.

THE INORGANIC SALTS OF URINE. URINARY DEPOSITS.

Chlorides.—The chief base is sodium.

EXPERIMENT I. Add to urine a few drops of nitric acid, and then a solution of silver nitrate. A white precipitate (AgCl) is produced soluble in ammonia. The nitric acid prevents the phosphates being precipitated by the silver nitrate.

Chlorides are derived mainly from the food, and they have, therefore, very little significance in metabolism. The percentage of sodium chloride in the blood is a very constant one, and, if it should rise above its normal level (as by taking much common salt in the diet), the excess is at once drained off in the urine. In certain diseases where effusions occur into the tissues (as in pneumonia or pleurisy) the percentage in the blood falls below normal, and less is excreted in the urine, but afterwards, when the effusion is being reabsorbed into the blood, the percentage again rises and an excess appears in the urine.

Phosphates.—These are mostly alkaline phosphates (NaH_2PO_4, Na_2HPO_4 and similar salts of potassium), but there are also earthy phosphates (*i.e.* of calcium and magnesium).

EXPERIMENT II. **To detect the Total Phosphates.**—Add to urine an equal bulk of nitric acid, and then half its bulk of an acid solution of ammonium molybdate; warm to 55° C.; a yellow crystalline precipitate of ammonium-molybdo-phosphate separates out.

EXPERIMENT III. **To separate the Earthy from the Alkaline Phosphates.**—Make urine alkaline with ammonia, and allow to stand for about half an hour. A white precipitate of calcium and magnesium phosphates settles down; filter, dissolve the precipitate in nitric acid, and add to this solution some molybdate of ammonium and warm—a yellow precipitate results.

Most of the urinary phosphates come from the food, but a certain proportion is derived from the metabolism of nuclein. In order to determine these nuclein phosphates, it is necessary to estimate the amount of free phosphates given in the food, and then to deduct this from the amount excreted in the urine and faeces, and the difference (*i.e.* the excess excreted to that administered) corresponds to that derived from the decomposed nuclein. If the alloxuric bodies be simultaneously estimated, it will be found that their amount bears a constant ratio to that of the phosphates, since they are both derived from the same source.

Sulphates.—These have a bitter taste, in consequence of which they are not taken to any extent in the diet, so that those excreted in the urine are derived from the sulphur of broken down tissue proteid. It will be remembered that proteid contains 1 per cent. S and 16 per cent. N, and, if it be true that both these elements in the excreta represent end products of proteid metabolism, a similar ratio should exist in the urine. This is approximately the case, the ratio of H_2SO_4 to N being about 1-6.[1] A determination of the sulphates provides us, therefore, with a very valuable control in estimating proteid metabolism, and is, indeed, the *only* means by which we can determine the influence of non-proteid nitrogenous bodies on the metabolism of tissue proteid. About 90 per cent. of the sulphuric acid is excreted in combination with alkalies—the **inorganic sulphates**; and about 10 per cent. in combination with organic bodies absorbed from the intestine—the **ethereal sulphates.**

EXPERIMENT IV. To about 5 c.c. of urine add an equal bulk of alkaline barium chloride solution (a mixture of 2 vols. $Ba(OH)_2$ and 1 vol. $BaCl_2$). A precipitate of barium sulphate separates out. This corresponds to the inorganic sulphates. Filter, acidify the filtrate with hydrochloric acid, and boil—a second precipitate, not so marked as the

[1] Theoretically, the ratio should be a little over 1-5, some of the S being contained in the urine, not as sulphates, but organically bound up in such bodies as cystin.

first one, separates out. This corresponds to the ethereal sulphates, which have been decomposed by boiling with the acid.

To determine the total sulphates, it is, therefore, necessary to boil the urine with hydrochloric acid before adding the barium salt.

The organic radicles with which the acid combines are chiefly phenol and indoxyl, which, it will be remembered, are produced in the intestine by the action of bacteria on proteids. Only a fraction of these is absorbed into the blood, where, on account of the fact that they are poisonous, they are at once combined with sulphuric acid to form an acid salt, and this then combines with the alkalies to form a neutral salt which is non-poisonous, and is excreted as such in the urine. The excretion of these ethereal sulphates becomes enormously increased where excessive bacterial digestion is taking place in the intestine *e.g.* in intestinal obstruction, typhoid fever, etc. (See Advanced Course, p. 452.)

Carbonates.—These are never found in the acid urine of a normal diet, but if the diet be purely vegetarian, and as a consequence the urine react alkaline, they occur in considerable amount. They are detected by the fact that they cause the urine to effervesce on the addition of a mineral acid. They are derived from the carbonates and organic acids contained in vegetables. They are usually combined with the alkalies, but if they should be combined with the alkaline earths they cause a cloudiness in the urine.

URINARY DEPOSITS.

As it is voided the urine is quite clear, but, on standing some time, a sediment usually separates out, and this varies somewhat under different conditions.

Acid urine *from a healthy person may deposit the following* :

1. **Urates** (see p. 256).—The sediment has a chalky appearance and is usually tinged reddish. It disappears on warming the urine. Examined microscopically it is generally amorphous—*quadriurates*—but may show a crystalline structure—*acid urates*—usually as needles, or as balls with spines projecting from them (Fig. 157).

2. **Uric Acid.**—This may be split off from the urates as described on p. 258. It appears as a cayenne pepper-like sediment, and has a definite crystalline appearance under the microscope (Fig. 156). The crystals may vary much in shape, but are always large and tinged a reddish colour. The most usual shapes for the crystals to assume are "sheaves," "whetstones," "rhombic tables," and sometimes "dumb-bells." The presence of the crystals does not necessarily indicate an

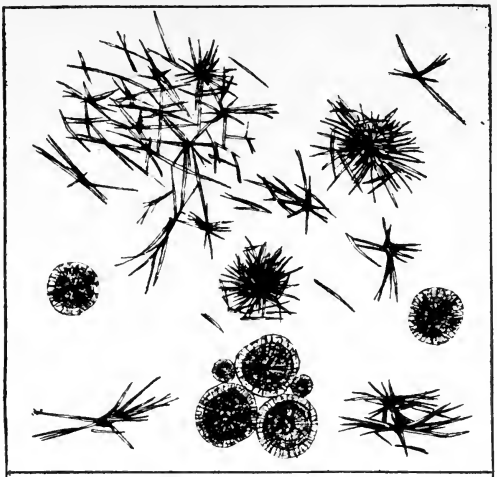


FIG. 157.—Sodium urate. × 350.

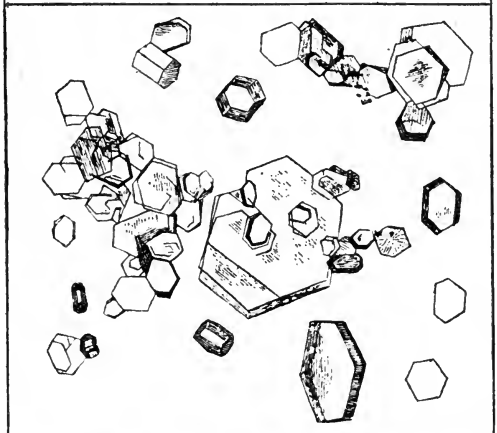


FIG. 158.—Cystin. × 350.

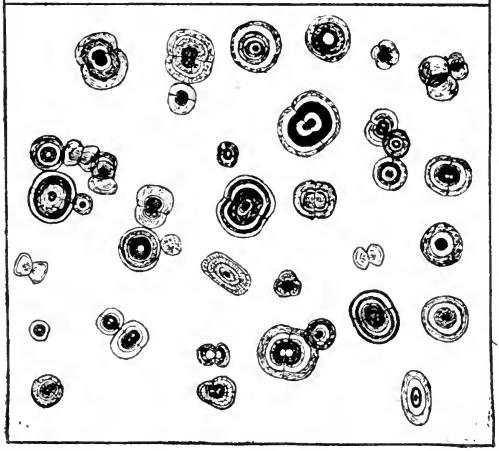


FIG. 159.—Calcium carbonate (from human urine). × 400.

increased excretion of uric acid, but is usually due to a more rapid decomposition of urates.

3. **Calcium Oxalate.**—This is usually a scanty deposit, adhering to irregularities on the surface of the glass of the urine jar, or forming a glistening layer on the top of the mucous deposit, that settles at the bottom.

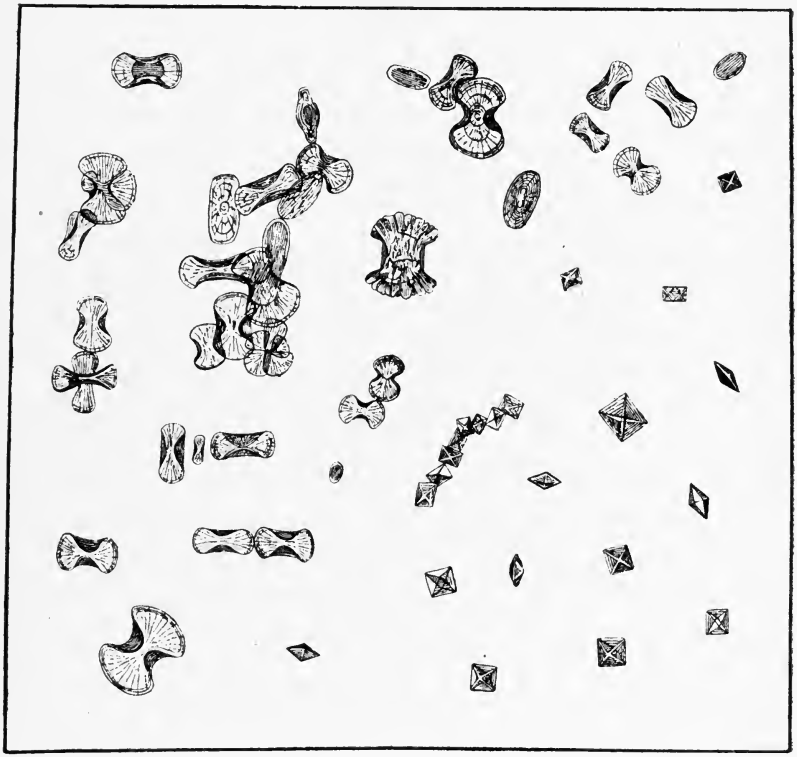


FIG. 160.—Calcium oxalate. × 500.

The crystals are insoluble in acetic acid, which reaction distinguishes them from phosphates or carbonates. They are also insoluble in ammonia, which distinguishes them from urates.

Microscopically they are seen to be very small octahedra, often flattened along one axis, so that they appear like squares with diagonal lines (hence called "envelope" crystals, Fig. 160).

4. **Stellar Phosphates** (Fig. 161).

Acid urine from a person suffering from disease, or during the administration of certain drugs, may deposit:

1. **Cystin.**—This forms a deposit somewhat like that of quadriurates.

Microscopically, however, it shows a distinct crystalline structure consisting of hexagonal colourless plates or slabs (Fig. 158).

2. **Leucin and Tyrosin.**—Though very rarely, these two bodies sometimes occur in urine (*e.g.* in severe hepatic disease), where their appearance is similar to that in a pancreatic digest (see Fig. 148).

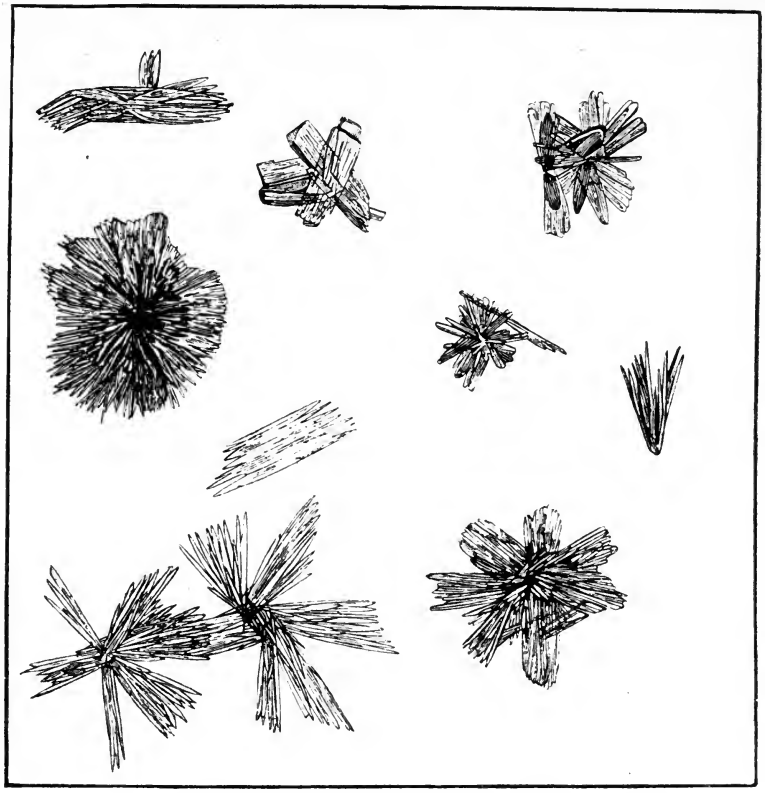


FIG. 161.—Stellar phosphate of calcium. × 500.

3. **Hippuric Acid.**—This may appear in urine during the administration of benzoic acid. It crystallises in four-sided prisms. It is quite common in the urine of herbivora.

In **Alkaline Urine** *the following may occur*:

1. **Phosphates.**—Of these there are two kinds, viz., phosphate of calcium and ammonium-magnesium phosphate.

(*a*) Phosphate of calcium.—The sediment is chalky and never pigmented; it clears up on adding a few drops of nitric acid; it is increased by boiling. Microscopically it is usually amorphous, but may exist as long prismatic crystals arranged in star-shaped clusters,

hence called *Stellar Phosphates* (Fig. 161). The crystalline form may also occur in faintly acid urines.

(*b*) Ammonium-Magnesium Phosphate.—When urine gets stale and ammonia develops in it, a white sediment and a white surface film form. Under the microscope these are seen to be made up of large clear crystals like "*knife rests*," or, if excess of ammonia be present, they may look like "*feathery stars*." This latter type can be easily got by adding ammonia to normal urine (Fig. 162).

2. **Biurate of Ammonia.**—This looks like the biurate of soda crystals, but is associated with crystals of phosphates, and occurs in an alkaline urine.

3. **Carbonates.**—In the urine of vegetarians these are not uncommon. The urine effervesces on adding acetic acid. Microscopically the sediment is usually amorphous, but may exist as biscuit-shaped crystals or as dumb-bells (Fig. 159).

CHAPTER XIX.

PATHOLOGICAL URINE.

I. **Proteids in the Urine—Albuminuria.**—Traces of mucin or nucleo-albumin may be added to the urine in its passage along the urinary tract, but otherwise, healthy urine does not contain any proteid. Where the kidneys are diseased, however, a certain amount of the plasma proteids leak into the urine, where they can be recognised by certain tests, the condition being called *Albuminuria.*

Also when proteids other than *serum* albumin and globulin gain access to the blood, they are at once excreted into the urine. It is on this account that albuminuria results after the consumption of a large number of raw eggs (egg flip), because the intestinal epithelium allows a certain amount of the unchanged albumin into the blood, where, however, it is foreign (in being egg- and not serum-albumin), and is consequently immediately picked out by the kidneys. In disease of the red bone marrow, a body somewhat similar in its reactions to an albumose, is added to the blood and is excreted by the urine (Bence Jones' Albumosuria).

Although globulin may occur along with albumin in the urine, or even in some cases independent of it, it is of no practical importance to distinguish between them, so that the tests about to be described include both bodies.

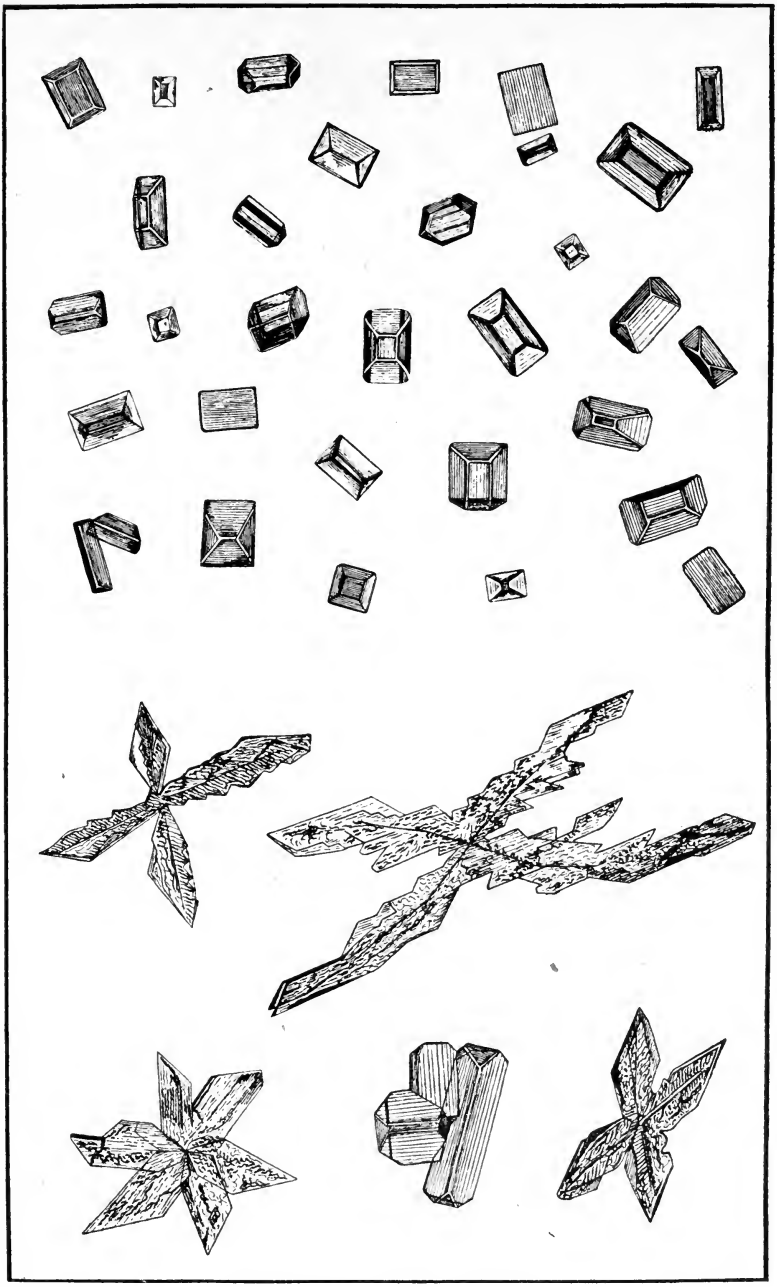


FIG. 162.—Triple phosphates. × 400

The tests employed, depend on certain of the reactions described under proteids. It is obvious, of course, that the colour reactions will not be applicable to the urine, those employed depending on the production of coagula. The most important of these are:

1. **Heat Coagulation.**—EXPERIMENT I. Place some *clear* urine in a test tube, and boil. A white turbidity or coagulum indicates that either albumin or phosphates are present (earthy phosphates are precipitated by boiling). To the boiling solution, whether it show a turbidity or not, add 3-4 drops of concentrated nitric acid. If due to phosphates, the turbidity will disappear, but will remain if due to proteid. In nitric acid any acid- or alkali-albumin which the urine may contain is insoluble. Where there is doubt as to the occurrence of a haze, the test tube should be about three-quarters filled, and only the upper layer should be boiled, the test tube being meanwhile held low down. By holding it against a dark background the slightest haze becomes very evident by this method, on account of contrast with the unboiled layer beneath.

2. **Heller's Test.**—EXPERIMENT II. Place some clear urine in a test tube. Hold the test tube in a slanting position, and allow concentrated *pure* nitric acid to run *very slowly* down the side, so that it forms a layer underneath the urine. Where the two meet, a sharp white ring (of coagulated acid albumin) is formed. The test may also be done by placing the nitric acid first in the test tube, and covering this with the urine slowly delivered from a pipette. The ring does not disappear on warming. A similar ring may be obtained when proteoses are present, but in this case the ring clears up on gently warming the test tube, and reappears on cooling. In warming, very great care must be taken that no mixing of the two layers occurs. When mucin is present in excess *a diffuse haze* may be produced in the portion of urine next the acid. Also when the urine is very concentrated acid urates or urea nitrate crystals may develop and simulate the reaction. In these cases, the urine should be diluted with two or three times its bulk of water, and the test reapplied, when very little doubt will remain as to the reaction.

3. **Salicyl-Sulphonic Acid Test.**—This is perhaps the most delicate of all the tests.

EXPERIMENT III. Add to about 10 c.c. of urine a drop or two of a saturated solution of pure salicyl-sulphonic acid. A white precipitate results, which on boiling changes into a number of coagula.

This reaction occurs in a dilution of 1-230,000 albumin. The only other body with which this reagent produces a precipitate is proteose, in which case, however, the precipitate disappears on warming.

The reagent, if pure, keeps indefinitely. If impure, however, it turns red on keeping. It has the great advantage over nitric acid in being non-corrosive, and therefore easily carried about (MacWilliam).

There are numerous other tests, but their application is superfluous if the above be properly applied.

Proteoses are detected by the precipitates produced by nitric and salicyl-sulphonic acids clearing up on heating the urine, and returning when it is cooled. The so-called "albumose" in Bence Jones' albumosuria is coagulated by moderate heat, but redissolves on boiling the urine. Albumose can best be separated from albumin by adding salicyl-sulphonic acid, boiling and filtering. The coagulated proteid remains on the filter paper, and the proteose is gradually precipitated in the filtrate as the latter cools.

FIG. 163.—Esbach's albuminometer.

Quantitative Estimation of Albumin.—For clinical purposes this is done by means of *Esbach's albuminometer* (Fig. 163). The determination is made by measuring the depth of the coagulum produced by adding Esbach's reagent to the urine (a mixture of 10 grms. picric acid and 20 grms. citric acid in 1000 c.c. distilled water).

EXPERIMENT IV. Place clear urine, filtered if necessary, in an Esbach's tube up to the mark *U*. If the reaction be alkaline, render slightly acid by the addition of acetic acid, and if the specific gravity be above 1008 dilute it with water till this density or something below it is obtained.[1] Now add the reagent up to the mark *R*. Close the tube with a tightly-fitting cork and invert several times, so as to mix the fluids thoroughly. Allow to stand in an upright position for twenty-four hours, and then read off the graduation corresponding to the top of the precipitate. This gives the number of grammes of dried albumin per litre of urine used. If the urine has been diluted the necessary calculation must be made in order to obtain the percentage in the original urine.

II. **Sugars in the Urine.**—In the *disease* known as diabetes mellitus, the most important symptom consists in the detection of dextrose (or glucose) in the urine, or, in other words, of the presence of **glycosuria**. This condition can also be produced *experimentally*: (1) *By puncture of the floor of the fourth ventricle.* The cause of the glycosuria in this

[1] These corrections should be made before the urine is measured into the Esbach's tube.

condition being an excessive glycogenesis in the liver, whereby the percentage of dextrose in the blood rises above the normal, the excess being excreted into the urine by the kidneys. The glycosuria ceases when all the liver glycogen has been used up, and it cannot be produced by a similar experiment in animals which have been previously starved, so as to clear their livers of glycogen.

(2) *By extirpation of the Pancreas.*—If the whole of the pancreas be removed in dogs, glycosuria is at once established, and the blood will be found to contain an excess of dextrose. So far, then, the cause of the glycosuria is the same as in the previous condition, viz., an excess of sugar in the blood. If, after the condition has existed several days, the liver be examined it will be found to be glycogen-free, but, unlike the previous condition, the glycosuria still continues, and in a few days it will be noticed that the animal has become markedly emaciated. The cause of the emaciation is that the proteid tissues are undergoing dissolution. That such is actually the case is seen by a determination of the urea excretion, which will be found to be enormously increased in amount. In the course of a few weeks the animal dies of emaciation.

These results show us that the pancreas must possess, besides its digestive function, some controlling influence on the metabolism of carbohydrates. The probable nature of this influence has recently been suggested in certain experiments by Cohnheim. The expressed intracellular juice of a pancreas has no, or only a very feeble power of decomposing dextrose. The same is true of the expressed juice of a muscle. If, however, these two juices be mixed and allowed to act on dextrose, the latter is very quickly decomposed.

This means that for the efficient combustion of dextrose in the muscles the muscle zymases must be activated by a substance supplied by the pancreas; in other words, by an internal secretion of the pancreas. This mechanism is analogous to that of entero-kinase on trypsinogen (see p. 236).

It is of importance to note, that in severe cases of natural diabetes the pancreas is frequently diseased.

(3) *The administration of certain drugs more especially of Phloridzin.*—The administration of this drug is immediately followed by glycosuria, which, however, ceases after a few days. If the liver be examined at this stage it will be found that a large proportion of its glycogen has disappeared. If a second dose be administered the glycosuria will reappear, and will persist so long as the drug is administered, and even after all glycogen has been used up. After some time, however, the animal becomes very emaciated, this being accompanied by excessive urea excretion.

Unlike the two previous forms the percentage of sugar in the blood is normal, or even sub-normal. On this account, it is supposed that phloridzin produces glycosuria by disturbing the controlling mechanism of the kidney, whereby the latter allows too much dextrose to escape into the urine, in consequence of which the percentage tends to become sub-normal in the blood. Increased demands are therefore made on the stored up glycogen, which at last becomes used up, and then the supply has to be furnished by the proteids, and these break down. In both pancreatic and phloridzin diabetes, therefore, proteid is split into its nitrogenous and carbonaceous moieties, and these appear in the urine, the former as urea, the latter as dextrose.

The other sugars which the urine may contain are **lactose** and **pentose**. The former of these is sometimes found in the urine of nursing mothers, and the latter appears in the urine whenever pentoses (Wood Sugars, p. 417) are given in the food.

Tests for Dextrose in the Urine.—The tests for dextrose, as described, can, with slight modifications, be applied to its detection in urine.

The most important of these are:

EXPERIMENT V. **Trommer's Test.**—Make 5 c.c. urine strongly alkaline with strong KOH solution, then add copper sulphate solution to it a drop at a time, shaking vigorously between each addition, until a trace of cupric hydrate remains undissolved. Heat to near boiling. A red precipitate of cuprous oxide forms if dextrose be present.[1]

EXPERIMENT VI. **Fehling's Test.**—Boil 5 c.c. of Fehling's solution in order to ascertain that the Rochelle salt which it contains, has not decomposed into reducing bodies. If no reduction occur, add a drop of the suspected urine and boil again. If no result, go on adding small quantities, boiling between each addition, till 5 c.c. have been added. If no reduction now ensue *no pathological amount of sugar can be present.*

A negative result with either of these tests excludes the presence of dextrose, although a positive result does not necessarily imply that it is present, since there are several other bodies in urine, *e.g.* uric acid, creatinine, glycuronic acid, etc., which are capable of reducing metallic oxides in alkaline solution.

[1] The slight excess of cupric hydrate also becomes reduced. If the urine contain much ammonia (*e.g.* after standing some time) it will dissolve a large amount of cupric hydrate, so that this part of the reaction (the resolution of cupric hydrate) is no indication of the presence of dextrose. In such urines, too, the cuprous oxide formed by reduction will also become dissolved, and a colourless solution instead of a red precipitate will result.

The percentage of these bodies in normal urine is, however, too low to cause the reduction, and it is only where a concentrated urine is employed that there is any chance of confusion. If the tests are applied as above described, the chance of error is very much lessened. There are, however, certain tests which are given only by dextrose, and these are:

1. **The Fermentation Test.**—EXPERIMENT VII. Place some diabetic urine in a small beaker, and boil it on a sand bath for ten minutes (this expels any air it may contain). Cool the urine and test its reaction; if alkaline, render faintly acid with a weak solution of tartaric acid. (This precaution is necessary in order to prevent putrefaction, which would lead to the evolution of ammonia.) Add a small piece (about the size of a split pea) of German yeast, and stir it in the urine until a milky solution is obtained. Now transfer the fluid to a Doremus ureometer (Fig. 164) so that the upright limb is completely filled with fluid. Place this in an incubator, or in a warm place, as on the mantelpiece, over night when it will be found that gas—CO_2—has collected in the upper portion of the vertical limb.[1]

FIG. 164.—Ureometer.

Two control tubes—one with a weak solution of dextrose and yeast, the other with normal urine and yeast—should be arranged so as to prevent any fallacy due to inactive or impure yeast

Instead of using a Doremus' ureometer a test-tube inverted in a trough of mercury may be employed.

2. **The Phenyl Hydrazine Test.**—The method of employing this is described in the Advanced Course (see p. 417).

Quantitative Determination of Sugar.—Two reactions of sugar are taken advantage of in order to estimate the amount of it present in any solution. The one depends on its reducing power, and the other on its power of rotating polarized light. In this country it is usually the former of these which is employed, so that it is the only method which we will describe here. The principle and technique of the polarimetric method is described on p. 421.

To determine the reducing power of any fluid, the latter, diluted if necessary, is run from a burette into a measured quantity of boiling Fehling's solution, which is made of such a strength that 10 c.c. correspond to 0·05 grammes of dextrose. When all the blue colour of the Fehling's solution has been discharged, the number of c.c. of fluid required is read off, and the percentage calculated (Fig. 165).

[1] Tubes, similar in construction to Doremus' ureometer, are prepared in which the vertical limb is graduated in percentages of dextrose.

Experiment VIII. Dilute 5 c.c. of diabetic urine with 95 c.c. of distilled water, and place the solution in a burette. Place 10 c.c. of Fehling's solution, diluted with four or five times its bulk of water, in a porcelain basin, and bring it to the boil. Now run the diluted urine into the Fehling's solution, stirring all the while, until the blue colour has quite disappeared. To tell the exact moment at which this occurs, allow the basin to stand for a minute or so with the flame removed; the precipitate of cuprous oxide settles to the bottom, and it can easily be seen, by tilting the basin slightly, whether any blue tint remains in the supernatant fluid. When reduction is complete read off the number of c.c. of diluted urine required, and divide by 20. The result gives the number of c.c. of undiluted urine which contain 0·05 grm. dextrose. How much will 100 c.c. contain?

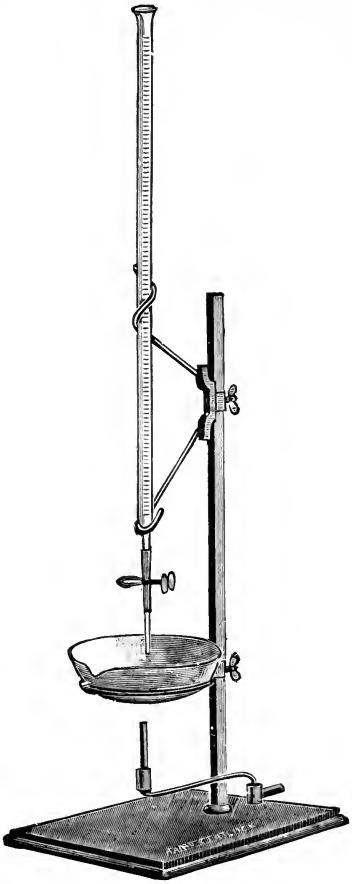


Fig. 165.—Apparatus for the estimation of sugar in urine.

It requires several trials before anything like constant results can be obtained by this method, the difficulty being to hit the exact moment when the blue colour disappears. It is desirable always to apply a preliminary determination in order to find out approximately, where special care will be required in a second titration for the exact result.

The chief difficulty in the determination of the end reaction is due to the presence of the red precipitate, suspended in the pale blue solution. In order to obviate this difficulty Pavy adds to the Fehling's solution strong ammonia, which has the power of dissolving the cuprous oxide as it forms, the resulting solution being colourless. The end reaction is then easily seen, but the method has the disadvantage that fumes of ammonia are evolved, and the solution very easily absorbs oxygen from the air, and reforms the cupric salt. On account of these facts the

titration has to be carried out in a flask through the cork of which pass two tubes, one from the burette, and the other to allow the ammonia and steam to escape.

Both these disadvantages are obviated by employing, instead of ammonia, a solution of *potassium cupro-cyanide*. This has the power of dissolving cuprous oxide, to form a colourless solution, and the reduced oxide absorbs oxygen so slowly from the air that the titration may be carried out in a basin.

The solution of potassium cupro-cyanide is prepared by adding to 10 c.c. of boiling Fehling's solution, diluted with 40 c.c. of water, a 5 per cent. solution of potassium cyanide until the blue colour just disappears. What happens is that the double cyanide of potassium and copper is formed, which is colourless. Now, this salt is *not* reduced by dextrose, but if it be mixed with more Fehling's solution it in no way interferes with the reducing power of this, and at the same time it dissolves the cuprous salt whenever it is formed. To carry out the titration the above solution of potassium cupro-cyanide is mixed with 10 c.c. Fehling's solution, the further technique being exactly the same as described in Experiment VIII., the end reaction in this case being the moment at which the solution becomes colourless.

The solution, with the excess of Fehling's solution added, keeps very well, and for clinical purposes, at least, should be prepared in stock.[1]

For very accurate determinations of sugar, the **Allihn-Soxhlet method** should be used. The titration methods described above are not quite accurate, because an excess of copper in a mixture of Fehling's solution and glucose increases the reducing power of the sugar. When a sugar solution is gradually run into a fixed quantity of Fehling's solution, therefore, the reducing power of the sugar becomes less and less as the titration proceeds, and the final result is apt to be too low. This error is obviated in the Allihn-Soxhlet method where a small quantity, 25 c.cm. of the sugar solution, which must be of *a percentage less than one*, is discharged at once into a large volume of diluted Fehling's solution (60 c.cm. + 60 c cm. water), and the mixture boiled in an evaporating dish for *exactly* two minutes. The cuprous oxide thus produced is quickly collected on the asbestos weighing filter (p. 492) connected with a suction filter flask, the basin thoroughly washed on to the filter with boiling water, the precipitate washed with alcohol and ether, and then dried.

The cuprous oxide is now reduced to metallic copper by passing a

[1] The potassium cupro-cyanide may also be prepared by adding pot. cyanide solution to a solution of cupric sulphate. For details of preparation of stock solution see Sutton's *Volumetric Analysis* (ed. 1896), p. 317.

stream of pure hydrogen gas[1] through the weighing filter, meanwhile warming the tube. When reduction is complete, the weighing filter is cooled in a desiccator containing hydrogen gas and then weighed. From the amount of copper found the sugar can be read off in specially prepared tables (see *Arch. f. d. ges. Physiol.* (Pflüger), Vol. XCVI., p. 105).

Blood in the Urine—Haematuria.—If blood be mixed with urine in the kidney it colours the urine brown, since, by being mixed for some time with an acid fluid, the haemoglobin has been changed into methaemoglobin. If the urine be examined by means of the spectroscope the band in the red, which is almost characteristic of methaemoglobin, will be seen.

If the haemorrhage be from the bladder or urethra the urine will be coloured red, and will show the spectrum of oxyhaemoglobin.

The sediment in both these cases will show blood corpuscles under the microscope. Sometimes, however, blood pigment occurs in the urine without corpuscles being present. Such a condition is called **Haemoglobinuria**, and, since the pigment escapes from the blood in the kidney, it has become changed into methaemoglobin before the urine is voided. Besides the spectroscopic and microscopic tests, there is a very delicate chemical test for blood called the *guaiac test.*

Experiment IX. To about 5 c.c. of urine add two drops of tincture of guaiac. A white precipitate of resin is obtained. Now add about 5 c.c. of ozonic ether, and note that a deep blue colour develops at once where the two fluids meet. The reaction is said to be due to oxidation of the guaiac, the haemoglobin carrying the oxygen from the ozonic ether to the guaiac.

Apply also the appropriate microscopic and spectroscopic tests for blood in urine.

Bile in the Urine.—When the bile duct is blocked by a calculus, or by swelling of its mucous membrane from catarrh, the bile, which accumulates in the bile channels, is reabsorbed into the blood-vessels and carried to the tissues, which become stained with bile pigment. Under these conditions the urine contains bile constituents, the most easily recognised of which are the bile pigments.

Experiment X. Apply Gmelin's test (see p. 234) to the urine of a jaundiced patient. Where only a trace of bile is present, the reaction may be made more delicate by first of all filtering the urine through white filter paper, and, when all has filtered through, applying a drop of fuming nitric acid to the stain on the filter paper. The play of

[1] The hydrogen is purified by bubbling it through an acid solution of $KMnO_4$, and then passing it through a pumice stone and H_2SO_4 absorption tube (p. 146).

colours is then very distinct. Also apply Matthew Hay's test (see p. 233).

Acetone in the Urine.—In severe diabetes this body occurs in the urine. It is also present in the urine in experimental diabetes, when this is accompanied by the breaking down of proteid tissues. As intermediate bodies *aceto-acetic acid and β-oxybutyric acid* are formed. The relationship between these three bodies will be seen from the following equations :

(1) β-oxybutyric acid = $CH_3—CH<^{OH}_{CH_2COOH}$.
(2) Aceto-acetic acid $CH_3—CO—CH_2COOH$.
(3) Acetone $CH_3—CO—CH_3$.

β-oxybutyric acid is the first formed and soon becomes oxidised to form aceto-acetic acid, which then splits into acetone and carbonic acid. There are simple tests for aceto-acetic acid and acetone ; β-oxybutyric acid, for which there is no simple test, always occurs along with aceto-acetic acid.

Experiment XI. **Test for Aceto-acetic Acid.**—Add to the urine dilute ferric chloride, allow the precipitated ferric phosphate to settle. The supernatant fluid becomes red if aceto-acetic acid be present.

Experiment XII. **Test for Acetone.**—The urine has a peculiar fruity odour. Add a few drops of caustic potash, warm and add, drop by drop, a saturated solution of iodine in potassium iodide till a brown colour is produced. Now add more caustic potash and boil ; a smell of iodoform is given off.

There are numerous other bodies which may occur in the urine in disease, and their description will be found in text-books on clinical medicine. There are two of these, however—glycuronic acid and homogentisic acid—which are of great physiological interest, first of all, because they both have the power of reducing metallic oxides in alkaline solution, and may, accordingly, be confused with dextrose ; and secondly, because they both indicate an unusual form of metabolism.

Homogentisic Acid is di-oxyphenyl-acetic acid $C_6H_3<^{OH}_{OH}<_{CH_2COOH}$.

When present in the urine it causes the latter to become of a dark-brown colour on standing, or this change in colour may be hastened by adding some alkali. It is present in the urine during the whole life, and it has been noticed that persons who exhibit it are almost invariably the children of first cousins. It can be easily separated from the urine by adding a solution of lead acetate, filtering off the precipitate

of inorganic salts which at first forms and allowing the filtrate to stand, when large needle-shaped glancing crystals of the lead salt separate out. If these be collected and treated with sulphuretted hydrogen, so as to remove the lead, the acid is obtained in a pure state.

Glycuronic Acid.—Chemically this is dextrose in which the end—CH_2OH—group has become oxidised to form COOH, or carboxyl. It has, accordingly, the formula $COOH—(CH.OH)_4—CHO$. It is an intermediate body in the metabolism of dextrose, and usually becomes further decomposed in the organism, to yield carbonic acid and water. Sometimes, however, it unites with the aromatic bodies (plenol, skatol, etc.) absorbed from the intestine to form a salt. In this combination it takes the place of sulphuric acid (see p. 263). In very small amount, it seems to be always present in the urine, but under certain conditions (as after the administration of certain drugs) it becomes increased to such an extent as to impart to the urine a very considerable power of reducing metallic oxides in alkaline solution. When this is the case it is apt to be confused with dextrose. The only absolute test whereby it may be distinguished from dextrose is that it does not ferment with yeast.

Cystin (see also p. 230).—In rare pathological conditions the urine may contain a body having the formula—

$$\begin{array}{llll} CH_2 & -S- & S & -CH_2 \\ | & & & | \\ CH & -NH_2 & NH_2 & -CH \\ | & & & | \\ COOH & & & COOH, \end{array}$$

and consisting, therefore, of two cystein molecules (see p. 230). It also occurs in the urine of dogs poisoned with phosphorus. It is very insoluble and tends, when present, to form calculi. It forms peculiar crystals (see p. 265). It is of interest in the present connection, because it is an unusual end product of proteid metabolism. Its main chemical test depends on its containing sulphur.

NOTE TO ADVANCED COURSE.—Many elementary details concerning experiments are omitted from this portion of the work. References to these will be found in the text or in the index.

PART III.

ADVANCED EXPERIMENTAL PHYSIOLOGY.

MUSCLE AND NERVE. CIRCULATION. RESPIRATION. ANIMAL HEAT. CENTRAL NERVOUS SYSTEM AND SPECIAL SENSES. (Advanced Course.)

THE PHYSIOLOGY OF MUSCLE AND NERVE.

CHAPTER I.

EXTENSIBILITY AND ELASTICITY OF MUSCLE WHEN AT REST AND CONTRACTED. COMPARISON WITH RUBBER.

Muscle is both extensible and elastic, that is, it can be stretched beyond and will return more or less to its original length when the extending force is removed. These are important properties; for, unless muscle were readily extensible the sudden contraction of one set of muscles would in the body be liable to rupture their antagonists.

In the study of these properties a gastrocnemius preparation may be used, but a muscle whose fibres run more nearly parallel to each other is preferable, such as a sartorius preparation from a large frog or better still a gracilis-semimembranosus preparation.

A **gracilis-semimembranosus preparation** consists of the two large internal thigh muscles (Figs. 20, 21). The gracilis is a large muscle lying along the inner side of the sartorius; it arises from the ischial symphysis and is inserted into the head of the tibia. The semimembranosus is a bulky muscle behind the gracilis on the posterior aspect of the thigh; it also arises from the ischial symphysis and is inserted into the back of the head of the tibia. To make the preparation, isolate these two muscles from those surrounding them near their points of insertion, cut through the tibia below this point and through the femur just above the knee joint. Holding this piece of bone, separate the two muscles up to the symphysis and remove with them the bone from which they arise. If a larger or longer muscle still is required, a double preparation may be made with the muscles of both thighs and the two hung side by side, or one below the other, united in the middle by the piece of the symphysis.

The following experiments should be performed. The bone at the

upper end of the preparation is rigidly fixed in a clamp and to the lower end is attached by a short thread or pin a brass mm. scale, having its zero at the bottom. The lower end of the scale has a small tray to carry weights or a hole by which weights can be hooked on. A pointer carried by a separate stand is placed opposite the zero of the scale. A weight of 10 grms. is attached to the scale and the amount of extension read off; then another 10 grms. is added and so on until the load is 100 grms. or more. It will be found that the length to which the muscle is extended is not proportional to the weight used, but that, by each increase of weight the muscle is stretched rather less, the greater the previous extension. By removing the weights one by one the elasticity of the muscle is observed; it is not complete; for when all the weights have been removed the muscle does not at once return to its original length. An 'extension-remainder' is present, and this is the more marked the more the muscle is fatigued by the degree and duration of the extension. Therefore the observations should be made as rapidly and on as fresh a muscle as possible. It is probable that muscle in the body with its circulation intact is completely elastic.

If the muscle is replaced by a suitable piece of rubber band and the same observations are repeated on it, it will be found that the series of elongations are more nearly proportional to the weights used, thus conforming nearly to Hooke's Law, which states that the successive increments in length produced by equal increments of weight are, in a perfectly elastic body, equal. Also, as the weights are successively removed, it will be found that the elasticity of rubber is more nearly perfect. But, if the extension be great and of long duration, an 'extension-remainder' does appear and only gradually disappears.

Another method of demonstrating the same properties is to fix the upper end of a muscle-preparation in the clamp of a simple myograph and to attach its lower end to the lever by a bent pin. Attached to the lever vertically below the muscle is a scale-pan or hook to which weights can be suspended. The writing point of the lever is brought on to the surface of a stationary smoked drum and a zero line described by rotating the drum by hand. The drum is rotated back so that the point of the lever is 5 mm. from the beginning of the zero line, a weight of 10 grms. is attached to the lever, the muscle will be extended and the writing point will record a new vertical line on the drum. Turn the drum by hand so that the writing point will describe a horizontal line 5 mm. long,[1] attach

[1] By thrusting the points of a pair of fine forceps through a thin piece of cork a means of measuring off equal distances is obtained; there is a mm. scale on the induction-coil.

another 10 grms. and repeat the process until 100 grms. or more are extending the muscle. In the same way reverse the process and remove the weights of 10 grms. one by one. If now the lower ends of the vertical lines drawn by the fall and rise of the lever are joined, a curved line will be formed, showing that the extension of the muscle becomes less and less for each additional weight. Further, when all the

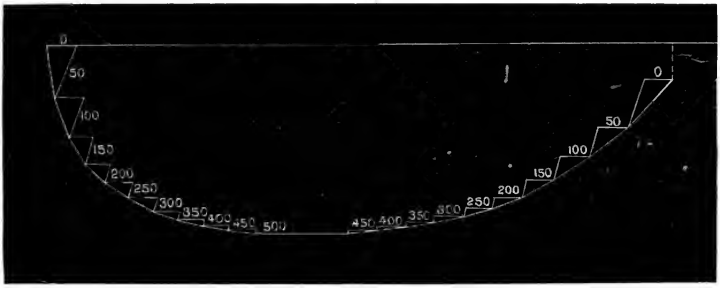


Fig. 166.—Curve of extensibility and elasticity of gastrocnemius. The figures on the curve are weights in grms. Temp., 15° C. (A.P.B.)

weights have been removed, the writing point will be below the original zero line, showing an 'extension-remainder' (Fig. 166). It will also be seen that the line corresponding to the elasticity of the muscle is a flatter and more gradual curve than that corresponding to the extension; this is caused by the long continued load impairing the elasticity of the muscle.

Fig. 167.—Elasticity curve of quiescent muscle. To be read from right to left. The figures on the curve are for weights in grms. (M.S.P.)

If the experiment be repeated on a piece of rubber band, the line joining the lower ends of the vertical lines will be nearly straight, and little or no 'extension-remainder' will be seen. Figs. 167, 168 show a comparison of the lines thus described for a muscle and piece of rubber loaded from 0 to 500 grms. and then gradually unloaded again.

A contracted muscle is more extensible than a resting one. This is of importance in the body; for, otherwise, a sudden and powerful contraction of a muscle, trying to lift a heavy weight, would be liable to rupture either the muscle itself, or its tendon, or the bones to which it

is attached. As a matter of fact, of these three structures muscle, owing to its increased extensibility during contraction, is the least often ruptured. In order to demonstrate this properly the muscle-preparation is attached to the clamp and lever, as in the last experiment. Arrange the apparatus for stimulating the muscle directly with single

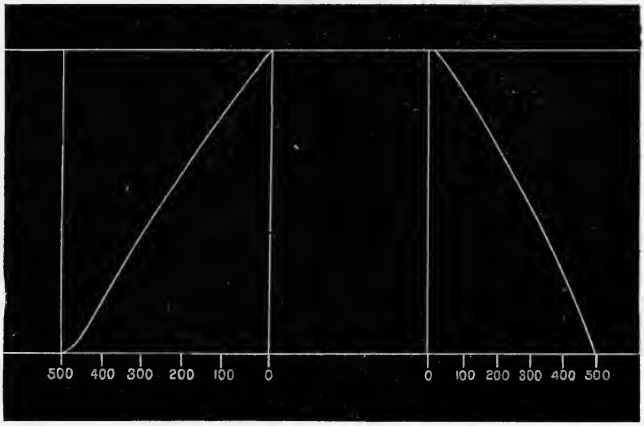


Fig. 168.—Elasticity curve of rubber tubing. The figures represent weights in grms. (M.S.P.)

maximal induction-shocks, using a spring-key in the primary circuit. Bring the writing point on to a stationary drum and, with the muscle weighted only by the lever, describe an abscissa line corresponding to the resting muscle. With the writing point again at the beginning of this line, stimulate the muscle once and, from the top of the ordinate so marked, draw another abscissa line corresponding to the muscle when contracted. Rotate the drum by hand, so that the writing point is now 5 mm. along the 'resting' abscissa line; hang 20 grms. on to the lever and stimulate, so as to record a second ordinate 5 mm. from the first. Repeat this process, increasing the weight by an equal amount each time. In this way Fig. 169 was produced. It is clear that the distance of the lowest point of each ordinate below the 'resting' abscissa line represents the extension of the resting muscle by a given weight, and that the distance of the top of the same ordinate below the 'contracted' abscissa line represents the extension, by the same weight, of the muscle when contracted. If the lowest and then the highest points of the ordinate are joined, two curved lines are produced which represent respectively the curves of extension of resting and contracted muscle (Fig. 169). It will be seen that the extensibility of contracted muscle is absolutely greater, and increases more rapidly, than that of resting muscle. Hence, if the observations were carried

far enough, the two curve lines would ultimately cross; this means that if a muscle were loaded by a weight greater than it could lift, it

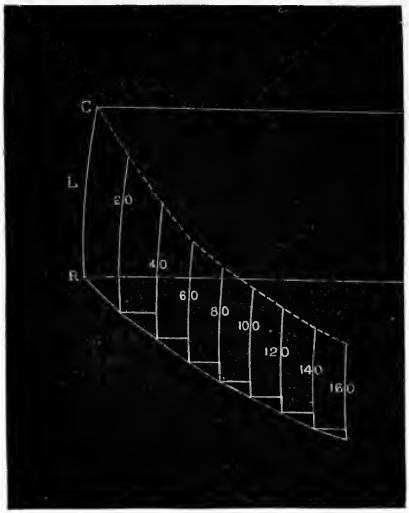


FIG. 169.—Comparative extensibility of resting and contracted gastrocnemius. Temp. 12° C. Magnification, 5. Figures represent actual weights in grms. R is the 'resting' and C the 'contracted' abscissa line. (A.P.B.)

would during its stimulation actually lengthen (Weber's paradox). If this were not so, we should, when trying to lift a load greater than the muscle could move, run a great risk of rupturing our muscles.

CHAPTER II.

LOAD AND AFTER-LOAD. WORK DONE WITH INCREASING LOADS.

MUSCLES may be loaded in two ways; the load may be applied before the muscle has begun to contract, or only after it has already begun to contract; this latter method, in order to distinguish it from the former, is called 'after-loading.' Most of the muscles in the body are both loaded and after-loaded; that is, they are constantly loaded by the pull of their antagonists, and it is only after they have already begun to shorten that the main load—the weight of the limb, etc.—is applied to them. The deltoid, however, is an instance of a muscle constantly loaded by the weight of the arm; the ventricle of the heart, on the other hand, is a muscle which is only after-loaded.

The effect of load, and of its method of application on a single muscular contraction, will be studied in the following ways: (*a*) the contraction given by a muscle loaded and after-loaded with the same weight will be compared; (*b*) a constant load will be thrown on to a muscle as an after-load later and later in its period of shortening, and the effect on the contractions noted; (*c*) the muscle being just completely after-loaded, the height of contraction, with increasing loads, will be measured and the work done with each calculated.

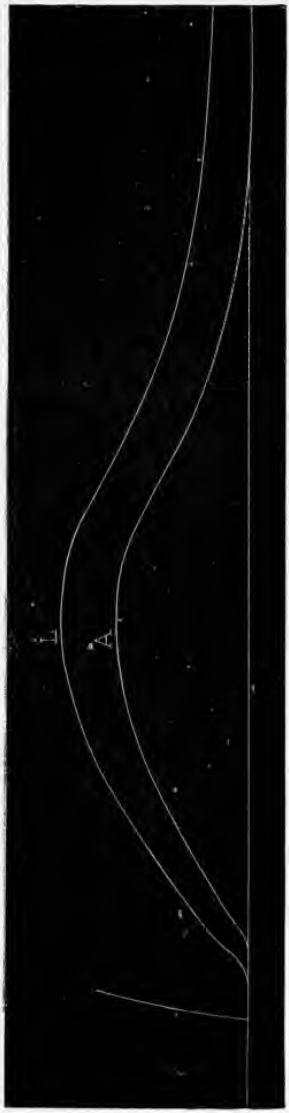


FIG. 170.—Contractions of the same muscle when loaded, L, and when after-loaded, A. Actual load on muscle was 10 grms. Temp., 10° C. Magnification, 5. (A.P.B.)

Comparison of the Contractions of a Loaded and After-loaded Muscle.—Arrange the apparatus for stimulating a muscle with single maximal induction shocks, using the drum as a key in the primary circuit. Fix a gastrocnemius preparation to a myograph lever, provided with an after-loading screw; by raising the screw the metal part of the lever can be supported at any level (Fig. 25). Hang a weight of 50 grms. near the axis and raise the screw until the whole of the weight is just after-loaded; this point can be ascertained by supporting the weight with the finger, and when the muscle no longer tends to raise the lever off the after-loading screw, the muscle is unstretched by any load. Arrange the apparatus so that with the screw in this position the lever is horizontal. Record a single contraction of the muscle on a rapidly revolving drum, mark the point of stimulation, and draw an abscissa. Then lower the after-loading screw until the muscle is loaded with the whole weight, and super-impose on the same abscissa and with the same point of stimulation a contraction of the loaded muscle (Fig. 170).

The main differences between these two curves are—in the purely after-loaded muscle there is an appreciable lengthening of the latent period owing to the muscle in its unstretched condition having to take in 'slack'; a diminution in the height of the contraction, owing to the absence of tension on the muscle before the contraction began. In other words, moderate initial tension increases the power of a muscle to do work.

Progressive After-loading of a Muscle.—With the same arrangement of apparatus as in the preceding experiment, record a single con-

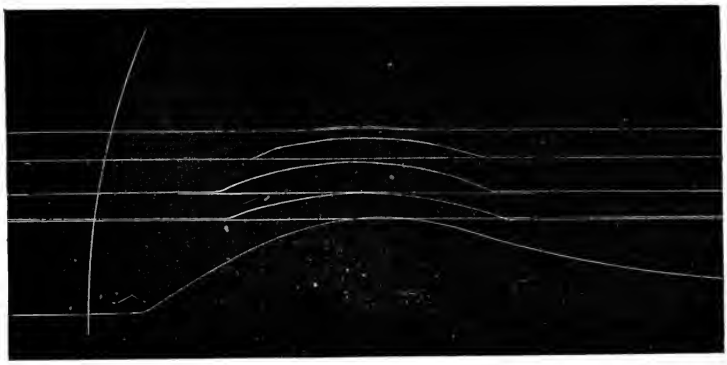


FIG. 171.—Effect of progressive after-loading of a gastrocnemius. Actual load on muscle, 4 grms. Magnification, 5. Temp., 10° C. (A.P.B.)

traction of the muscle when just after-loaded, draw a base line and mark the point of stimulation. Now raise the after-loading screw until the writing point is on a level with the highest point of the preceding curve; draw a fresh abscissa at this level and record a contraction; the point of stimulation will be the same as before. Repeat this process until the muscle can no longer lift the lever off the after-loading screw (Fig. 171).

From this experiment we see that, in a series of contractions each more after-loaded than the last, a muscle is able to undergo a little further shortening each time until it reaches its maximal shortening; for the explanation of this see page 294. Also by measuring the heights of the contractions above their respective abscissae, we learn that the longer after stimulation it is before the muscle meets the resistance of a given weight, the less is the muscle then able to overcome that resistance and raise the weight. In other words, as a muscle contracts its extensibility progressively increases, and its absolute contractile force decreases, until at the height of its contraction its extensibility is greatest and its absolute contractile force

nil. Hence a muscle would contract under the most favourable circumstances, if the load, as it was raised, progressively decreased.

Relation of Load to Work done during Contraction.—In order to record the height of contraction for a large range of weights, it is more convenient to record on a stationary drum simply the heights of a series of twitches than to super-impose a large number of curves. The apparatus is arranged for stimulating the muscle with a single maximal induction-shock, using a simple key in the primary circuit. A weight is hung near the axis of the lever of such a size that the actual load on the muscle is 50 grms.; the method of calculating this weight has been already given on p. 29. The muscle is just completely after-loaded throughout the experiment in order to get rid of the effect of alterations in the initial tension. With the lever horizontal, the muscle is stimulated, and the height of its contraction recorded on a stationary drum. The drum is rotated a short distance by hand; an additional load of 50 grms. is hung from the lever, and another contraction recorded. The process is repeated until the muscle is no longer able to raise the load off the after-loading screw. Fig. 172 gives the result of such an experiment; in it the magnification was 5, and the actual load on the muscle half of the weight hung near the axis of the lever. The following table gives in grm. mm. the work done by the muscle with the various loads.

Actual load in grm.	Actual lift in mm.	Work in grm. mm.
50	4·0	200
100	3·2	320
150	2·2	330
200	1·8	360
250	1·2	300
300	1·0	300
350	·8	280
400	·5	200
450	·4	180
500	·3	150
550	·2	110
600	·1	60
700	0	0

From the last column in this table we see that, although the height of the contractions diminishes continuously, the actual work done by the muscle increases at first rapidly and then more slowly, until it reaches its maximum with a load of 200 grms. After that point the work done begins to decrease slowly, and then more rapidly until at 700 grms. a load is reached which the muscle is unable to

lift. This weight represents the 'absolute contractile force' of this muscle, that is, the load which, brought to bear on the muscle at the instant of contraction, is just able to prevent it from shortening. Although the muscle is unable to lift this load, and therefore, when stimulated, does no visible mechanical work, it nevertheless liberates energy chiefly as heat.

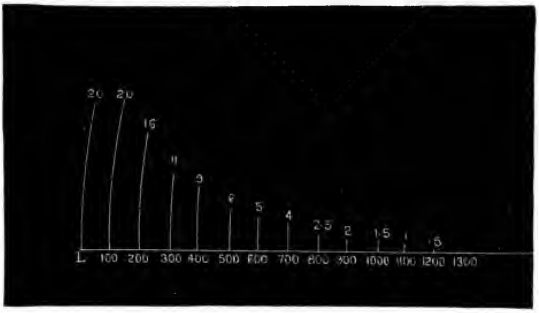


FIG. 172.—Height of contractions of gastrocnemius with increasing load. The number above each contraction is its observed height in mm. Magnification, 5. The number below each contraction is the weight in grm. hung at the axis of the lever; the actual load on the muscle was half of this number. (A.P.B.)

We are now in a position to recapitulate, so far as load is concerned, the conditions necessary to obtain an optimal contraction of a muscle and to see how far they exist in the living body. Initial tension, we have seen, decreases the latent period and increases the power of the muscle to do work. In the body the muscles are constantly loaded to a slight extent, and are thus kept stretched and free from 'slack.' In this way movements with a short latent period, and with an absence of jerkiness are obtained; and the muscles by being stretched are kept irritable, awake and fit for sudden work. On the other hand we see that a muscle, when purely after-loaded, is at a disadvantage for doing work; yet in the body the main load is thrown on as an after-load. The advantage of this arrangement depends upon the increased extensibility of contracting muscle; for, in this way liability to rupture is reduced; further, there is a saving of energy in pulling at a dead weight through an elastic spring, instead of through an inelastic cord, since some of the energy expended would be lost in a sudden jerk, but, in the case of the spring, is stored up in it and given out again as its elastic recoil. Thus smoothness is imparted to even the most sudden movements. We have also seen that as a muscle shortens its absolute contractile force decreases; therefore, it is clear that the after-load should be thrown on to the muscle at the instant of contraction, when the contractile

force of the muscle is at its maximum, and not later; this is the arrangement in the body. Further, it would be an advantage if the load decreased as the contractile force of the muscle during its contraction decreased; this is not usually the case in the body, but it does occur in certain movements, as, for instance, in jumping or when, with the upper arm horizontal, a weight in the hand is raised by flexing the forearm on the elbow.

CHAPTER III.

COMPARISON OF ISOTONIC AND ISOMETRIC CONTRACTION.

We have already seen (p. 27) that in order to obtain an accurate record of the change in form of a contracting muscle by means of a myograph-lever, it is necessary to use a light lever, and to weight it as near its axis as possible. By this arrangement the inertia of the movable system is reduced to a minimum, and the tension on the muscle throughout its shortening and relaxation remains nearly constant. This method is therefore called isotonic; it registers the development of that part of the energy liberated by the muscle which appears as the mechanical energy of the change in form. On the other hand, if the muscle is made to pull against a strong spring, the muscle will undergo but slight change in length, and the energy, which would otherwise have appeared as change in form, will now be converted into tension and stored in the spring. This is the so-called isometric method. If the movement of the spring is recorded by a lever attached to it we get a record of this conversion into tension of part of the energy liberated by the muscle when stimulated. Further, by allowing the spring to exert tension on the muscle before stimulation, it is possible to investigate the effect of initial tensions on the subsequent liberation of energy.

Fig. 173 shows a lever which can be used for either the isotonic or isometric method. The only part which needs description is the axis; it consists of a stiff steel wire. About 2 mm. from one end of the axis the lever is rigidly fixed to it, and the small projecting piece of wire fits loosely into a small socket in the brass support. The other end of the axis is carried by piercing a rigid brass arm, in which it can work loosely for the isotonic method, or to which it can be firmly clamped in any position for the isometric method. In this latter case contraction of the muscle, instead of leading to much shortening, produces torsion of the axis, and the excursion of the lever is proportional to the tension

exerted by the muscle. The length of wire which undergoes torsion can be adjusted by altering the horizontal length of the brass arm carrying the axis; the smaller the muscle the greater this length must be.

Arrange the apparatus for stimulating a muscle directly with a single maximal induction-shock, using the drum as a key in the primary circuit. Prepare a single or double gracilis-semimembranosus prepara-

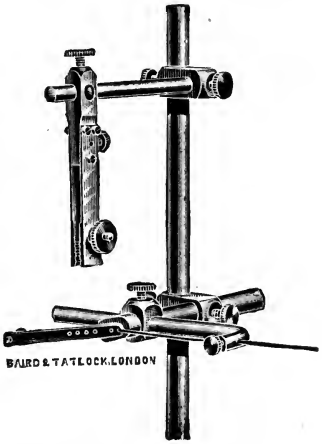


Fig. 173.—Isotonic and isometric lever.

tion (p. 281), fix the bone of one end in the muscle clamp, and the lower end, by means of a bent pin or very short thread, to the lever one inch in front of its axis. Release the screw fixing the axis and see that it works smoothly. Attach a weight of 50 grms. to the lever near its axis and adjust the muscle-clamp vertically so that the lever is horizontal. Record a single contraction on a rapidly revolving drum, mark a base line and the point of stimulation. Swing the writing point off the drum, but do not move the base of the stand carrying the myograph. Now remove the weight and attach the muscle to the lever just in front of its axis. The muscle-clamp will need horizontal adjustment, so that the muscle again lies in a vertical straight line with its point of attachment to the lever. Adjust the length of the wire to the size of the muscle, and with the lever horizontal firmly clamp the axis. The muscle-clamp will probably need vertical adjustment, so that the muscle may be taut but under no initial tension. Swing the writing point on to the drum; it should exactly coincide with the old abscissa line, and, if the base of the stand has not been moved, the point of stimulation

will be the same. Let the drum revolve, open the Du Bois key, and record a single isometric contraction.

Now remove the muscle and attach to the lever at the same spot a thread which passes over a pulley held by the muscle-clamp. Tie on to the free end of the thread a weight of 20 grms.; this will raise the lever a certain amount; draw a line across the isometric curve on this level

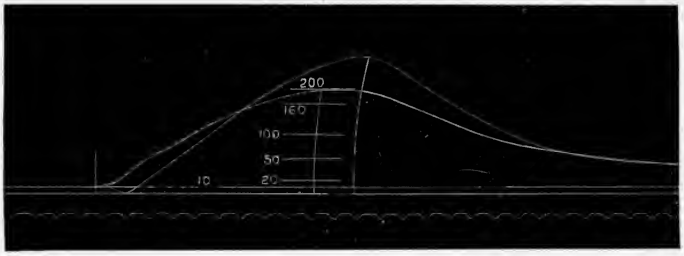


FIG. 174.—Comparison of an isotonic and isometric contraction of a single gracilis and semimembranosus preparation. For the isotonic curve 50 grms. were hung at the axis of the lever, the actual load on the muscle being a third of this, and the magnification was 5. For the isometric curve the magnification was 15, and the muscle started to contract under an initial tension of 10 grms. Time marker, 100 per sec. (A.P.B.)

parallel to the abscissa line. Repeat the same process with weights of 50, 75, 100 grms., and so on until the level of the top of the curve is reached. In this way the tension or resistance which the muscle has overcome is measured.

Fig. 174, obtained by this method, shows the main differences between an isometric and isotonic curve; these are, that in an isometric curve the highest point is sooner reached and the curve has a flatter top. In other words, a muscle reaches its maximal tension sooner and maintains it longer than its maximal shortening.

The effect of initial tension on an isometric curve may also be investigated. With the lever horizontal, and the muscle-clamp adjusted so that the muscle is under no initial tension, a zero-abscissa line is drawn. The muscle-clamp is now raised so as to slightly elevate the writing point; by raising the lever the wire axis has undergone torsion, and the muscle is under an initial tension. Describe a fresh abscissa line at this level, mark the point of stimulation, and record a single isometric twitch. By removing the muscle and substituting the pulley and weights, the value of the initial tension and of the various parts of the curve in grms. may, as before, be estimated. It will be found that the effect of an initial tension is to increase slightly the latent period and total time of the twitch, but a more important result is that it increases the maximal tension or resistance overcome by the muscle when stimulated.

From the two curves in Fig. 174 certain rough but important deductions may be made. The initial tension on the muscle in the two cases was not quite the same, but this may be neglected. In the isometric contraction the observed height of the curve is 14 mm., but the magnification was 15, and therefore the muscle really underwent a shortening of $\frac{14}{15} = \cdot 933$ mm. Supposing muscle were a perfectly elastic body the 'contractile stress' or pull exerted by it at any point during a contraction may be calculated by the formula, $\tau = \frac{1}{2} lw$; where τ is the pull, l is the amount of shortening in mm., and w the resistance in grms., whether that of a load or of a spring, which the muscle has overcome. In the isometric curve the maximal pull exerted by the muscle was $\frac{1}{2} \times \cdot 933 \times 200 = 93 \cdot 3$ grms. In the isotonic curve the load on the muscle was $\frac{50}{3}$ grms., and the pull exerted by the muscle when it had again shortened by ·933 mm. was $\frac{1}{2} \times \cdot 933 \times \frac{50}{3} = 7 \cdot 7$ grms.

The comparison of an isometric with an isotonic curve, and of isometric curves having different initial tensions, shows really the effect that resistance to contraction has upon the liberation of energy by a muscle; for it shows that a muscle which has shortened to a given length will be exerting a far greater pull when its effort to shorten has been resisted than when it has reached the same length during an isotonic or unresisted contraction; and this is especially true of resistance during the first part of the muscle's period of effort. In the body all muscles even at rest are extended by an initial tension, and their efforts to shorten during contraction are more or less resisted; and we find that this resistance, so far from decreasing, actually increases the efficiency of the muscle for doing work. We further see that the pull exerted by a muscle during its contraction is not determined simply by the strength of the stimulus reaching it, but also by the mechanical conditions of tension and load under which the muscle finds itself before and after it has begun to respond to the stimulus. For, we have found that with the same strength of stimulus a muscle responds with a pull which increases directly as the resistance it has to overcome. This power of skeletal muscles to adjust their liberation of energy to the work which they find they have to do, must clearly be an enormous saving to the body.

CHAPTER IV.

COMPARISON OF THE SHORTENING IN A SINGLE CONTRACTION AND IN TETANUS.

At first sight there seems to be a great difference between the form and height of a tetanus curve and of a single twitch in response to a maximal stimulus; since the record of a voluntary muscular contraction closely resembles a tetanus curve in form and height, it would seem to follow that a voluntary contraction must be of the nature of a tetanus and cannot be a single contraction. This difference between the height of a single contraction and of a tetanus curve is, however, more apparent than real, and is at any rate not a fundamental distinction between the two.

In order to investigate this point, arrange the apparatus for stimulating a gastrocnemius-sciatic preparation with single maximal induction-

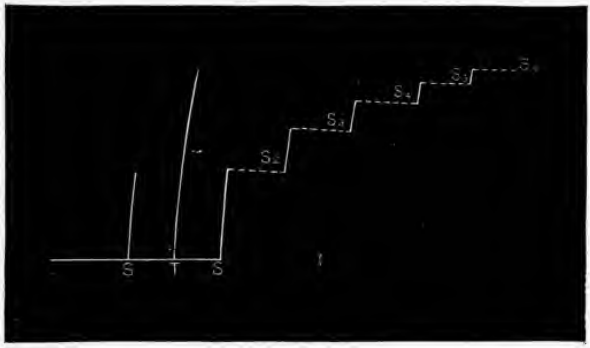


Fig. 175.—Comparison of the shortening of a gastrocnemius during a single isotonic contraction S, during complete tetanus T, and during a series of progressively after-loaded contractions S to S_6. (A.P.B.)

shocks, using a simple key in the primary circuit, and a myograph-lever provided with an after-loading screw. Place a weight of 20 grms. near the axis of the lever, bring the writing point on to a stationary drum and record a base-line by rotating the drum by hand. Stimulate the muscle with a single maximal shock. Rotate the drum on a short distance and stimulate the muscle by a tetanising current for a second or two; a contraction of about double the height of the single contraction will be recorded. Rotate the drum again and repeat the single stimulus; the contraction will be of its former height. Now raise the after-loading screw until the writing point is just at the top of the

contraction, rotate the drum and stimulate. The lever will be raised a less distance than before, but still it will be raised above the level of the previous single contraction. Repeat this process of supporting the muscle at the level of the top of the previous contraction until the muscle is no longer able to lift the lever off the after-loading screw. It will be found that the highest point reached by a series of single contractions taken in this way will be of about the same height as that of the tetanus (Fig. 175).

The reason why the single isotonic contraction of a weighted muscle is not so high as a tetanus curve seems to be as follows. The electro-chemical change in muscle begins directly the stimulus reaches the muscle and has already culminated when the change in form is beginning; that is, the change in form being a relatively slow process continues after its real cause, the electro-chemical change, has ceased. It is quite at the very beginning of the period of shortening, that the process, by which chemical energy is transformed into muscular force, is at its maximum, and then it rapidly declines. Consequently, if the inertia of the mass to be moved is great and is not overcome by the muscular force as rapidly as this force develops, the weight lags behind, the change in form, therefore, can only take place more slowly, and the muscle has ceased to pull at the weight before it has had time to accomplish its maximal shortening. In complete tetanus, however, before the pull of the muscle in response to one stimulus is at an end, the muscle again begins to pull in response to a second stimulus and so on, consequently the muscle has ample time to undergo its greatest shortening in spite of the inertia of the weight.

That it really is the inertia of the load which is the principal cause of the difference in height between these two curves can be shown by the fact that a muscle, weighted only by a very light lever, will, in response to a single maximal stimulus, shorten almost as much as it can do in tetanus.

In the case of the muscle with its load supported until later and later into its period of shortening, it is clear that the more the period of shortening is passed through by the muscle in its unloaded condition, the less will the inertia of the weight be able to cause delay in the process of shortening, and, consequently, the muscle in the same time will be able to undergo more and more nearly its full shortening.

It follows, too, from what has been said, that, if it were possible to slow down or prolong the chemical changes in muscle in response to a stimulus, and consequently to prolong the period during which the muscle exerted an active pull, it would enable the muscle to undergo more nearly its complete shortening, by providing a longer period

during which to overcome the inertia of the load. It is possible in several ways to prolong the period of active pull; only two will be mentioned. (1) By cooling the muscle. Record on a rapidly-revolving drum the contraction of a gastrocnemius preparation, stimulated directly by a single maximal shock, and weighted with 20 grms. near the axis of the lever, first at 15° C., and then after ice has been in contact with

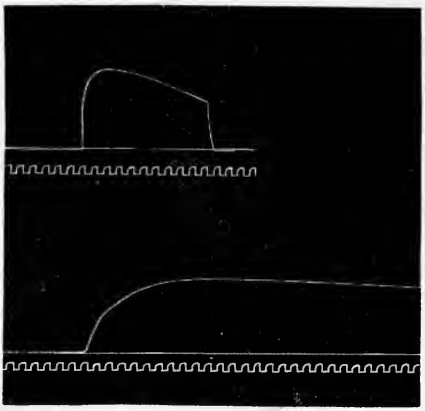


FIG. 176.—Comparison of the height of contraction of hyoglossus during tetanus, upper curve, and of the same muscle in response to a single maximal stimulus after thorough poisoning with veratrine. Time marking in seconds. (A.P.B.)

the muscle for some minutes. (2) By veratrine. On a slowly-revolving drum record first a tetanus curve of a hyoglossus preparation, and then the contraction of the same preparation in response to a single maximal stimulus, after thorough poisoning with veratrine (see p. 30). It will be found that the two curves are practically of the same form and height (Fig. 176).

CHAPTER V.

SUMMATION OF STIMULI.

IN a previous chapter the subject of summation of contractions has been dealt with. This summation of 'effect' must be distinguished from the summation of stimuli, by which an inadequate stimulus, if repeated sufficiently often, becomes first adequate and then for a time increasingly effective. This is a summation of 'cause,' and probably plays an important part in the life of all living matter.

In order to demonstrate the summation of stimuli, arrange the apparatus for stimulating a gastrocnemius muscle directly with single

induction-shocks, using a simple key in the primary circuit. Place the secondary coil at such a distance from the primary that the break-shocks are just subminimal. Repeat the stimulus every 5 seconds. It will be found that sooner or later the summed excitations will cause a contraction, and, if the contractions are recorded on a slowly revolving drum, that a well-marked 'stair-case' effect is produced (Fig. 177).

In dealing with the response of muscle to two successive stimuli, it has been seen that, when the second stimulus falls within the latent period of the first, the muscle is refractory, so far as being able to

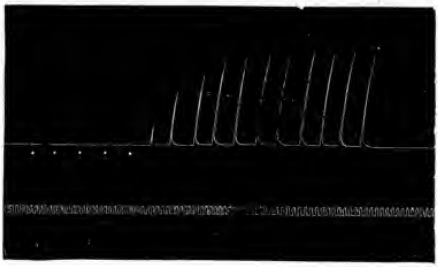


FIG. 177.—Effect of subminimal stimuli repeated every 5 seconds on gastrocnemius stimulated directly. The dots mark the points at which stimuli were sent in before they became obviously effective. Time marking in seconds. (A.P.B.)

respond with a second contraction is concerned; but it is not true that a muscle during its refractory period always entirely ignores a second stimulus.

In order to investigate this point, the apparatus is arranged as in demonstrating the effect of two successive stimuli (p. 42). The two 'strikers' are placed at such an angular distance apart that the second stimulus falls well within the latent period of the first; the muscle is stimulated directly. The secondary coil is placed at such a distance from the primary that when, by rotating the drum by hand, one of the strikers is made to pass over the naked wire, a minimal or submaximal break, but no make contraction is obtained. A tuning fork is arranged to write under the myograph-lever, the drum is allowed to make one revolution at a rapid rate, a base line is drawn, and the points of stimulation corresponding to each 'striker' are marked. Swing the lever away from the drum, but do not alter the position of the base of the stand carrying the myograph. The single contraction so recorded is the response of the muscle to two break shocks. In order to determine whether the muscle has been in any way influenced by the second stimulus, raise the second 'striker,' so that it will no longer touch the naked wire, and record the contraction due to the first stimulus alone (Fig. 178). It will be found that the contraction in

response to the single stimulus is not so great as that due to the two stimuli. In other words, there has been a summation of stimuli during the refractory period. In the same way subminimal stimuli can be summated, but two maximal stimuli are summated only when they follow each other after an interval of less than $\frac{1}{500}$th second.

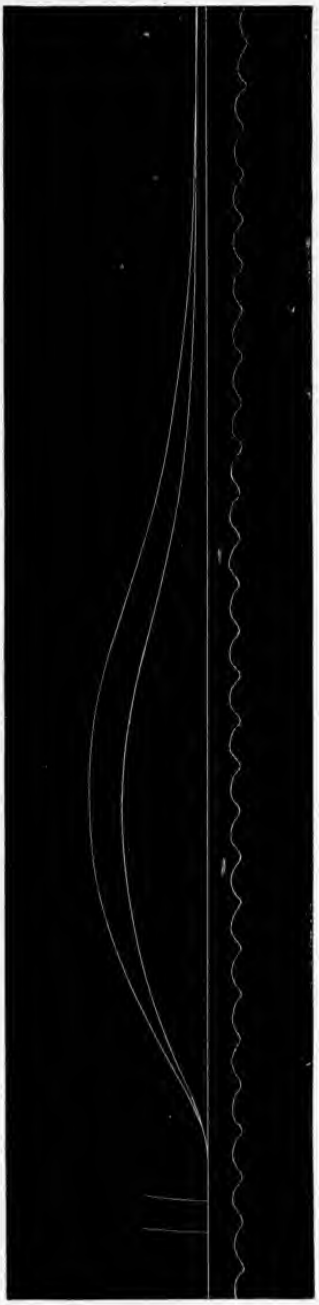


FIG. 178.—Effect of two submaximal stimuli on gastrocnemius, the second stimulus falling well within the latent period of the first. Upper curve represents the combined effect of both stimuli, the lower curve the effect of the first stimulus alone. Time marker, 100 per sec. (A.P.B.)

As has been pointed out on p. 25, when a 'striker' passes over the naked wire, there is both a make and break of the primary circuit; consequently in these experiments the muscle really receives four induction-shocks, of which, according to the position of the secondary coil, all four might be individually subminimal, or the two break-shocks might be alone effective, or all four might be effective. In order to deal with the summation of two break-shocks alone, it is usual to perform these experiments with the following special piece of apparatus.

The Spring or Trigger Myograph (Fig. 179).—It consists of a heavy metal base which is clamped to the bench. The essential part of the apparatus is an oblong metal frame carrying a smoked glass plate, the recording surface, which is shot on two horizontal wires past the writing points. In order to prepare the apparatus for use, the frame is pulled to one side by one of the arms attached to it; this compresses a spring on the other arm, and the frame is held in position by a catch or trigger. When the catch is released the spring gives the frame and

glass plate a rapid and uniform horizontal motion, and the momentum carries the recording surface across until stopped by the buffers at the opposite side. The frame carries on its under surface two pins which knock over two vertical keys and so breaks two primary circuits (Fig. 180). K_1 is fixed, but K_2 is movable horizontally, and its position can

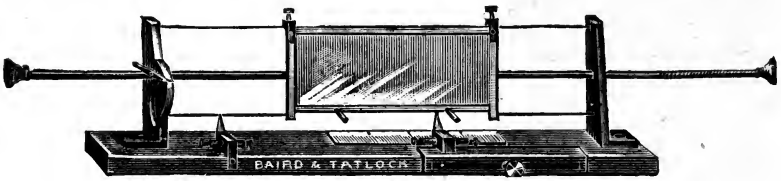


FIG. 179.—The spring myograph.

be adjusted so that it will be knocked over at any desired interval after K_1. A pointer is attached to K_2, and when this is opposite the zero of the scale this key will be knocked over at the same instant as K_1; therefore, in order that K_2 may be knocked over after K_1 and that the second

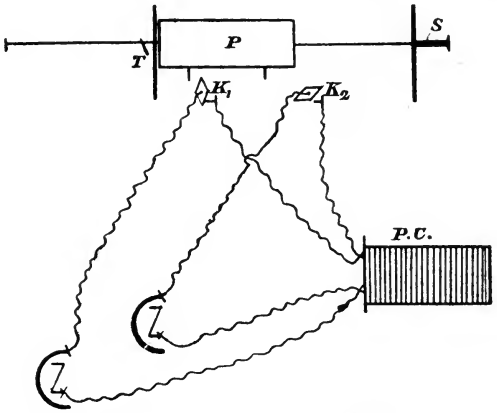


FIG. 180.—Diagram of the spring myograph in circuit.

stimulus may still fall within the latent period of the first, it is necessary to move K_2 a short distance along the scale from K_1. Place both keys in the primary circuit of the same coil and arrange the secondary coil at such a distance from the primary as to give submaximal break-shocks. With the spring compressed, the catch down and both keys vertical, the writing points of the lever and tuning fork are placed against the recording surface at its spring end in order that the whole contraction may be recorded. Release the catch. The frame is then pulled back to its original position, both keys are made vertical

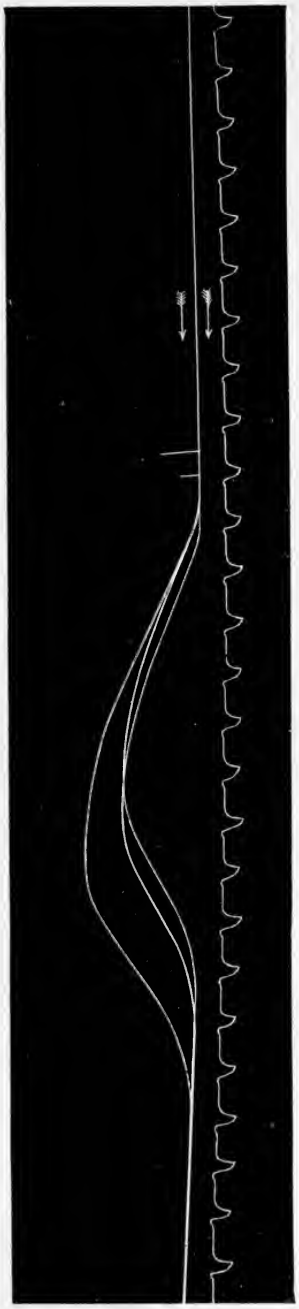


FIG. 181.—The effect of two stimuli upon the gastrocnemius muscle. The vertical lines show the moments of stimulation; the arrows indicate that the induction shocks were in the same direction. The uppermost curve represents the combined effect of both stimuli; the other curves the effect of the separate stimuli. Time marker, 100 per second. The curves, which were taken with the Pendulum Myograph, should be read from right to left. (M.S.P.)

again, and the pins on the frame are slowly brought up against the two keys in turn and the points along the curve marked at which the two stimuli entered the muscle; the second stimulus should have fallen well within the latent period of the first. Reset the apparatus, leaving K_2 horizontal, but placing K_1 vertical, and record the contraction due to the first stimulus alone. This second contraction will be found to be smaller than that caused by the summation of the two sub-maximal stimuli.

Fig. 181 shows the contractions obtained by a **Pendulum Myograph** which is fundamentally the same as a spring myograph, and differs only in that the smoked plate, instead of being shot horizontally across by a spring, swings across at the end of a long and heavy pendulum and describes an arc of a circle.

The glass plate in either case is varnished in the ordinary way, and, when dry the curves are reproduced by exposing to daylight sensitive paper covered by the smoked plate.

CHAPTER VI.

EFFECT OF DISTILLED WATER AND OF VARIOUS SALTS ON MUSCLE.

THE various tissues of the body are all bathed in the same fluid, the lymph, which so far as the water and salts it contains are concerned, has a uniform composition. The tissues, although immersed in the same fluid, show different and characteristic properties owing to their difference in structure and chemical composition. If, however, the composition of the fluid, in which any given tissue is immersed, be altered, the composition and consequently the properties of its protoplasm must also be altered. The first effect on living matter of such a change is to cause its stimulation, and then if the change be sufficiently profound and long-continued to produce its death.

Only two changes in the tissue fluids will be considered here, namely—(*a*) Gross change in the osmotic pressure of the fluid, by using distilled water or a strong saline solution; and (*b*) Change in the ions in solution without alteration in the osmotic pressure of the fluid, by using solutions of various salts isotonic with frog's blood-plasma.

Effect of Distilled Water.—Dissect out a gastrocnemius muscle and place it, without a 'trouser' of skin, in a watch-glass containing distilled water. For a few minutes the muscle may show irregular contractions, then it becomes opaque, swollen and incapable of responding to a stimulus with a contraction. The muscle is said to have passed into a condition of 'water-rigor.' Test the muscle with induction shocks and demonstrate that it will no longer contract.

By placing the muscle into distilled water two effects are produced—the inorganic salts in the muscle diffuse out into the water, and water is attracted by osmosis into the muscle so that each fibre becomes greatly distended with fluid. The first effect of these changes is to produce stimulation, but, as the muscle fibres are distended with fluid, they become incapable of contracting, and finally there are not enough salts left in the muscle to keep the globulins in solution; hence the muscle becomes gradually opaque and dies.

Effect of Strong Saline Solutions.—This effect will be exactly the opposite of that due to distilled water; for water will be abstracted from the tissue, and large quantities of the salt will diffuse into the muscle.

The effect on a tissue of mere abstraction of water from it is best seen by allowing a nerve to dry. Make a gastrocnemius and sciatic

preparation, keep the muscle and lower half of the nerve just moist with tap-water saline, but allow the upper half of the nerve to dry. As the nerve begins to dry, irregular contractions of the muscle come on which are stopped by moistening the nerve; showing that loss of water acts as a stimulus to nerve. If the drying is allowed to continue, the dry portion loses its irritability and dies.

Now place upon the muscle a few crystals of NaCl; irregular contractions will soon appear. These are partly due to the abstraction of water, but also, as we shall see in the next experiments, to the stimulatory effect of NaCl.

The above experiments show that, in order to keep muscles and nerves irritable and in good condition, they must be moistened with a fluid which will neither give up nor abstract water from the tissue, *i.e.* which is isotonic with the animal's lymph. For this purpose a ·7 per cent. solution of NaCl in distilled water has frequently been used. This solution, although isotonic with frog's blood, does not contain the calcium and potassium salts found in blood-plasma and lymph; and the question arises whether this alteration of the ions in solution affects in any way the properties of muscle.

In order to investigate this point, prepare two sartorius preparations with their bony attachments and without injury to their muscular fibres. Place one muscle in Biedermann's solution (·5 grms. NaCl, ·2 grms. Na_2HPO_4, 2·04 grms. Na_2CO_3 in 100 c.c. distilled water), and the other in ·7 per cent. NaCl in distilled water.

The muscle in Biedermann's solution, especially if the solution be cool (3°—10° C.), will after a shorter or longer interval begin to show fibrillary twitches and may even contract regularly and rhythmically as a whole. As soon as the result has been obtained, transfer the muscle to a solution made by adding to 100 c.c. of ·7 per cent. NaCl solution in distilled water, 10 c.c. of a saturated solution of $CaSO_4$, or of a 10 per cent. solution of $CaCl_2$ in distilled water. The spontaneous contractions will soon cease.

The other muscle placed in the pure NaCl solution may remain quiescent; very often it will show fibrillary twitchings and irregular contractions, which are rapidly stopped by transferring the muscle to the solution containing a calcium salt as well as NaCl. Should the muscle, however, remain perfectly quiescent[1] it can still be shown that it is no longer in a perfectly normal condition. After it has remained in the solution for half an hour, remove it and connect it

[1] Frog's muscle differs somewhat in its behaviour in any given solution according to the time of year, there being a marked difference between muscle in the autumn and spring.

to a myograph lever and stimulate it with a single maximal break shock. The contraction recorded on the drum will be no longer an ordinary single contraction, but a series of tetanic twitches of abnormal height and duration. Now remove the muscle, immerse it for ten minutes in the solution containing the added calcium salt, and again record its response to the same stimulus. A normal single contraction will be obtained. It is clear that sodium salts, when acting alone on skeletal muscle, have a powerful stimulatory effect, and that this can be neutralised by adding a certain proportion of calcium salt. For this reason 'normal' saline solution is always made with tap-water instead of with distilled water. Some tap-waters, however, do not contain nearly enough calcium to bring about complete neutralisation of the sodium salt.

From the above experiments we learn certain facts of considerable practical importance. We see that tissues are greatly affected by changes in the osmotic pressure of the fluid surrounding them. Care must therefore be taken not to expose the tissues of an animal or man to fluids which are not isotonic with the blood-plasma. In man the solution of NaCl isotonic with the blood-plasma is only just under 1 per cent., and therefore differs widely in strength from the solution for a frog; it is very necessary to bear this in mind when injecting fluid into veins or under the skin, and when irrigating the peritoneal cavity during operations. We further see that, when isotonic solutions of electrolytes are used, the tissues are by no means indifferent to the ions in solution. A really 'normal' saline solution would, therefore, be one which contained the same salts in the same proportion as the animal's own blood-plasma. Ringer's[1] fluid is an attempt to make such a solution for the frog. Since in man it would often be difficult to obtain such a solution when wanted, it might be preferable, instead of using an imperfectly 'normal' saline solution, to use an isotonic solution of a non-conductor, such as dextrose. A 5·8 per cent. solution of dextrose is isotonic with human blood-plasma.

In all the above experiments it has been found that skeletal muscle responds to the abnormal constant stimulus by an activity which is not constant, but intermittent or rhythmical. This raises the question whether the rhythmical contraction of the heart may not be the normal response of that particular kind of muscle to the constant chemical stimulus of the blood-plasma, and the same might be also partly true of the rhythmical activity of the respiratory and vasomotor centres.

[1] A modified Ringer's solution contains NaCl ·7 per cent., $CaCl_2$ ·0026 per cent., and KCl ·035 per cent.

CHAPTER VII.

FATIGUE OF A VOLUNTARY MOVEMENT AND OF A MUSCLE-NERVE PREPARATION WITH ITS CIRCULATION INTACT.

When a voluntary movement is repeated sufficiently often fatigue is produced. The seat of this fatigue has to be investigated; it might be in some part of a neurone in the central nervous system, or in some part of the peripheral nerve and muscle: in other words, the fatigue might be primarily central or peripheral. As the result of certain ergographic experiments it has been answered that this fatigue is of central origin. The experiments consisted in lifting a heavy weight suspended over a pulley by flexing a finger and registering the height of each successive lift. When the movement had been repeated until the muscle was no longer able to lift the weight at all, it was found that electrical stimulation of either the nerve supplying the muscle or of the muscle itself caused the weight to be again lifted, but to a less height than before. When the electrical stimulation had in turn fatigued the movement it was found that a voluntary contraction of the muscle was again able to lift the weight, owing, it was supposed, to the resting of the cells in the central nervous system. From these experiments it was argued that the fatigue of a voluntary movement is purely central.

The methods used in the above experiments are open to grave objections, and it is necessary to touch upon some of these in order to avoid them. The use of a heavy weight is open to the objection that the muscle, when no longer able to lift that weight, is still capable of contracting, and could well lift a lighter weight; therefore, it is better to make the muscle bend or pull on a spring, which will enable the feeblest as well as the strongest pull exerted by the muscle to be recorded. Again, electrical stimulation of a nerve or a muscle can be a much more powerful stimulus than that resulting from the maximal discharge of a motor nerve-cell; consequently the fact that peripheral stimulation can make the muscle again lift the weight after voluntary impulses fail, is no proof that the fatigue was central. Further, when a nerve or muscle is stimulated by electrodes placed upon the skin, it is impossible to produce equal stimulation of all fibres; some muscle-fibres will receive a maximal and others only a sub-maximal or minimal stimulus, and the pull of the muscle as a whole will be equivalent to that of a weaker muscle. When the muscle appears to be fatigued by peripheral stimulation, then a return to volitional stimulation, by pro-

ducing equal stimulation of every fibre, leads to an apparent recovery of voluntary power. In this way is to be explained the apparent paradox, that a muscle fatigued by either voluntary or peripheral stimulation shows a recovery of power when stimulated in the opposite way.

In order to investigate this subject we shall compare the curve of voluntary fatigue taken with a spring ergograph from the human abductor indicis, with the curve obtained from the frog's gastrocnemius, with its circulation intact and stimulated through the sciatic nerve.

The Spring Ergograph.—A simple form of this instrument is shown in Fig. 182 to consist of a rigid upright iron bar which is clamped to

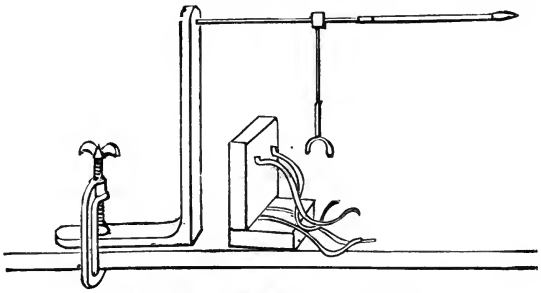


Fig. 182.—Spring ergograph. (Porter.)

the table. From the upper end of this projects a horizontal straight steel spring, the free end of which carries an ordinary writing point. The spring carries on its under side a short vertical steel arm, the lower end of which fits over the distal end of the second phalanx of the index finger. When the abductor indicis contracts the spring is pushed up; by sliding the vertical arm along the spring the magnification of the movement and the strength of the spring can be altered. The hand is placed along the vertical side of the wooden support and the three outer fingers tied to it, leaving the thumb and index finger free. The forearm should be fixed to the bench in some form of support, but care must be taken not to tie down the arm sufficiently tightly to interfere with its circulation.

The subject of the experiment should sit comfortably and with his eyes shut, should not be spoken to nor in any way have his attention diverted, but should confine himself to giving a maximal contraction of his muscle every time he hears the beat of a metronome, which is set to give a beat every second. The observer takes the time of the experiment in minutes and so calculates the number of contractions recorded, further he has to see that the vertical arm does not slip

out of position along the finger. In this way take 300 to 600 contractions on a drum revolving at an extremely low rate (Fig. 183).

At first sight the most striking feature of the curve is the more or less rhythmical waxing and waning in the height of the contractions; this seems to be purely central in origin and to be due to variations in the strength of the voluntary impulse communicated to the muscle. Practice to a large extent does away with this rhythm. When the height of the contraction is measured it will be found that the average height decreases during the first 180 contractions and then attains a fairly constant level, which represents about 85 per cent. of the height of the original contractions. The initial decrease is better marked in Fig. 184, and here the fatigue-level was only about 45 per cent. of the original height. The characteristics of an ergographic fatigue-curve, therefore, are an initial fall which takes place during a variable number of contractions, and the attainment of a fairly constant level, which represents varying percentages of the height of the original contractions. This curve strongly suggests that during a series of contractions two processes are at work; one by which available combustible material is being used up and the products of katabolism are accumulating, and the other by which both these defects are made good by the circulation. During the early part of the curve the first process preponderates over the second and the height of the contraction decreases, but as soon as the two processes exactly balance each other a uniform level is maintained for hundreds of contractions. The probable seat of these processes will be referred to after the next experiment has been performed.

In order to obtain a record of the contractions of the gastrocnemius with its circulation intact, arrange the apparatus for stimulating the sciatic nerve with maximal induction shocks, using a simple key in the primary circuit. The cerebrum of the frog must be destroyed and the muscle-nerve preparation made without causing bleeding. The cerebral hemispheres are destroyed by compression, leaving the medulla and spinal cord intact, and the gastrocnemius is prepared in the usual way. A string ligature is placed beneath the gastrocnemius and tied tightly round the upper part of the tibio-fibula and the remaining muscles; the leg is then cut through below the ligature. The whole frog is placed belly downwards on the myograph-board, a strong pin is pushed through the lower end of the femur and driven firmly into the cork. A piece of moistened flannel is then pinned down over the trunk to prevent the contractions of the muscles of the trunk from disturbing the lever connected with gastrocnemius. The skin over the middle of the thigh is divided longitudinally for a short distance, the muscles carefully

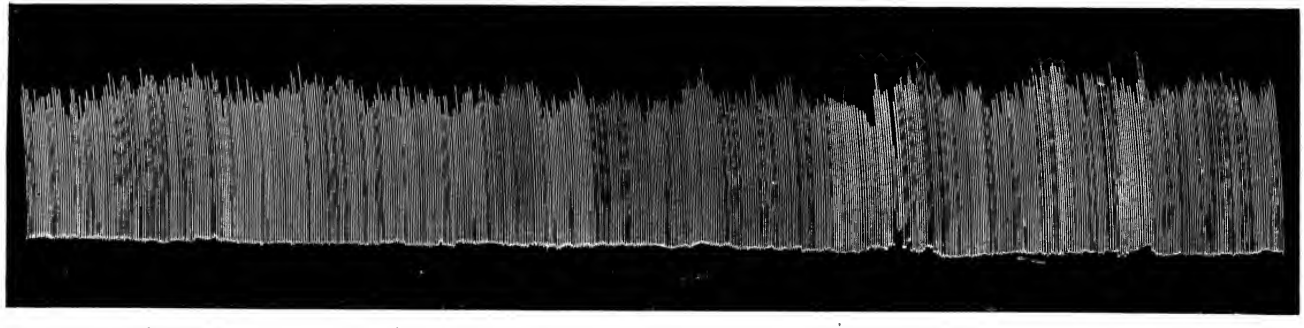


FIG. 183.—Ergographic tracing of abductor indicis taken with a spring ergograph. The tracing is to be read from right to left and represents 600 contractions performed at the rate of one per sec. (A.P.B.)

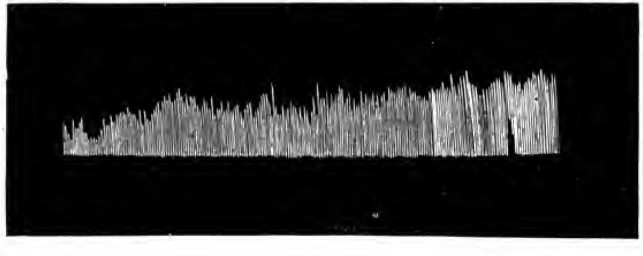


FIG. 184.—Ergographic tracing of abductor indicis of an ill-nourished boy. The tracing is to be read from right to left and represents 150 contractions performed at the rate of one every 2 secs. (A.P.B.)

separated and the sciatic nerve exposed and freed; the nerve is gently raised by slipping a thread beneath it and the electrodes, insulated

FIG. 185.—Exhaustion curve of gastrocnemius with its circulation intact. Muscle was just after-loaded and was stimulated indirectly once every 5 seconds. This part of curve extended over a period of 45 minutes and was broken off temporarily owing to the frog moving. (M.S.P.)

FIG. 186.—Continuation of same experiment, extending over a period of 15 minutes. (M.S.P.)

from the underlying muscles by a small piece of cork, are placed beneath the nerve. It is essential that the nerve should not be injured and

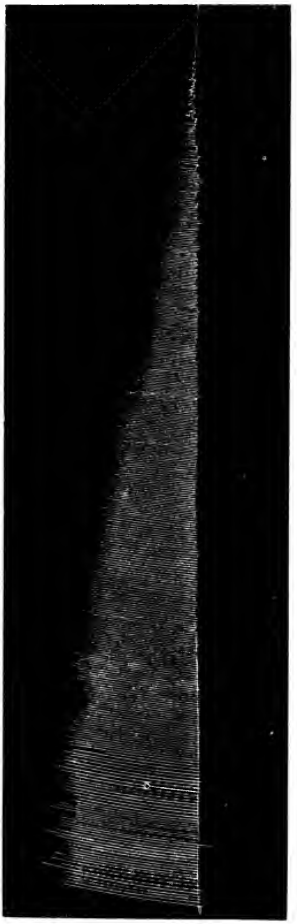


FIG. 187.—Continuation of same experiment, after the leg had been cut off. The whole curve represents a period of about 40 minutes. (M.S.P.)

FIG. 188.—Exhaustion curve of gastrocnemius just after-loaded and stimulated once every 5 secs. indirectly. First part of curve represents 15 minutes, then follow 5 minutes' rest and a short recovery curve. After another 7 minutes' rest a further recovery curve is shown with the muscle loaded instead of after-loaded. (M.S.P.)

should be kept properly moistened throughout the experiment. The muscle is suitably weighted and just after-loaded. The nerve is stimulated by a maximal shock every 5 secs., and the contractions recorded on a drum revolving at the slowest possible rate (Figs. 185, 186).

It will be seen that the height of the contractions, although increasing at first, gradually falls off until at the end of about 200 contractions it reaches a uniform level, which represents about 85 per cent. of the original height and was then maintained with scarcely any alteration for three-quarters of an hour. This curve, therefore, is identical in general form with that obtained by the ergograph. Here again we see an initial fall and then a constant level of contraction, representing probably the equilibrium between two opposite processes, which must in this case be affecting some part of the peripheral nerve and muscle. The actual seat of this peripheral change is not absolutely certain (see further Expts. on p. 330).

Now cut through the leg in the middle of the thigh, so as to destroy the circulation through the gastrocnemius and continue the stimulation (Fig. 187). It will be seen that the height of the contractions rapidly and continuously decreases, and that at the end of about 320 contractions the muscle is no longer able to lift the lever off the after-loading screw.

We conclude, then, that a comparison of an ergographic tracing with one obtained by the artificial stimulation of the motor nerve of a muscle, whose circulation is intact, does not demonstrate the existence of central apart from peripheral fatigue.

CHAPTER VIII.

THE RATE OF TRANSMISSION OF A NERVOUS IMPULSE.

THE rate at which an impulse is transmitted along a nerve is important because it throws some light upon the nature of the impulse. It travels much more slowly than an ordinary electric current, and, although it is accompanied by an electric change, it is something more complex. Its rate of propagation is 27 metres per second (88½ feet per sec.) in the frog's sciatic nerve, and 33 metres per second (108 feet per sec.) in the motor nerves of man.

(*a*) **In the Motor Nerves of the Frog.**—The following experiment should be performed for the determination of the velocity of the nervous impulse in the sciatic nerve of a frog:

A recording drum is arranged with a 'striker' for completing the circuit of the primary current of the induction-coil. To the secondary coil are attached two Du Bois keys in the manner shown in the diagram (Fig. 189); from these pass two pairs of electrodes, one of which will be applied to the upper portion of the nerve, the other to the lower

portion. The entire length of the sciatic nerve is dissected out, and the gastrocnemius muscle is connected with the lever of a myograph; the drum is arranged for rapid revolution, and a maximal shock is to be used for excitation. The latency of the muscular contraction (Chapter III., p. 22) is then determined, first for stimulation by the upper pair of electrodes, the lower pair being short-circuited by closure of its Du Bois

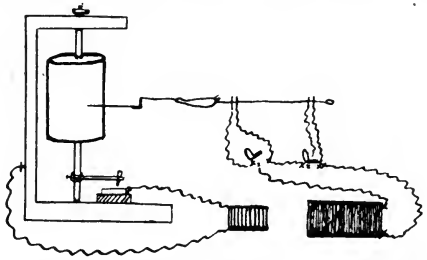


FIG. 189.—Diagram of the experiment on the rate of transmission of a nervous impulse.

key; then the experiment is made with the lower pair of electrodes for the exciting point. The time of this latency is determined by recording underneath the curves the vibrations of a tuning fork with 100 vibrations per second; the difference in time between the moment of stimulation and the resulting contraction in the two cases represents the time taken for the nervous impulse to pass along the length of nerve between the two pairs of electrodes (Fig. 189). This piece of nerve is measured in millimetres,[1] and then the velocity of the transmission of the nervous impulse is calculated.

For the accurate determination of the rate of propagation of a nervous impulse a very rapid rate of movement of the recording surface is required; for this reason the spring-myograph (Fig. 179, p. 299) or the pendulum-myograph may be used with advantage in the place of the drum.

(*b*) **In the Motor Nerves of Man.**—The velocity of the transmission of a nervous impulse in the motor nerves of man can be determined in the following way: A thick-walled india-rubber ball, similar to that used with a photographic 'shutter,' is connected with a recording tambour. Two clinical electrodes are moistened with strong saline solution in order to improve their conduction and contact with the skin; the large flat electrode is fastened to the leg of the subject, and the small electrode placed above the clavicle will be pressed over the brachial nerves. These electrodes are connected with the secondary

[1] There is a millimetre scale upon the slide of the induction-coil.

coil of an inductorium, and in the primary circuit is interposed the 'trigger' key of the spring-myograph.

The india-rubber ball is held between the middle finger and the thumb, and the contraction of the flexor muscles will be recorded by the lever of the tambour, when the nerve is excited. The moment of stimulation is determined in the usual way (p. 25), and then the experiment is again performed, but with the small electrode pressed over the median nerve at the bend of the elbow. The moment of stimulation is again determined, in order to show that the resting position of the point of the lever has not been changed. The difference between the latency in the two contractions is measured by a tuning-fork vibrating 100 times per second, and the length of nerve between the two points of stimulation is estimated; from these data the rate of transmission of the nervous impulse can be calculated.

CHAPTER IX.

THE POLARISATION OF ELECTRODES AND UNPOLARISABLE ELECTRODES.

Polarisation of Electrodes.—Ordinary metal electrodes in contact with a muscle or nerve will be surrounded by lymph, and in this fluid electrolysis will take place during the passage of an electric current. The ions resulting from this electrolysis will be positive and negative respectively; if, therefore, the circuit of this seat of chemical and electrical change be suddenly made or broken, a shock will be produced, for the wires of the electrodes surrounded by the electrolysed fluid will form a minute battery. This can be demonstrated by the following experiment: A pair of electrodes, connected with a Du Bois key, is placed under the sciatic nerve, which has been exposed in the thigh of a pithed frog. Making or breaking the circuit causes no contraction. The two wires of a Daniell battery are connected with each side of the Du Bois key, and the current is allowed to pass through the nerve for several seconds. Then these two wires are rapidly disconnected from the battery and key; the key is closed and opened, and each time a contraction of the muscles of the leg is caused. This make and break can be repeated several times with a similar result, until the polarisation has disappeared.

This experiment shows the necessity of unpolarisable electrodes in experiments upon the effects produced in nerve and muscle by the

passage of a constant electric current, and also the necessity of using a Du Bois key as a bridge to short-circuit the electrodes.

Unpolarisable Electrodes.—The preceding experiment has shown that the electrolysis occurring around the ordinary metal electrodes may easily act as an exciting electric current, and thus cause errors in experiments. In order to avoid this unpolarisable electrodes are used. The electric current from the battery is conducted through media which are not liable to polarisation.

The structure of Burdon-Sanderson's electrodes is shown in the following diagram (Fig. 190). A smooth amalgamated zinc rod dips into a saturated solution of zinc sulphate, which in turn conducts the current by means of a plug of kaolin or china clay, made into a thick paste with normal saline solution (·75 per cent. sodium chloride). The plug rests upon a small glass tube with a flange; this delays the spread of the zinc sulphate into the kaolin. The nerve or muscle can be placed in contact with the plug of kaolin, or may be connected thereto by threads saturated with normal saline solution and kaolin. The plug must be kept moist with normal saline solution, for the electrodes have a high resistance.

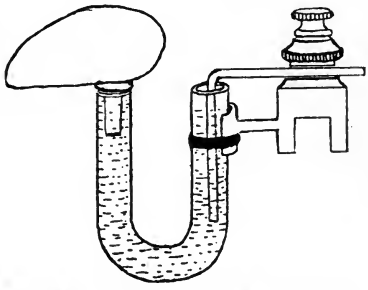


Fig. 190.—Unpolarisable electrode. Burdon-Sanderson's pattern.

The electrodes must be set up with clean hands and material, otherwise polarisation will occur. The solution of zinc sulphate must not be allowed to touch the tissue, for chemical excitation would occur. Kaolin and normal saline solution do not stimulate muscle and nerve.

The previous experiment on the polarisation of electrodes should be repeated with the unpolarisable electrodes. The result will be negative if the electrodes have been well and truly made.

CHAPTER X.

TRANSMISSION OF A NERVOUS IMPULSE IN BOTH DIRECTIONS.

The excitatory state produced by stimulation of a nerve can be transmitted in both directions. This can be shown by the following experiments.

Sartorius Experiment.—The sartorius muscle is dissected out and its

iliac end is divided into two portions (Fig. 191). Stimulation with a weak induction shock at (*a*) or (*a'*), when there are no nerve-fibres, will produce a contraction of the one half of the muscle. Excitation, however, at (*b*) or (*b'*), where there are nerves, will evoke a contraction of both halves.

Gracilis Experiment.—The gracilis muscle of the frog is in two portions completely separated by a tendinous intersection (Figs. 21, 192). Both halves of the muscle are supplied by a single nerve, the individual fibres of which divide and supply both halves of the muscle. Stimula-

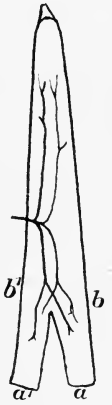


FIG. 191.—Diagram of the sartorius experiment to show the transmission of a nervous impulse in both directions.

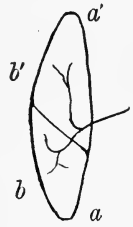


FIG. 192.—Diagram of the gracilis experiment to show the transmission of a nervous impulse in both directions.

tion of any kind at (*a*) or (*a'*), where there are no nerve-fibres causes only the corresponding half of the muscle to contract; but excitation at (*b*) or (*b'*), where the nerves lie, will cause both halves to contract.

With this last experiment should be *contrasted* the so-called "paradoxical contraction."

Paradoxical Contraction.—At the knee of the frog the sciatic nerve divides into the peroneal and popliteal nerves, but the individual fibres of the two nerves arise separately from the central nervous system and lie alongside each other in the sciatic nerve (Fig. 193). As much of the popliteal nerve (*a*) as possible is carefully dissected out; the nerve is divided and stimulated by a galvanic shock; the muscles supplied by the peroneal nerve (*b*) will contract. This is due to the electrotonic current, set up in nerve (*a*), stimulating the fibres of (*b*) where the two nerves lie close together at (*c*). It is *not* due to the transmission of a nervous impulse in both directions.

Mechanical stimulation of the nerve (*a*) produces no polarisation and therefore will not cause a contraction of the muscles supplied by the

nerve (*b*). On the other hand the effect of electrical stimulation of the nerve (*a*) upon the nerve (*b*) is not due to simple escape of electrical current, for if a moist thread be tied at (*a'*) no contraction of the muscles supplied by the peroneal nerve will be obtained when the nerve is stimulated at (*a*). A moist thread will not block the passage of an electrical current, but it destroys the physiological continuity of the nerves and thus prevents the extension of the electrotonic current.

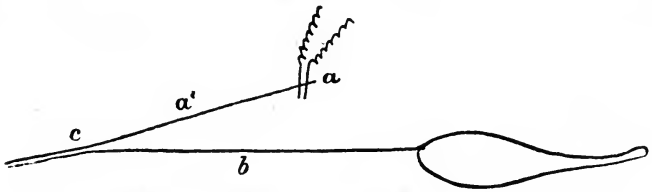


FIG. 193.—Diagram of the experiment known as "paradoxical contraction."

In certain cases, when the tissues are very excitable, the so-called "paradoxical contraction" appears to be due to the excitation of the nerve-fibres (*b*) by the action-currents of the adjacent nerve-fibres at (*c*). The current of action of one nerve stimulates another nerve.

CHAPTER XI.

THE RELATION BETWEEN MUSCLE AND NERVE. THE INDEPENDENT EXCITABILITY OF MUSCLE.

IN addition to the experiments which have been described in the elementary course (page 48), the following experiment upon the sartorius muscle should be performed.

The sartorius muscle lies on the ventral surface of the thigh (Fig. 21), and its outlines can be made distinct by sponging it with the frog's heart full of blood. The muscle is carefully dissected out and will contract when its nerve, which passes into the muscle at the middle of its inner border, is cut across by the scissors. If the muscle be placed between two glass-slides and examined under a microscope, the distribution of its nerve can be seen to resemble that shown in the diagram (Fig. 194). The finer branches of the nerves and even the end-plates can be more readily seen if the muscle be treated with acetic acid. There are no nerves in the terminal

FIG. 194. — Diagram of the sartorius muscle to show the distribution of its nerves.

portions of this muscle, which consists of fibres running in a direction parallel with its length.

The sartorius muscle is dissected from the other thigh and the nerveless parts are stimulated by a pinch with a pair of forceps or by an electrical shock; they contract, the muscle possesses independent excitability.

The absence of nerves from the terminal portions can also be shown in the following way. The muscle is suspended from its tibial end and is lowered until the cut iliac end touches some strong glycerine contained in a watch-glass; it does not contract. A thin transverse slice is cut away and the muscle is again lowered into contact with the glycerine; there is still no contraction. This procedure is repeated until the nerves are cut across and on contact with the glycerine are stimulated and make the muscle pass into a contracted condition.

CHAPTER XII.

THE EFFECT OF TEMPERATURE UPON THE EXCITABILITY AND CONDUCTIVITY OF NERVE. THE MOIST CHAMBER.

LIVING structures contain about 80 per cent. of water, and upon the presence of this water largely depend many of the physical and chemical changes which underlie the phenomena of life. It is important, therefore, that in exact experiments precautions should be taken to prevent the drying of living tissues. For this purpose the moist chamber is used. It consists of a glass case to cover the myograph or other apparatus employed for holding the tissue, and it is kept moist by the presence of a piece of tow or cotton-wool soaked in water.

The **excitability** of nerve is altered by changes of temperature. Local cooling raises the excitability for most stimuli, but in order to demonstrate this special precautions, as Gotch has shown, are necessary. For, when a moist electrolyte is cooled its resistance is increased and therefore with an electrical stimulus the physiological increase in excitability may be completely obscured by the physical increase in resistance. In order to eliminate this source of error it is only necessary to stimulate the nerve by a galvanic current passing through a large external resistance, 100,000 ohms; in this way any change in the resistance of the nerve will be, when compared with the total resistance of the circuit, so small as to be negligible.

A freshly prepared muscle and nerve preparation is placed in the moist chamber and under one portion of the sciatic nerve is placed a small glass tube through which a current of warm or cold water can be circulated. The make of a descending current is used as the stimulus so that the kathode is upon that portion of the nerve

which is subjected to the change of temperature (Fig. 195). A minimal stimulus is obtained for the nerve when the temperature is that of the room, 15° C. The height of the contraction is recorded on a stationary drum, which can be moved by hand before the next contraction. A current of cold water, 5° C., is passed through the tube; the stimulus will now produce a maximal contraction. Warming the nerve will have an opposite effect. If, however, the experiment be performed with very short stimuli, such as are produced in an induction-apparatus with the core removed from the primary coil, it will be found that cooling the point of excitation *diminishes* the excitability.

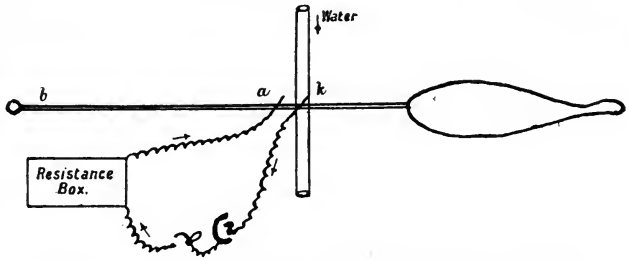


FIG. 195.—Diagram of the experiment to show the effect of temperature upon the excitability of nerve.

A similar degree of cooling has no effect upon the **conductivity** of the frog's sciatic nerve. This can be readily shown by stimulation of a portion of the nerve central to the cooled area.

CHAPTER XIII.

THE INFLUENCE OF CARBON DIOXIDE, ETHER AND CHLOROFORM UPON NERVE.

THE effects of the above agents upon the excitability and conductivity of nerve can be shown by the following experiment. The sciatic nerve of a muscle- and nerve-preparation is passed through two notches in the cork of a small gas-chamber,[1] the holes are closed around the nerve by kaolin moistened with normal tap-water saline solution, and the top is closed by a glass cover-slip fastened down with kaolin (Fig. 196). In this way the action of the gas or vapour is confined to one portion of the nerve. The preparation is placed in a moist chamber after two pairs of electrodes (*a*) and (*b*) have been arranged in the way shown in the diagram. By means of glass tubes connected with long pieces of rubber tubing the gas or vapour can be conducted through the gas-

[1] A small gas-chamber can be easily made out of a large cork. With a large borer the centre is cut out and a slice of the plug so removed is reinserted to form the floor. Two needles are pushed through the walls of the chamber to form the pair of electrodes (*b*).

chamber without any escape into the moist chamber. Minimal stimuli are obtained for the nerve lying on the two pairs of electrodes, and the contraction is recorded upon a stationary drum. A stream of carbon dioxide is passed through the gas-chamber; the minimal stimulus is no longer effective at (*b*), but is still adequate at (*a*). The carbon dioxide has locally lowered the *excitability*, but has not affected the *conductivity* of the nerve. Fresh air is drawn through the gas-chamber; the stimulus at (*b*) will again become effective.

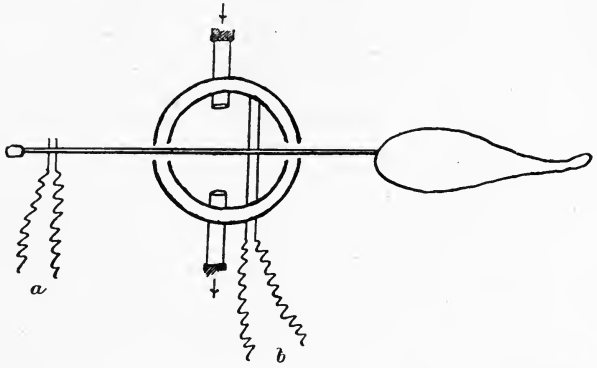


FIG. 196.—Diagram of the experiment upon the influence of carbon dioxide, ether and chloroform on the excitability and conductivity of nerve.

This experiment is repeated with ether or chloroform, and it is found that both the excitability and conductivity are diminished. The removal of the vapour by a current of fresh air will restore both the excitability and conductivity. Prolonged action of the anaesthetic will produce poisoning, and in this respect chloroform is much the more toxic of the two.

CHAPTER XIV.

THE EFFECT OF A CONSTANT ELECTRICAL CURRENT UPON THE EXCITABILITY AND CONDUCTIVITY OF NERVE.

THE passage of a constant current produces changes in the excitability of nerve, at the anode there is a condition known as **anelectrotonus**, the excitability is diminished; at the kathode there is an increase in excitability, a state of **katelectrotonus**. The conductivity is also affected, there is a fall in both the anodic and kathodic regions. These effects can be shown by the following experiment.

One Daniell battery is connected by two wires with a Pohl's reverser whereby the direction of the current can be changed; from the reverser the wires pass by means of a Du Bois key to a pair of unpolarisable electrodes. This is the polarising circuit. The stimulating circuit is set up separately for the production of single induction-shocks (Fig. 197). A preparation of the sciatic nerve and gastrocnemius muscle is carefully made from a recently pithed frog, and is placed in a moist chamber; a pin is fixed through the lower extremity of the femur, and the tendo Achillis is connected by a thread with a lever.

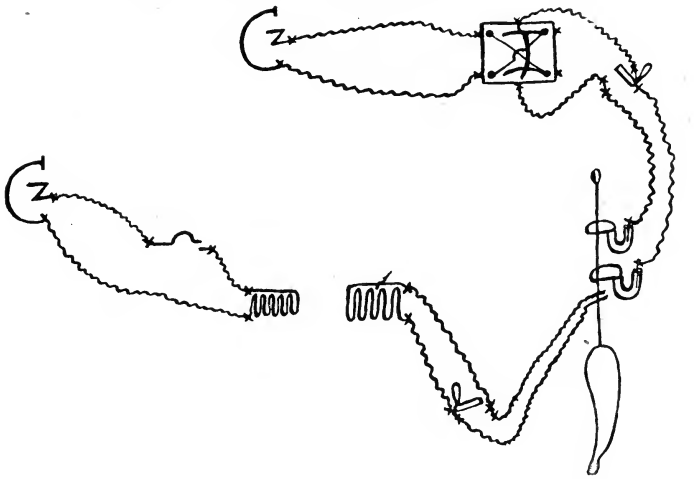


FIG. 197.—Diagram of the experiment on the effects of a constant electrical current upon the excitability and conductivity of nerve.

The sciatic nerve is placed across the kaolin plugs of the unpolarisable electrodes. The drum can be moved by hand. A minimal stimulus for the nerve is obtained, care being taken to use only the break or make-shock. The minimal contraction is recorded on the stationary drum.

The current from the polarising circuit is closed in an ascending direction, so that the current enters the nerve on the side near the muscle and immediately above the stimulating electrodes, which are connected with the inductorium. The nerve around the point of entry or anode of the polarising current is depressed in its excitability, and the application of a minimal, or even stronger, stimulus is no longer effective (Fig. 198). The polarising current is short-circuited by the Du Bois key, and by means of the reverser is changed in its direction, so that on opening the Du Bois key the current is descending, and the area of nerve near the stimulating electrodes passes into a condition of

katelectrotonus. The minimal stimuli now become maximal, owing to the increase in the excitability of the nerve at the kathode.

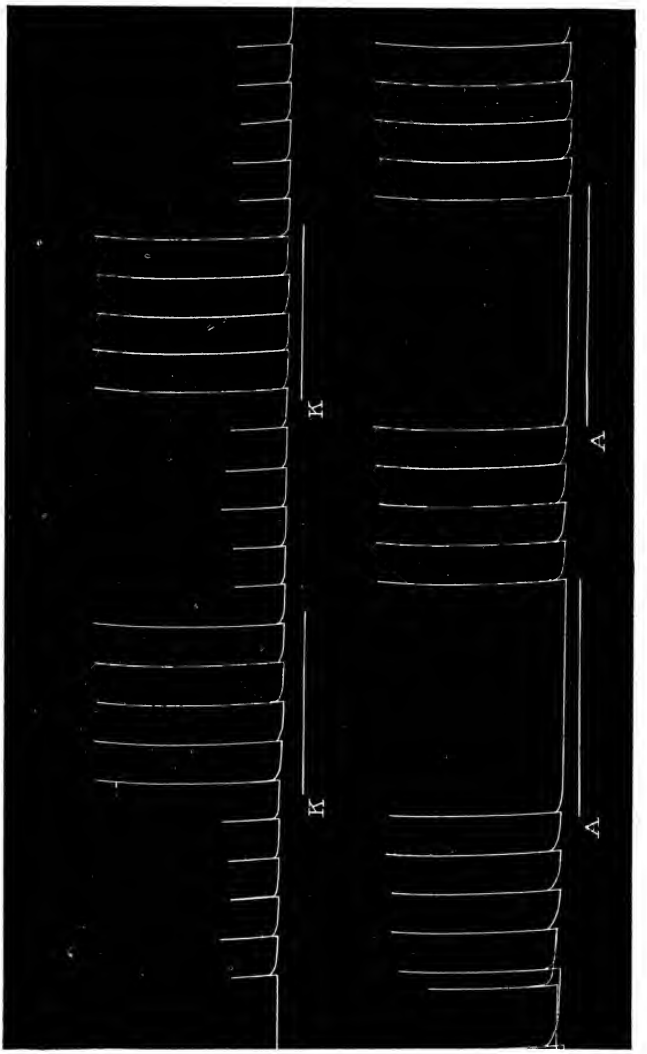


FIG. 198.—The effect of a constant electrical current upon the excitability of nerve. (1) The nerve was stimulated at regular intervals by single induction shocks almost strong enough to produce a maximal contraction. The horizontal lines marked A show the time during which the polarising current was closed in an ascending direction, that is the duration of anelectrotonus; the single induction shocks were no longer effective. (2) The nerve was stimulated at regular intervals by minimal induction shocks. The horizontal lines marked K show the time during which the polarising current was closed in a descending direction, that is the duration of katelectrotonus around the exciting electrodes; the minimal shocks become maximal. The curve should be read from left to right. (M.S.P.)

The above experiments can be repeated with a crystal of common salt placed in the position of the stimulating electrodes. The salt causes chemical excitation, and the muscle shows incomplete tetanus, which is quelled by anelectrotonus, and augmented by katelectrotonus (Figs. 200, 201).

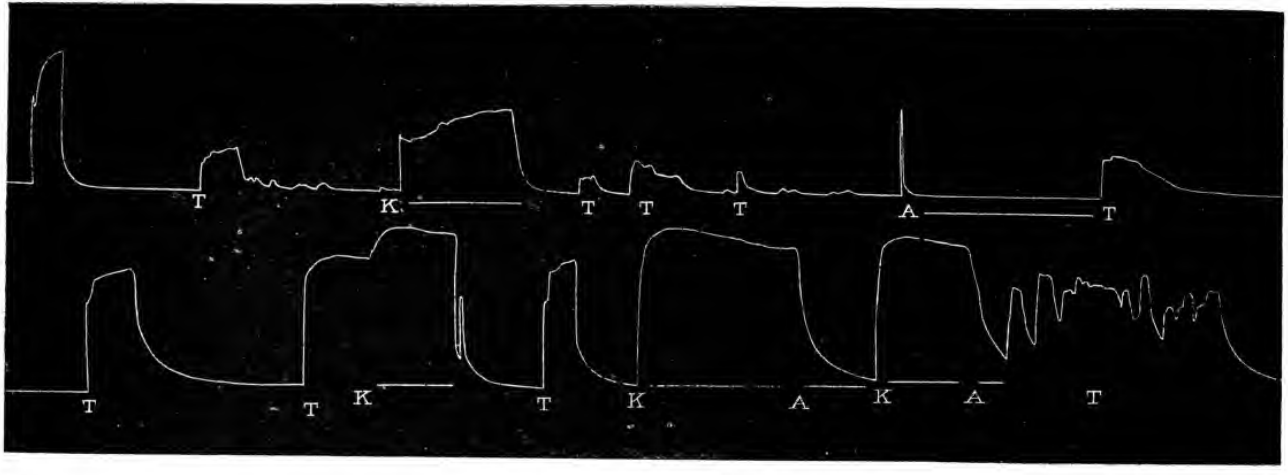


FIG. 199.—The effect of a constant electrical current upon the excitability of nerve. The nerve was stimulated by weak tetanising shocks obtained with Helmholtz's arrangement; the incomplete tetanus T was recorded. The production of a condition of katelectrotonus, K—— increased the effect of the stimuli and thus augmented the tetanus. The production of a condition of anelectrotonus, A·——, around the exciting electrodes abolished the effect of the stimuli; the excitability of the nerve was diminished, the tetanus ceased. The curves should be read from left to right. The tracing has been reduced in size. (M.S.P.)

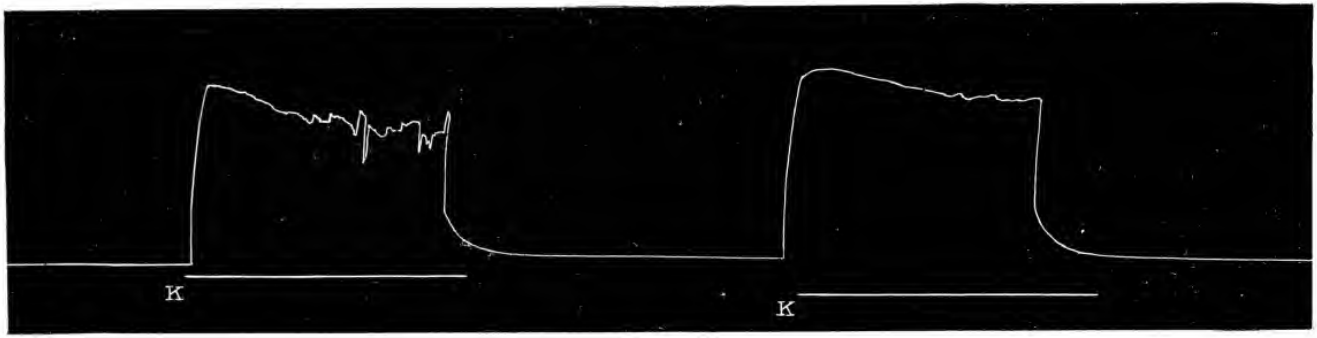


FIG. 200.—The effect of a constant electrical current upon the excitability of nerve. A crystal of common salt was employed for the stimulation of the nerve; the chemical stimuli were subminimal, but became effective directly the nerve around the crystal of salt was thrown into a condition of katelectrotonus by the closure of the polarising current. During this period of katelectrotonus, K——, the chemical stimulation of the nerve caused incomplete tetanus of the muscle. The curve, which has been slightly reduced in size, should be read from left to right. (M.S.P.)

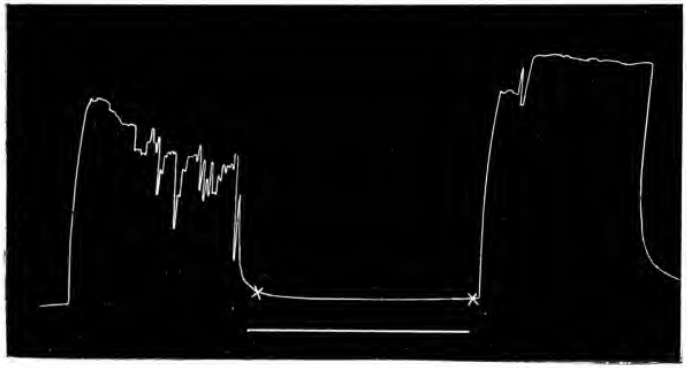


FIG. 201.—The effect of a constant electrical current upon the excitability of nerve. This tracing is a continuation of that in Fig. 200. The chemical stimulation of the nerve from the penetration of the salt produced incomplete tetanus, which could be abolished or quelled by throwing the stimulated portion of the nerve into a condition of anelectrotonus. The beginning, duration and end of the stage of anelectrotonus are indicated by the stars and horizontal line The curve should be read from left to right. (M.S.P.)

The conditions of anelectrotonus and of katelectrotonus spread on each side of the respective electrodes, so that between the poles there is a decrease in excitability near the anode, an increase of excitability near the kathode, and no alteration in excitability at a point about midway between the poles. These changes in the interpolar region are best studied by means of a mechanical stimulus, such as Tigerstedt's hammer, which is worked by an electromagnet. The results of such an experiment are shown in Fig. 202.

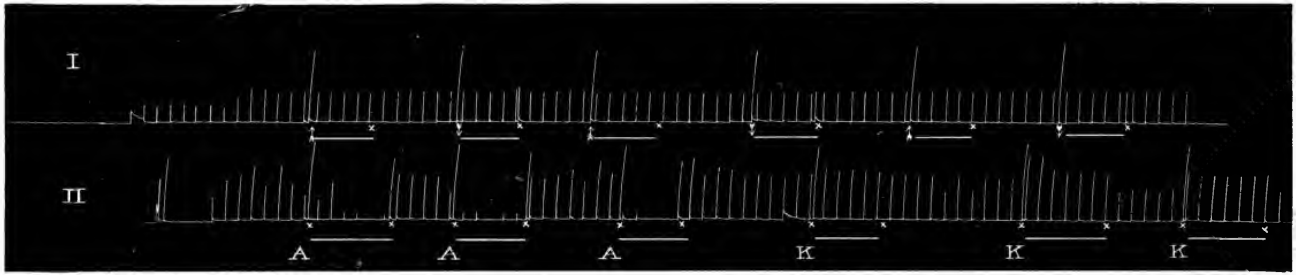


FIG. 202.—The effect of a constant electrical current upon the excitability of nerve in the interpolar region. I. The stimuli produced by the vibrating hammer were applied at the *middle point* between the polarising electrodes, The moment of closure of the constant current is shown by the big contraction, its duration by the horizontal line and stars, and its direction, ascending or descending as the case may be, by arrows. The contractions are practically equal whether there is or is not a polarising current present; the excitability of the middle point is unchanged. II. The vibrating hammer was applied in the interpolar region but near the electrode, anelectrotonus A was produced, the stimuli became subminimal; then katelectrotonus K was produced, the effectiveness of the original stimuli was increased. The moment of closure of the constant current is shown by the big contraction, its duration by the horizontal line and stars. The curves, which have been reduced to one half the original size, should be read from left to right. (M.S.P.)

The effect of the constant current upon the conductivity of the nerve is determined upon the same preparation. The stimulating electrodes are placed upon the central part of the nerve; a minimal stimulus is found, and its effect is recorded upon the stationary drum. The polarising circuit is now closed through the nerve in either the ascending or descending direction, and then the minimal stimulus is again applied. It is no longer effective owing to the decrease in the conductivity of the nerve. This change in the conductivity of nerve is also shown in the third stage of Pflüger's Law of Contraction, and in the experiment upon the absence of fatigue in a stimulated nerve (Chapter XVII., p. 330).

CHAPTER XV.

LAW OF EXCITATION. PFLÜGER'S LAW OF CONTRACTION.

According to Du Bois Reymond's Law of Excitation the efficiency of an electrical current as a stimulus to a nerve depends upon the rapidity of the change in the strength of the current passing through a given sectional area of the nerve. The changes in the electrical current are most abrupt at the point of entry and the point of exit, the anode and kathode respectively. Excitation starts at these points; thus at the make of a galvanic current the excitation takes place at the kathode, and at the anode when the circuit is broken. Excitation is produced by a sudden rise in excitability produced by the *appearance of katelectrotonus*, or *the disappearance of anelectrotonus*. In both cases the molecular stability of the nerve is suddenly lessened.

The effect will vary according to the strength and direction of the galvanic current, for if there be an intervening region of anelectrotonus or of subsiding katelectrotonus, the conductivity of the nerve in this region will be diminished, and the transmission of the excitatory state will be blocked.

Pflüger's Law of Contraction, as stated below, can be deduced from the above facts and can be proved by simple experiments.

[Pflüger's Law of Contraction.

PFLÜGER'S LAW OF CONTRACTION.

Strength of Current.	Direction of Current. Ascending.		Descending.	
	Make.	*Break.*	*Make.*	*Break.*
Weak.	Minimal contraction.	No contraction.	Minimal contraction.	No contraction.
Moderate.	Maximal contraction.	Minimal contraction.	Maximal contraction.	Minimal contraction.
Strong.	No contraction.	Maximal contraction.	Maximal contraction.	No contraction.
Very strong and prolonged.	No contraction.	Ritter's tetanus.	Maximal contraction.	No contraction.

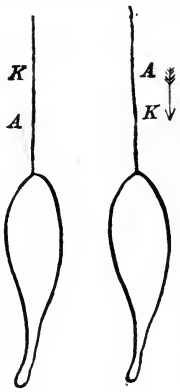


Fig. 203.—Pflüger's Law of Contraction.

Ritter's tetanus is due to a prolonged excitation at the anode, when the circuit of a very strong current is broken: injury of the anodic region of the nerve abolishes this effect.

In order to prove Pflüger's Law the following experiment should be made. A pair of unpolarisable electrodes are connected to a rheochord by means of a Pohl's reverser in the manner shown by the diagram (Fig. 204). From the Daniell battery two wires pass to the terminals of the rheochord. A muscle- and nerve-preparation is made and is placed in a moist chamber. The lever connected with the muscle is made to write upon a stationary drum.

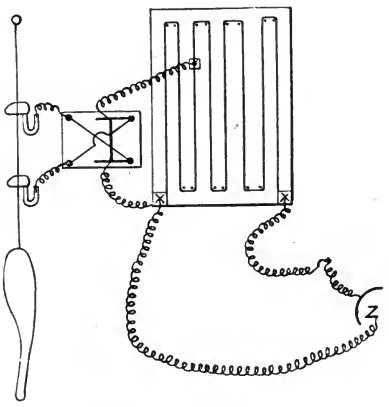


Fig. 204.—Diagram of the experiment upon Pflüger's Law of Contraction.

By closing a mercury-key a weak current is sent through the nerve in an ascending direction, the anode being the nearer to the muscle. A minimal contraction occurs at make, but there is no contraction at break, for the rise in excitability at the former anode is insufficient to cause an excitation (Fig. 205 (1), (2)). The current is reversed and the experiment is repeated.

Now by means of the rheochord the current is strengthened and the

results observed with the make and break of both ascending and descending currents. There is a contraction in each case, but the contraction at break is minimal for the disappearance of anelectrotonus is the less effective stimulus (Fig. 205 (3), (4)).

The rheochord is removed and the entire current of one or two Daniell batteries is used. There is now a maximal contraction at make

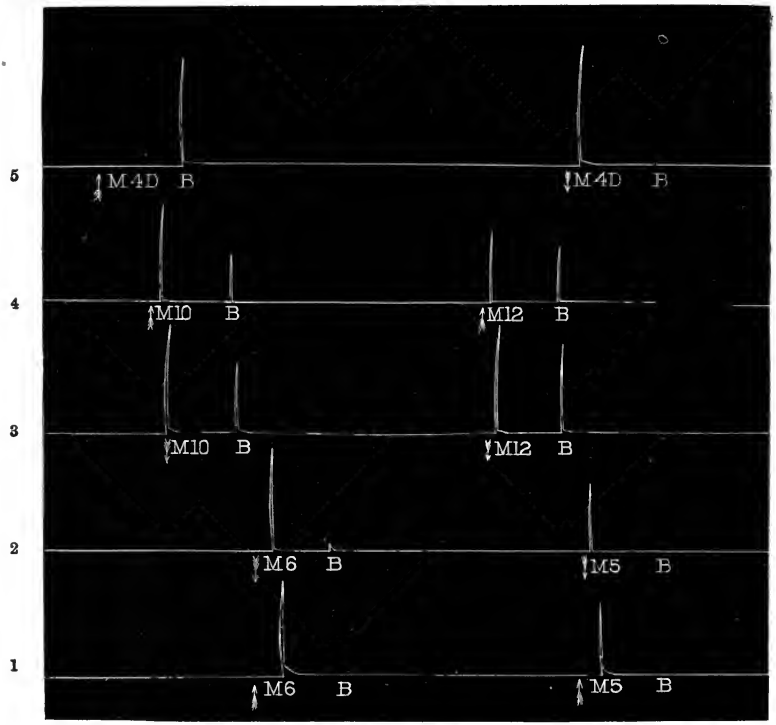


FIG. 205.—Pflüger's Law of Contraction. 1 and 2, Weak constant current. 3 and 4, Moderate strength of current. 5, Strong current. M = point at which current was made, B = point at which current was broken. The numbers 5, 6, 10, and 12 indicate the scale of the rheochord; 4 D indicates that the current was that of 4 Daniell cells. The arrows indicate the direction of the current, ascending or descending as the case may be. The curve should be read from left to right. (M.S.P.)

of the descending current, and at break of the ascending current (Fig. 205 (5)). When the ascending current is made the excitation at the kathode is blocked by the anode below; when the descending current is broken the excitation due to the disappearance of anelectrotonus is blocked by the diminished conductivity and excitability of the region below, in which the katelectrotonus has disappeared. To every action there is a reaction.

With four or five Daniell cells the experiment is again repeated. The

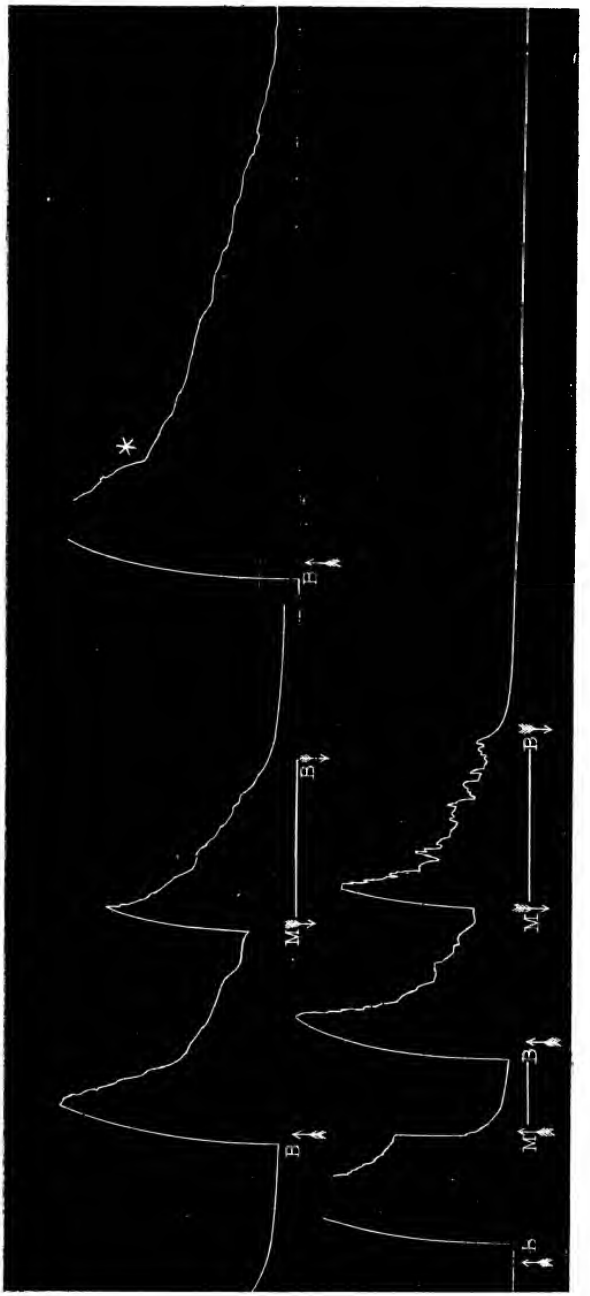


FIG. 206.—Curves of Ritter's tetanus. The arrows indicate the direction of the strong constant current, ascending or descending as the case may be. B = the moment the current was broken; M = the moment the current was made. The star indicates the moment when the nerve was cut and removed from the electrodes. The curve, which has been reduced in size, should be read from left to right. (M.S.P.)

current is allowed to flow in an ascending direction for ten minutes; breaking the circuit causes tetanus (**Ritter's Tetanus**). If the current be reclosed the tetanus ceases (Fig. 206 (2)). After a further period of ten minutes the current is again broken, tetanus occurs and will be increased if the current be closed in the descending direction (Fig. 206 (2)). If the nerve be divided on the far side of the anode during the tetanus, the twitches are not abolished for the excitation is occurring at the former anode (Fig. 206 (2)).

These results are more readily understood if it be remembered that a rise in excitability occurs with the *appearance of kalectrotonus* and the *disappearance of anelectrotonus*, and that a region of depressed excitability is found in the part in which *anelectrotonus is set up*, or in which *katelectrotonus has just disappeared*. A sudden rise in excitability produces a stimulation.

CHAPTER XVI.

THE EFFECT OF A CONSTANT ELECTRICAL CURRENT UPON MUSCLE.

It has been already shown that the make and the break of a constant electrical current stimulate nerve; this is also the case with muscle. *On the make of the current the contraction starts from the kathode; at break the contraction starts from the anode.* The appearance of katelectrotonus and the disappearance of anelectrotonus stimulate voluntary muscle; for cardiac muscle this has already been proved (page 50).

The following experiments should be performed upon the sartorius muscle.

The Clamped Sartorius.—A pair of unpolarisable electrodes are made, and threads which have been well soaked in normal saline solution, and covered with kaolin, are fixed to the kaolin plugs. The sartorius muscle is carefully dissected from a curarised frog (page 49); the muscle should not be cut away from its origin and attachment, but pieces of the pelvic girdle and of the tibio-fibula are left, so that ligatures may be tied to these without damage to the muscle. The sartorius is now very carefully clamped in the middle by Gaskell's clamp fastened to a vertical stand (Fig. 225, page 345); the muscle is held, but not so tightly as to damage its continuity and power of conduction. The pieces of bone at the ends of the sartorius are fastened by ligatures to two light levers fixed to the vertical stand, and then

the threads of the unpolarisable electrodes are tied gently, one to each end of the muscle. The conductivity of the threads is maintained by the saline kaolin. The unpolarisable electrodes are connected by a Du Bois key with a rheochord and Daniell cell.

On closure of the current the lever attached to the kathodic end will move first; the contraction starts in this portion of the muscle and spreads through the length of the sartorius. On break of the current the other lever, the one attached to the anodic end of the muscle, will move first. The direction of the constant current should be reversed and the experiment repeated, so that a control may be placed upon the results.

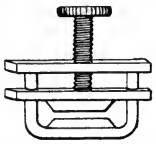


FIG. 207.—A screw clamp which can be made to serve the purpose of a Gaskell's clamp. Two small wedges of cork are fixed one to the lowest bar and the other to the moveable bar.

If a graphic record be taken, the levers should be of equal length so that their writing points will mark the one vertically under the other.

Engelmann's Experiment.—The other sartorius muscle of the curarised frog is dissected out with a piece of the pelvic girdle and is suspended by fixing a pin through the bone; the kaolin plugs of the unpolarisable electrodes are replaced by two new plugs so arranged that the constant electrical current can be made to pass transversely across the pelvic end of the muscle. When the current is made, the free end of the muscle moves towards the kathodic side; but towards the anodic side when the current is broken.

Biedermann's Experiment.—The sartorius muscle is carefully dissected from a pithed frog and is left attached to the pelvic girdle. The surface of the muscle is gently dried with filter-paper and then is striped transversely with equidistant black lines; for this purpose a fine paint-brush, or bristle, and black water-colour paint are used. The thread of one unpolarisable electrode is placed on the muscles of the frog's abdomen, the thread of the other electrode is brought into contact with a portion of the striped sartorius. The unpolarisable electrodes are connected with a Daniell cell by a Du Bois key.

In the first place let the electrode applied to the sartorius muscle be the anode. On closure of the electrical current the black lines can be seen with a lens to recede from one another; the muscle relaxes in the anodic region. Anelectrotonus causes a decrease in excitability; the tone of the muscle is diminished. The direction of the constant current is now reversed: on closure the black lines approach one another; the muscle contracts in the kathodic region. Katelectrotonus produces a rise in excitability; the tone of the muscle is increased; it contracts.

CHAPTER XVII.

THE ABSENCE OF FATIGUE IN A STIMULATED NERVE.

NERVES are not subject to fatigue, even if they be repeatedly stimulated for long periods of time. The following experiment not only demonstrates this fact, but at the same time shows that the passage of a constant electrical current through a portion of a nerve blocks the transmission of the excitatory state which is produced in the nerve by a stimulus applied above the polarising electrodes (page 324).

An induction coil is arranged for faradic shocks, and a pair of unpolarisable electrodes are connected by a Du Bois key with a Daniell cell. The two sciatic nerves of a pithed frog are dissected up to their points of exit from the vertebral column, which is then cut across above the nerves. The thighs are cut away above the knee, and the two legs with their nerves are placed in a moist chamber, and are fixed by pins pushed through the lower extremities of the femora. The stimulating electrodes, which are connected with the secondary coil by means of a Du Bois key, are placed under both sciatic nerves; the unpolarisable electrodes are placed under one sciatic nerve midway between the muscle and the stimulating electrodes. The induction shocks are now allowed to pass through both nerves for a few seconds; the muscles of both legs are thrown into tetanus. The stimulation is stopped and the polarising current is passed through the one sciatic nerve. The faradisation of both nerves is again commenced; the muscle in the one case will be sent into tetanus and quickly fatigued, but the other muscle shows no contraction, for the polarising current passing through its nerve blocks the passage of the nervous impulses evoked by the stimulating electrodes. When the first muscle is fatigued the polarising current should be broken; the block is removed from the course of the sciatic nerve of the other muscle, which is at once tetanised by the stimulation of its nerve.

CHAPTER XVIII.

THE ELECTROMOTIVE PROPERTIES OF MUSCLE AND NERVE.

THREE simple experiments upon the electromotive properties of muscle have already been described (page 51). The following experiments require more care and very excitable tissues.

Secondary Twitch from the Heart.—If a freshly prepared and very excitable nerve be laid upon the heart of a frog, so that the cut end of the nerve is on the base and the longitudinal surface upon the apex of the ventricle, a twitch of the muscle connected with the nerve is observed at each contraction of the ventricle. Each time the muscle-fibres of the ventricle contract, a "*current of action*" is produced and stimulates the nerve.

A fine glass rod should be placed under the middle portion of the length of nerve, which lies on the ventricle, so that the current may not be short circuited.

FIG. 208.—Diagram of the experiment to show the stimulation of a muscle by the "current of action" of another muscle.

Stimulation of a Muscle by the "Current of Action" of another Muscle.—The sartorius muscle is very carefully dissected on each side, and then the one muscle is placed overlapping the other; the contact of the two muscles is secured by gentle pressure with two pieces of cork (Fig. 208). Stimulation of one muscle will produce a contraction in both; the "*current of action*" in the first stimulates the second muscle.

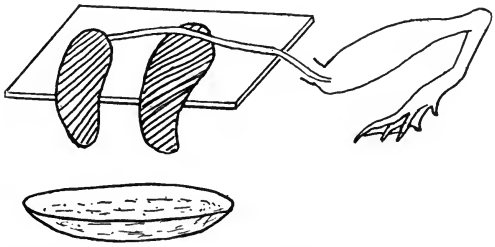


FIG. 209.—Diagram of the experiment to show the stimulation of a nerve by its own "current of injury."

Stimulation of a Nerve by its own "Current of Injury."—Two plugs of kaolin moistened with normal saline solution are placed upon a piece of glass, and the tails of the plugs are made to hang over the edge (Fig. 209). The sciatic nerve of a pithed frog is carefully dissected down to the knee, the thigh is cut across, but the leg and foot are left intact. The nerve is so placed that its cut surface is upon one plug and its longitudinal surface upon the other plug. A watch-glass filled with strong saline solution, which is a good conductor of electricity, is suddenly brought in contact with the ends of

the kaolin plugs; thus the circuit is suddenly made and can be suddenly broken by the removal of the watch-glass. If the preparation be very excitable, a twitch is observed at each make and break of the circuit; the nerve is stimulated when the circuit of its "*current of injury*" is completed or broken.

CHAPTER XIX.

THE ELECTROMOTIVE PROPERTIES OF MUSCLE AND NERVE—CONTINUED. THE GALVANOMETER AND THE CAPILLARY ELECTROMETER.

DEMONSTRATIONS.—The galvanometer and the capillary electrometer are delicate instruments which are easily damaged; they are employed to investigate the electromotive properties of muscle and nerve. The essential experiments upon that subject have already been performed by means of the so-called "rheoscopic frog." In this course,

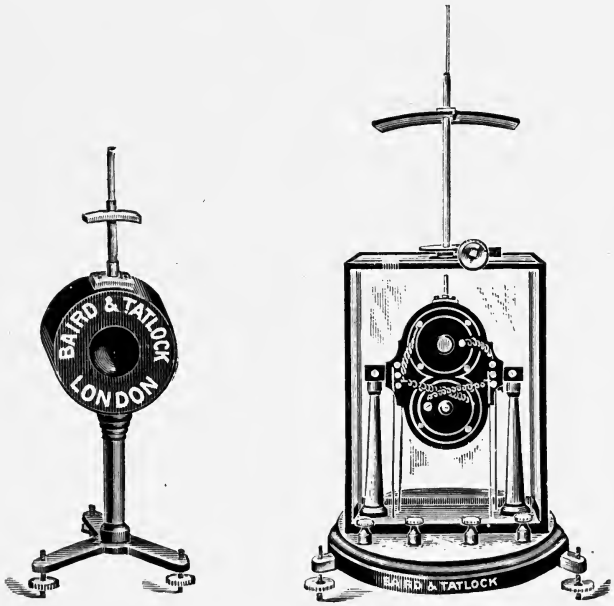


FIG. 210.—Galvanometers.

therefore, the experiments with the galvanometer and the capillary electrometer will be demonstrated to the student and only brief details will here be given.

The **Galvanometer** employed in these experiments is Kelvin's reflecting galvanometer. It consists of a suspended system of magnets so arranged as to make the system nearly "astatic"; the magnets are surrounded by coils of many turns of fine insulated wire. The resistance is high, from 5000 to 20,000 ohms. The movements of the mirror attached to the magnets are indicated by a spot of light upon the scale.

The amount of current sent through the galvanometer is regulated by means of a *shunt*, which is a resistance box whereby $\frac{1}{10}$th, $\frac{1}{100}$th, or $\frac{1}{1000}$th of the total current can be sent through the galvanometer

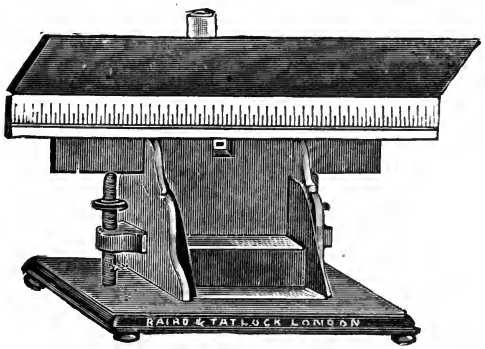


FIG. 211.—Scale and lamp for the reflecting galvanometer.

The electric current from the muscle or nerve is led off by means of unpolarisable electrodes, but before an experiment is performed the electrodes are tested, for in most cases they are not perfectly iso-electric. Any small deflection of the galvanometer due to this cause is compensated by a graduated current from a standard battery sent through the galvanometer in the opposite direction.

Perfectly uninjured muscle and nerve are iso-electric, but they are generally slightly damaged during the process of dissection and preparation. The deflection due to this **current of injury** or **demarcation current** (wrongly called the current of rest) is measured and is then increased by a more pronounced injury caused by touching one end of the muscle with a hot wire. The muscle is now stimulated by a tetanising current applied to its uninjured end; the deflection of the galvanometer is in the reverse direction, due to the **current of action** (formerly called the negative variation) which is produced when the muscle contracts.

The current of injury is, as Gotch pointed out, to be considered as a local current of action; around the injured portion the tissue is in a condition of excitation.

Similar experiments are demonstrated upon nerve.

Lippmann's Capillary Electrometer.—This instrument is a delicate electrical manometer, and is more suitable than the galvanometer for the investigation of the electromotive properties of the frog's heart; it responds to very rapid changes of electrical potential. It consists (Fig. 212) of a glass tube *C* drawn out at one end to a fine capillary tube; this is filled with mercury and is connected with a pressure apparatus by the rubber tubing *RT*. The capillary tube dips into a small trough filled with 10 per cent. sulphuric acid; the bottom of this vessel is covered with mercury *M* in order to provide good electrical conduction with the platinum wire. The movements of the column of mercury in the capillary tube are observed by means of a microscope fitted with a micrometer scale.

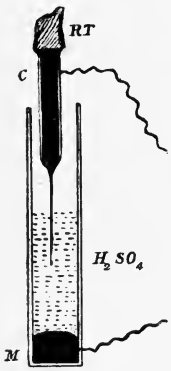


FIG. 212.—Diagram of the capillary electrometer.

The passage of an electrical current through the capillary tube alters the surface tension, and this alteration causes a movement of the mercury in the capillary tube. The movement of the column of mercury is from positive to negative, and the extent of the movement is roughly proportional to the difference in electrical potential. Based upon these facts are the determination of the direction of, and the measurement of the electromotive force of, the current which is under investigation.

With the capillary electrometer the *electromotive properties of the frog's heart* are demonstrated. The base and the apex of the ventricle are led off by unpolarisable electrodes to the electrometer: each time the heart contracts there will be a diaphasic variation, the contracted portion at first becomes negative and then positive to the uncontracted part.

THE CIRCULATION OF THE BLOOD.

CHAPTER XX.

THE HEART.

Bernstein's Experiment.—A ligature is drawn round the ventricle of a frog's heart half-way between the base and apex, and tightened sufficiently to destroy the physiological but not the physical continuity of the tissue at this point. The apex ceases to beat and remains red and full of blood. The bulbus aorterosus is now compressed so as to obstruct the outflow and raise the intra-ventricular pressure. The apex begins to beat rhythmically. The snail's heart ceases to beat when emptied of blood.

Engelmann's Experiment.—The ventricle is snipped on opposite sides so as to produce several interdigitating cuts. The wave of contraction still passes down the zigzag strip. All possible nervous channels of conduction are supposed to be divided by these means, and the experiment is taken as proving the conduction of the excitatory state from muscle cell to muscle cell.

The Action of the Valves and the Influence of the Diastolic and Systolic Pressures on the Output and the Absolute Force of the Heart.—A large frog or toad is pithed, and the heart exposed. A ligature is passed under each aorta, and the right aorta is tied. Ligatures are placed under each vena cava superior, and both these vessels are tied. The ligatures may be threaded through the eye of a needle, and the point of the needle stuck into a wooden handle. A ligature is also passed under the vena cava inferior. A V-slit is made into this with sharp scissors and a cannula inserted. The cannula is provided with a rubber tube on which a clip is placed. This tube is connected by a T-piece to a reservoir and a vertical tube. The latter is provided with a paper mm. scale (Fig. 214). The reservoir, tube, and cannula, are filled with Ringer's solution before the cannula is introduced. The left aorta is now slit and a fine cannula introduced. This is connected by a tube to a T-piece. One branch of the T-piece is closed by a clip and the other is joined to a vertical tube 1 metre in height. This tube is provided with a mm. scale. The aortic cannula and tube are filled with Ringer's solution before the cannula is introduced.

The reservoir is placed so that the level of the fluid in the venous manometer is 3 cm. above the heart, and the clip is then opened. The heart contracts and expels the fluid into the arterial manometer. Note the amount of each systolic output as indicated by the rise of level in this. As the pressure rises the output lessens. Note the competency of the auriculo-ventricular and aortic valves, and the elastic swing of the bulbus arteriosus (the dicrotic wave) which follows each systolic output. To investigate the competency of the auriculo-ventricular valves the reservoir must be temporarily clipped off from the venous manometer.

The work of the ventricle can be obtained by multiplying the volume of the output by the height of the lift. The absolute force of the ventricle is given by the height of

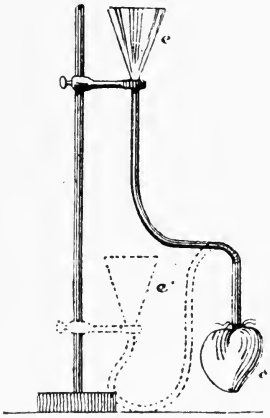


Fig. 213.--Method of showing the effect of tension on the apex-preparation. (Pitres.)

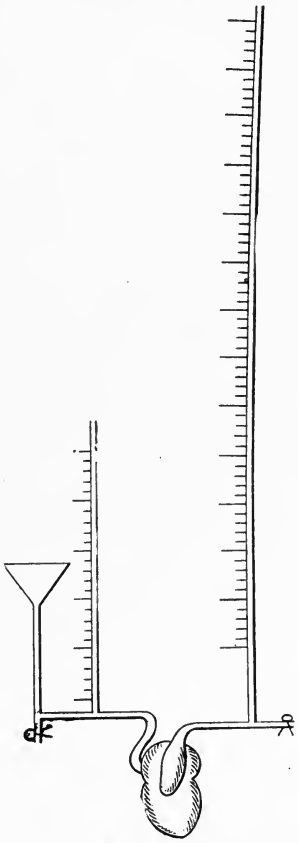


Fig. 214.—Method of investigating the action of the valves, the systolic force, output, etc., of the toad's heart.

the fluid, which the ventricle is just unable to lift. A vigorous toad's heart will lift the fluid up to a height of 1 metre. Let out the fluid in the arterial manometer and lower the fluid in the venous manometer to the level of the heart. The heart will not fill in diastole if the pressure in the latter is not positive. Raise the pressure in it to 10 and 20 c.m., and observe the effect of the increased diastolic pressure on the systolic output and on the absolute force of

the ventricle. The absolute force is increased by thus raising the diastolic pressure. Too high a filling pressure will throw the heart into diastolic paralysis. The auricle is paralysed much sooner than the ventricle.

The Isometric Contraction of the Heart (O. Frank's Method).—Fine cannulae are placed in the aorta and vena cava inferior and the cannulae are connected with the elastic manometers as in Fig. 215. The taps control the filling and output of the heart. The filling tube—a 1 c.c. pipette—is calibrated in $\frac{1}{100}$ c.c. The chambers of the manometers are made small and the levers light. The amount of fluid which must enter the manometer during the change of pressure is thereby made as small as possible, and the errors due to inertia are avoided. The outlet tap is opened and the heart is allowed to empty itself. This tap is then closed, and curves of ventricular and auricular systole taken.

A measured quantity of Ringer's solution is then admitted into the heart through the inlet tap, and new curves taken. This is repeated, and with each increase of diastolic expansion systolic curves are taken. The manometers are calibrated by placing them in connection with a mercurial manometer and a pressure bag or bottle. The following is an example of how the maximal tension is obtained:

Filling in c.c.,	0·0	0·09	0·22	0·36	0·48	0·68
Maximal tension,	12 mm. Hg.	48	66	70	67	64

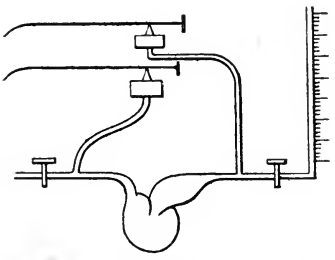


Fig. 215.—Isometric record of the heart.

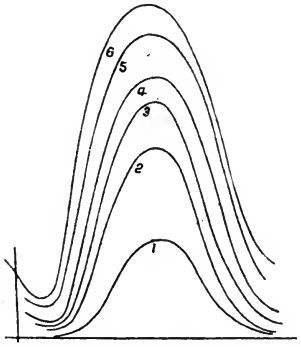


Fig. 216.—Effect of increasing diastolic pressures on the isometric curve of the frog's heart. (O. Frank.)

The tension of the heart-muscle obviously increases up to a maximum with the diastolic filling. Beyond a certain point it decreases. The maximal tension of the auricle is obtained by ligaturing the auriculo-ventricular groove. It is $\frac{1}{5}$ to $\frac{1}{8}$ of the maximal systolic tension.

CHAPTER XXI.

THE HEART—Continued.

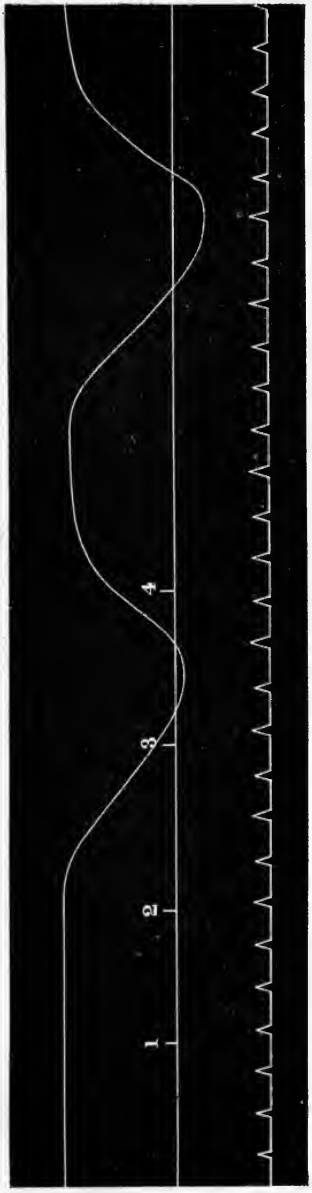


Fig. 217.—Record of contractions of a Stanniused heart. Excitations were given at the points marked 1, 2, 3, and 4. The second and third stimuli during systole were ineffectual. The fourth stimulus excited a second contraction. The contraction is represented by the down stroke. The time is marked in fifths of a second. (L.H.)

The Contraction Curve and Latent Time of the Stanniused Heart.—Expose the heart of a pithed frog. Pass a ligature under the two aortae, and draw the ends exactly round the white crescentic line which marks the sino-auricular junction. Tie the ligature. The sinus continues to beat, while the auricles and ventricle stand still. Pulling the heart up by the ligature, cut widely round the sinus and excise the heart. Place it beneath the heart lever (Fig. 59).

Two needle electrodes are inserted into the auricles, one on either side of the heart. The drum is set at a moderately fast rate, and the trigger key is placed in the primary circuit. A short circuiting key is placed in the secondary circuit, and the coil is arranged to give a break shock just perceptible to the tongue. Close the short circuiting key, and set the drum so that the striker is just beyond the trigger key. Then close the latter. Place the lever at a tangent to the drum, and bring the writing point lightly in contact. Then open the short circuiting key and start the

drum. Stop the drum immediately after recording the contraction. Close the short circuiting key, then close the trigger key; lastly open the short circuiting key. Bring the drum round slowly by hand until the striker just opens the trigger key. The heart will contract and the lever write a line marking the moment of excitation. Take another curve with the electrodes placed on either side of the base of the ventricle. The latent period will be less. In the first case the excitatory wave was delayed in the auriculo-ventricular groove. With the tuning fork (100 per sec.) take a time tracing just beneath the heart curves, and measure the latent period. It equals about 0·1 sec.

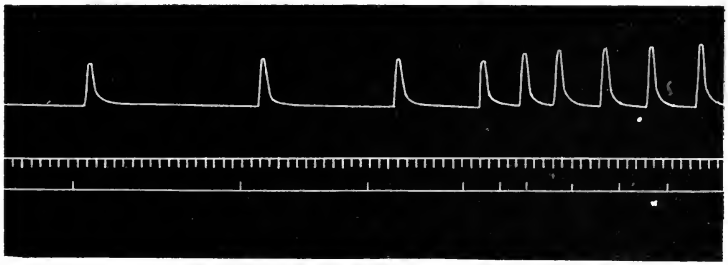


FIG. 218.—Stanniused heart. Staircase effect produced by excitations at the points marked on the lowest line. The time is marked in seconds. (L.H.)

The periods of contraction and relaxation will together last 2 sec. The contraction is much slower than that of striated muscle.

Any Stimulus, if effective, causes a Maximal Contraction.—Place a spring key and an electric signal in the primary circuit. Set the drum at the slow rate, and bring the heart lever and signal to write on the drum. Record the effect of excitation at intervals of a minute or more, with varying strengths of current. The heart gives 'all or nothing,' *i.e.* if excited at all it gives its full contraction.

The Refractory Period.—Record the effect of throwing in a second excitation (*a*) during the systole, (*b*) during diastole. The heart is *refractory* during the whole period of systole, *i.e.* it makes no response to a second stimulus. The excitability returns with diastole, and becomes greater as diastole proceeds. (Fig. 217).

Staircase Phenomenon.—A Stannius preparation is placed under the lever (Fig. 59), and excited with single induction shocks once in every five seconds. The stationary drum is moved on by 2 mm. between the excitations. The heights of the first four or five contractions form an ascending series. The heart responds to any stimulus which is effective by a maximal contraction. The height of the contraction depends on the condition of the heart muscle, not on the strength of the stimulus,

so long as the latter is effective. For the first few beats each contraction makes the heart more excitable. The same phenomenon is observed in the muscle of curarised frogs with intact circulation, and also in the

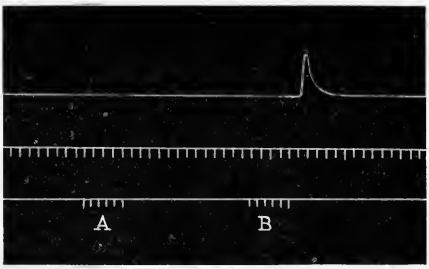


FIG. 219.—Stanniused heart. Summation of stimuli. A, ineffective, and B, effective stimuli. The time is marked in seconds. (L.H.)

galvanometric records of the action current of nerve. Waller attributes the staircase effect to the influence of CO_2 formed by the katabolism of the active tissue.

Summation of Stimuli.—Pull out the secondary coil until the break shock is just ineffective, and rhythmically stimulate the Stannius preparation with this inadequate stimulus. The heart will respond to the repeated excitation, and the first few beats will show the staircase phenomenon.

CHAPTER XXII.

THE HEART—CONTINUED. THE ACTION OF DRUGS.

The Suspension Method of Investigating the Action of Drugs on the Frog's Heart.—Pass a ligature under the vena cava inferior, where it is joined by the hepatic veins and enters the sinus. Make a V-shaped incision, and tie in a fine glass cannula. The cannula must be provided with a rubber tube ending in a syphon tube. The tube is provided with a clip, and the whole is filled with Ringer's solution, which is contained in a flask. Attach a hook to the ventricle apex, and record the heart by the suspension method. A slit is made into the aorta. Open the clip, circulate the Ringer's fluid, and record a series of contractions. Now replace the flask of Ringer's solution with one containing distilled water. The contractions will soon become less frequent, less forcible, and more prolonged. Finally the heart will cease to beat. It may be restored by Ringer's solution.

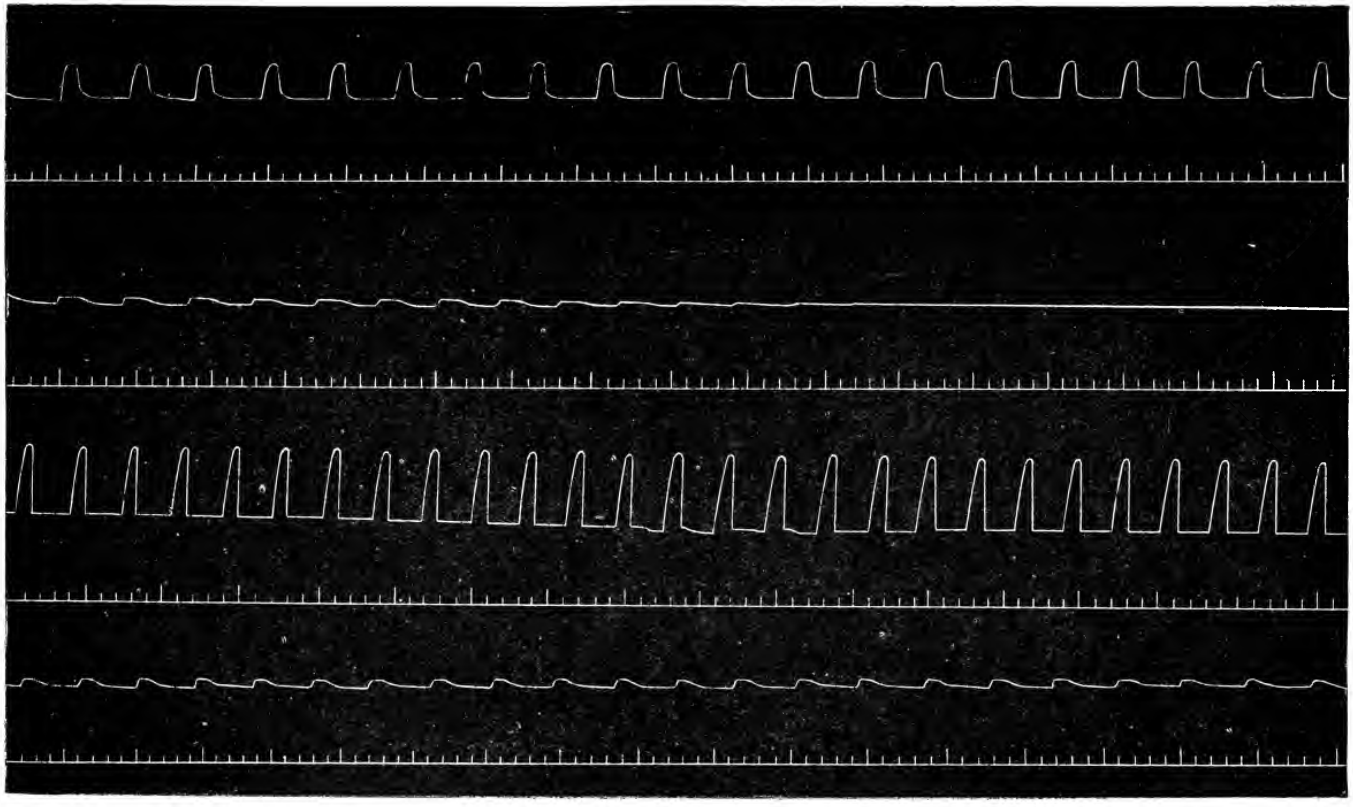


FIG. 220.—Contraction of the heart of the frog. I. Effect of distilled water. Temp. 16·25°. II. Contraction restored by normal tap-water saline. III. and IV. Repetition of the experiment. The time is marked in seconds. (Pembrey and Phillips.)

When the heart is completely restored replace the Ringer's solution with normal saline solution ·65 per cent. in distilled water. The heart will after some time become weaker, and finally stop in diastole. Water distilled in glass is less noxious than water distilled in copper or lead. Merely hanging a strip of copper foil in distilled water over-night increases its poisonous properties. It is calculated that there is not more than 1 part of copper in 70 million of the water. Tap-water contains traces of calcium salts, which are beneficial. Normal saline should therefore be made with tap-water. An isotonic solution of KCl 0·9 per cent. arrests the heart. A 2 per cent. solution of digitalin causes increased tone of the heart, vigorous systole, and incomplete diastole. The heart finally is arrested in a state of systolic contraction. Caffeine and veratrine also act tonically on the heart.

Supra-renal extract, or adrenalin, at first slows and then increases the tone and the frequency of the heart. It is an antidote to the effect of chloroform on the heart. Adrenalin is the active principle of the medulla of the supra-renal gland, separated by Takamine. A solution containing 1 part in 10,000 constricts vessels of the conjunctiva.

Weak solutions of acid bring the heart into diastolic arrest. Alkalies produce systolic arrest.

CHAPTER XXIII.

THE CARDIAC PLETHYSMOGRAPH. THE EFFECT OF TEMPERATURE ON THE HEART. CARDIO-PNEUMATIC MOVEMENTS.

Schäfer's Plethysmograph.—The double cannula is fitted with two rubber tubes, and clips are placed on these. The cannula and the tubes are filled with Ringer's fluid. One of the tubes ends in a syphon tube which dips into a flask containing Ringer's solution. The heart is now exposed, and a slit made in the left auricle with fine scissors. The scissors are passed into the ventricular cavity, and two or three snips are made so as to clear a way in the muscular meshwork within the cavity. The cannula is then inserted, and a ligature is tied round the auricles, including the cannula. The plethysmograph is filled with olive oil. The tap on the overflow tube is opened and the cannula is inserted. This tap is then closed, and the tap leading to the piston recorder opened. The clips on the cannula tubes are opened, and the beaker raised a few inches above the level of the heart so as to maintain an efficient flow to the heart. The piston records the contractions on the horizontal drum (slow rate)

If the heart does not beat spontaneously, it can be excited by attaching a wire to the cannula and another to the wire which passes through the bottom of the plethysmograph. The beaker can be replaced by other beakers containing Ringer's solution, to which a drug has been added. The instrument is not a very satisfactory one to use, as the piston is liable to stick. A simple form of plethysmograph can be made as follows:

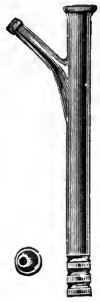


Fig. 221.—Byway cannula. The small figure represents a transverse section of the lower end of the cannula.

A chamber is cut in a large cork. Into the bottom of this passes the double-way cannula. The cannula is pushed out beyond the cork and inserted into the heart. The cannula is then withdrawn into the chamber. The latter is filled with Ringer's solution, and a piece of peritoneal membrane is tied over it. A disc of card attached to a lever rests on the membrane.

Effect of Heat and Cold on the Contraction Curve.—A Stanniused heart is cut out and the sinus pinned to a cork. The cork is attached

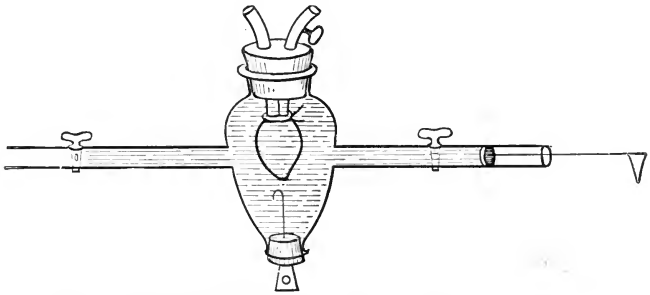


Fig. 222.—Schäfer's cardiac plethysmograph. (Pembrey and Phillips.)

to a T-piece beneath the lever. The heart is recorded by the suspension method. The heart is excited by electrodes pinned into the cork. Immerse the heart in Ringer's solution at 5° C. for about a minute, and then lower the beaker and record a contraction. Repeat the experiment with a solution heated to 15° C. and 25° C. The results will be similar to these obtained with striated muscle. Finally, record the heat contraction by immersing the heart in Ringer's solution heated to 45° C.

Cardio-pneumatic Movements.—One end of a glass tube is fitted into the nostril and the other end is connected with a delicate recording tambour. The other nostril and mouth are shut and the glottis kept open. The pressure falls during the period of the systolic

output of the heart. This is owing to the expulsion of blood from the thorax. Thus each systole aspirates air into the lungs. The same thing may be demonstrated by putting a U-shaped tube in the nostril filled with tobacco smoke. The column of smoke moves in and out with each systole and diastole of the heart.

CHAPTER XXIV.

THE TORTOISE HEART.

The Tortoise Heart.—Pith the brain of the land-tortoise (Testudo graeca), saw through the sides of the carapace, and remove the lower half of it. The heart will now be exposed. Pin out the head of the animal, and look for the vagus nerve which runs in company with the carotid artery on either side of the trachea. Record the heart by the suspension method, ligature the vagus, divide it, and excite the peripheral end. The heart will be arrested in diastole, and may remain in arrest for more than one minute. Excise the heart, cutting widely round the sinus, and lay it on a clean glass plate. Note

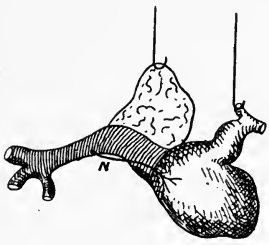


FIG. 223.—Diagram of the tortoise heart as suspended for recording. N = coronary nerve.

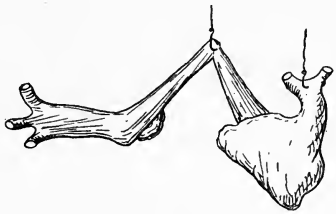


FIG. 224.—Tortoise heart with auricles slit so as to produce a block.

the sinus (Fig. 224), the two auricles, the single ventricle, and the 'sinus extension' which connects the ventricle with the sinus. The main groups of ganglion cells are found at the bifurcation of the large vagus nerve trunks in the sinus (corresponding to Remak's ganglion), in the sinus extension (V. Bezold's), and in the termination of this in the auriculo-ventricular ring (Bidder's). The auricles and the ventricle are ganglion-free, except in the neighbourhood of the sinus extension and auriculo-ventricular ring. One of the coronary veins runs on the surface of the sinus extension from ventricle to sinus

With this goes one of the nerve trunks which pass from the sinus to the auriculo-ventricular junction. Divide this 'coronary nerve.' The rhythm of the heart continues unaltered. In another tortoise heart, the muscular connection between ventricle and the rest of the heart may be divided while the coronary nerve is left intact. The ventricle stands still under these conditions.

Slit up the sinus extension on the left side so that the left auricle is entirely separated from sinus and right auricle, but remains attached to the ventricle. The heart will now beat in this order—sinus, right auricle, ventricle, left auricle.

Slit crosswise the right auricle until only a narrow bridge of muscle

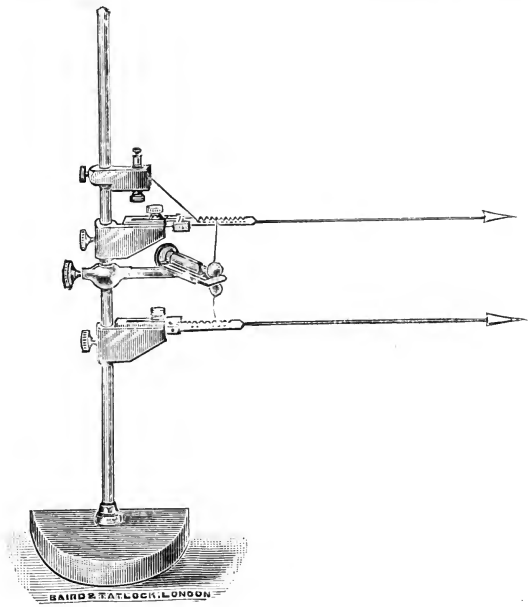


FIG. 225.—Gaskell's heart clamp and levers for recording the contraction of auricle and ventricle.

is left. The ventricle may now respond to only every second contraction of the sinus. The bridge is so narrow that the excitatory wave is partly blocked there.

Any strip of auricle and ventricle muscle cut from the tortoise heart may be taught to beat rhythmically by weak induction shocks. The rhythm, if once established, will continue for hours without artificial stimulation.

These experiments prove that the rhythm of the tortoise heart muscle is independent of the ganglion cells in the heart, and that the

excitatory wave travels by muscle and not by nerve from chamber to chamber.

Gaskell's Clamp and the Effect of Heat on Sinus and Ventricle.— The contraction of the auricle and ventricle are registered by means of

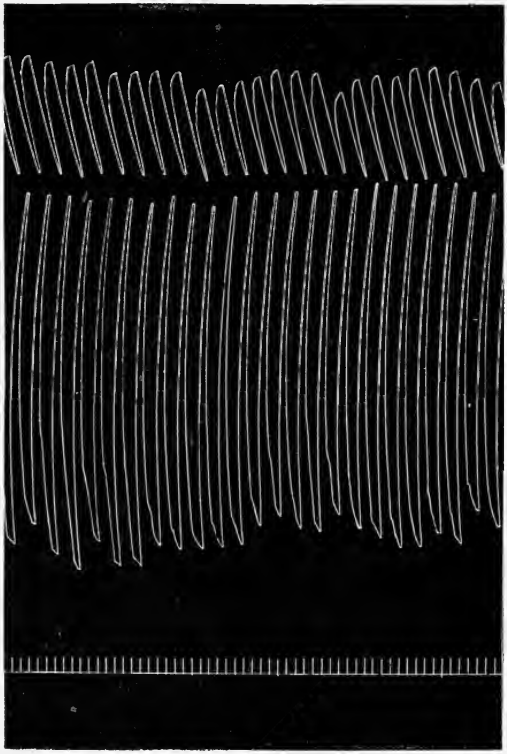


FIG. 226.—Record of the contraction of auricle and ventricle (toad) by the use of Gaskell's clamp and levers. The upper tracing is the auricle and here the contraction is represented by the down-stroke. The time is marked in seconds. (L.H.)

two levers which are attached by means of threads to the apex of the ventricle and auricle respectively; the one lever is pulled downwards against an elastic spring and the other upwards. The heart is held fast by means of a screw clamp in the auriculo-ventricular groove.[1] The clamp is provided with a fine screw, which can easily be adjusted so as to hold the heart firmly without injuring the tissue (Gaskell). In this way the contractions of auricle and ventricle are registered separately. Take a thick copper wire, bent into a hook at one end, and place the hook round the sinus. Warm the other end of the wire in a flame.

[1] A screw clip, to the bars of which cork wedges are fastened, will do for the clamp. See Fig. 207.

The result of warming the sinus is a great increase in the rapidity of the beats both of the auricle and ventricle.

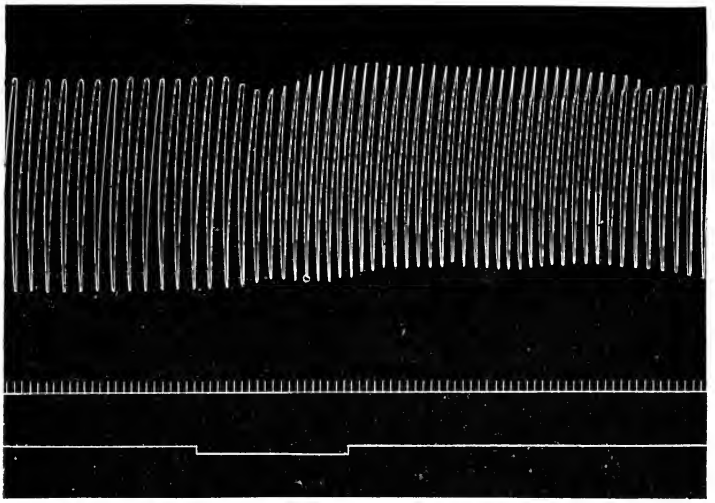


FIG. 227.—Record of the contraction of the toad's heart by the suspension method. Heat applied by the copper wire method. The signal in the third line shows the period during which the sinus was heated. Acceleration of the whole heart was produced. In this curve the down-stroke represents the contraction. The time is marked in seconds. (L.H.)

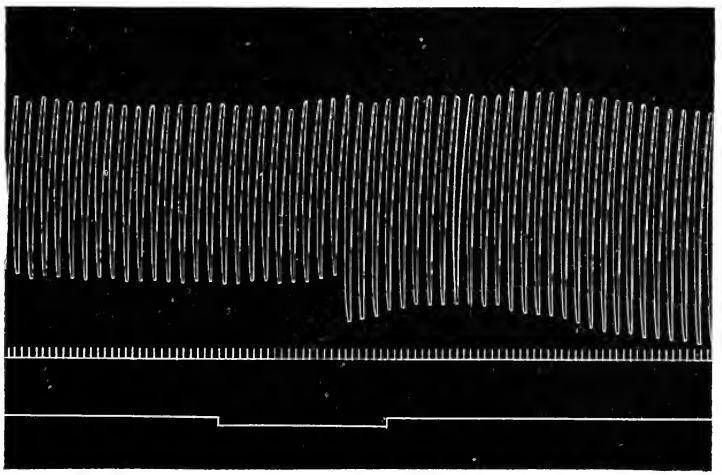


FIG. 228.—Continuation of Fig. 227. Ventricle heated. Augmentation of the ventricular contraction, but no change in frequency. (L.H.)

Now warm the ventricle in like manner. No alteration of rate of

rhythm is produced by heating the ventricle, but each ventricular contraction is augmented.

The observation of the local effect of warmth may be carried out equally well on a heart recorded by the ordinary suspension method.

CHAPTER XXV.

DISSECTION OF THE CARDIAC NERVES.

Dissection of the Cardiac Nerves in the Dead Rabbit.—After moistening the fur over the neck of a rabbit cut it short. Make an incision in the mid-line from larynx to sternum and pull apart the skin flaps. Separate the sterno-laryngeal muscles from the sterno-mastoid

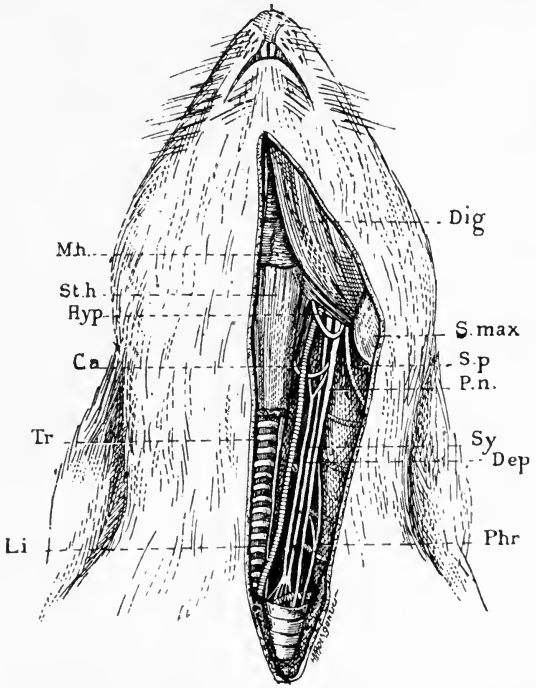


FIG. 229.—Dissection of the vagus, the depressor, and cervical sympathetic nerves in the rabbit. (Livon.)

along one side of the trachea and expose the carotid sheath. Look for three nerves running alongside of (Ca) the carotid artery: (P.n.) the vagus, this is the largest; (Dep) the depressor, a fine nerve which may be traced up to where it arises by two branches, from the superior

laryngeal nerve and from the vagus; (Sy) the cervical sympathetic, a slender thread, which must be traced up to the superior cervical sympathetic ganglion.

Follow the superior laryngeal nerve from the vagus to where it enters the larynx, and likewise trace (Li) the inferior laryngeal nerve.

Next divide the skin over the upper part of the sternum and reflect the left skin flap.

Pass threads round the sternal ends of the left first and second ribs. Tie these and divide the ribs between the threads and the sternum. Pull the ribs outwards by means of the threads, separate the intercostal muscles with the knife, and by cutting through the spinal attachments of these ribs remove them.

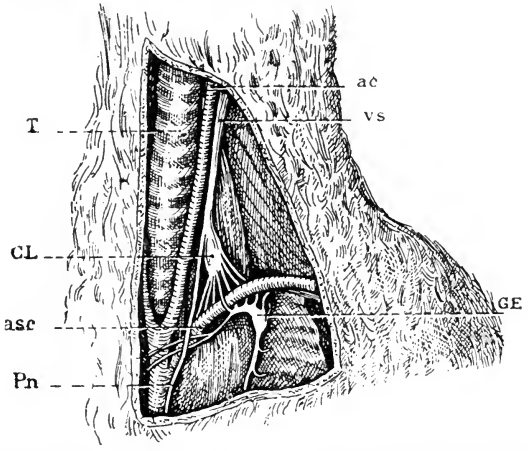


FIG. 230.—Dissection of the stellate ganglion (GE) and cardiac accelerators. The inferior cervical ganglion (CL) and vago-sympathetic (vs) are also shown. (Pn) vagus; (ac) carotid artery; (asc) subclavian artery. (Dubois.)

The stellate or first thoracic ganglion may now be found and cleaned from the surrounding adipose tissue. It lies just in front of the spinal attachment of the first rib. Branches enter the stellate ganglion from the first, second, and third thoracic roots. Below the sympathetic cord is attached to it, and above a nerve passes to it from the 8th cervical root. The ganglion sends off branches, which form the annulus of Vieussens, and pass to the inferior cervical ganglion. From the annulus and from the inferior cervical ganglion branches pass to the cardiac plexus. The stellate ganglion is the cell-station of these accelerator and augmentor fibres.

The stellate ganglion is also the cell-station of the fibres which pass to the brachial plexus (vasomotor, pilomotor, sudoriferous) and to the vertebral artery.

The cervical sympathetic fibres pass through the ganglion, and have their cell-stations in the superior cervical sympathetic ganglion.

Excitation of the (1) cervical sympathetic dilates the pupil, retracts the nictitating membrane, causes separation of pupils and projects the eye with the axis of the eyeball straight forwards.

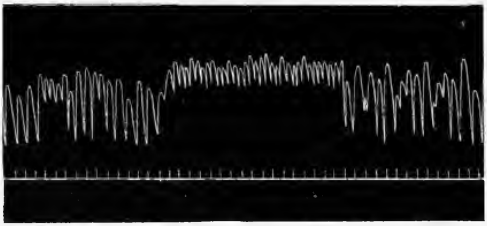


FIG. 231.—Arterial pressure. Effect of exciting the stellate ganglion (accelerator nerves). The time is marked in seconds. (L.H.)

It constricts the blood-vessels of the skin, glands, and mucous membrane of the head.

It dilates the vessels in the bucco-facial region of the dog.

It excites secretions of the glands of the head, both salivary and sweat glands.

FIG. 232.—Record of arterial pressure. Cardiac acceleration produced by excitation of the third dorsal root during the time shown by the signal line. (Bradford.) The time is marked in seconds.

It erects the hairs in the cat and monkey over certain regions of the face and scalp.

(2) The depressor nerve is an afferent nerve which runs from the heart to the spinal bulb, and causes general dilatation of the blood-vessels—especially in the splanchnic region. (See Fig. 112.)

It thus lowers the arterial pressure. The depressor is bound up with the vagus in the dog.

(3) The vagus is the inhibitory nerve to the heart, the motor nerve to the bronchial muscles.

It conveys both inhibitory and augmentory impulses to the alimentary canal.

It is a secretory nerve to the gastric glands and pancreas.

It contains afferent fibres from the heart which provoke reflex movements, pressor or depressor effects, and reflex cardiac inhibition.

The afferent fibres of the vagus coming from the lungs regulate the rhythm of respiration.

The superior laryngeal branch of the vagus is the motor nerve to the crico-thyroid muscles and the sensory to the larynx.

The inferior laryngeal branch is the motor nerve to the intrinsic muscles of the larynx.

CHAPTER XXVI.

THE PULSE.

The Velocity of Transmission of the Pulse Wave.—Two tambour sphygmographs are taken, and one is applied to the carotid, and the other to the radial or femoral artery. The recording tambours are brought to write exactly beneath one another on a fast drum, and a time tracing is taken with the tuning fork. The distance between the carotid artery and the radial or femoral is measured. The rate of transmission is about 5-8 metres a second. The rate of transmission increases as the coefficient of elasticity of the arterial wall. It is therefore greater with high than with low arterial pressure.

The velocity of transmission from carotid to radial may be lessened by placing the arm in water so as to produce vaso-dilatation. During the first six beats after vagus arrest of the heart, the velocity of transmission was 4·5, 4·5, 6·0, 7·5, 12, 13·5 metres per second respectively. The length of the pulse wave is the product of the velocity of transmission by the time occupied by the wave in passing any given point. Calculate this value from the record. It is about 5 metres, so the pulse wave reaches the periphery before it has left the aorta.

Impulse and Pulse Curves.—The cardiac impulse and the carotid pulse may be simultaneously recorded in man, and by this means the time relations of the cardiac cycle may be determined. The carotid pulse is recorded by means of a receiving tambour, which is strapped round the neck, and is provided with a button which rests on the

artery. The tambour is connected by means of a ⊥-tube with a recording tambour.

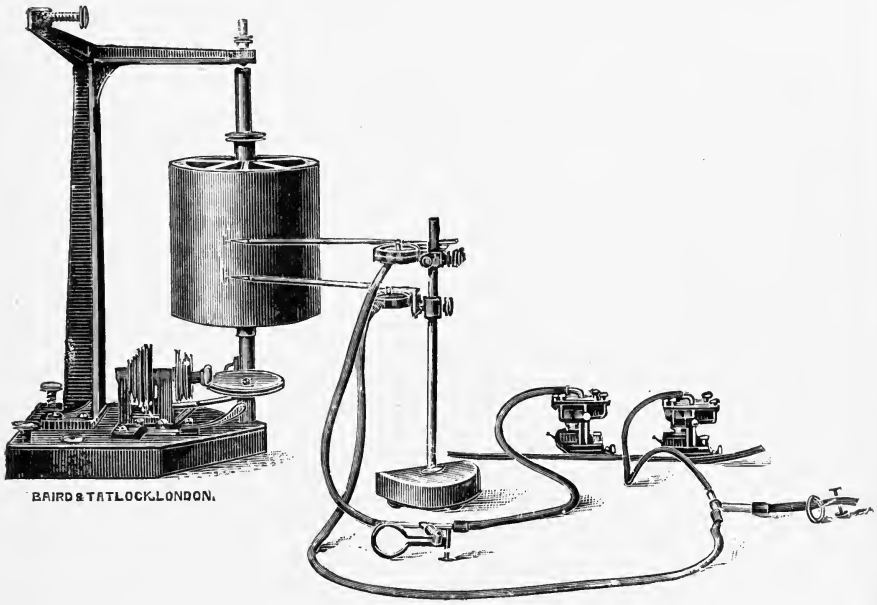


FIG. 233.—Tambour-sphygmographs arranged for measuring the velocity of transmission of pulse-wave.

The writing styles of the carotid and impulse tambours are arranged to write exactly beneath one another. A time tracing is taken beneath the curves. The beginning of the impulse curve marks the beginning

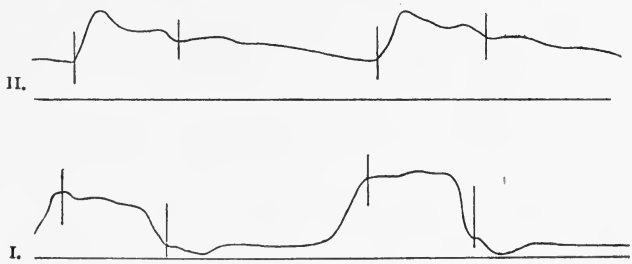


FIG. 234.—Impulse (I.) and pulse curves (II.). The vertical lines, marking the ascent of the pulse curve and the dicrotic notch, indicate the opening and closing of the semi-lunar valves.

of the ventricular contraction. The beginning of the carotid pulse curve marks the beginning of the period of systolic output and the opening of the aortic valves Between these points is the period of rising

tension, when the ventricle is raising the blood pressure up to that in the aorta. The beginning of the dicrotic notch corresponds with the closure of the aortic valves and the end of output. The time lost in the transmission of the pulse-wave from the heart to the carotid artery should be deducted in making these time measurements, but it is almost negligible. In a man with a pulse frequency of 70 the duration of systole was 0·379 sec., of diastole 0·483 sec. It is interesting to repeat the observations after the frequency of the heart has been accelerated by running up and down stairs. The diastolic period is shortened much more than the systolic period. When the pulse frequency varied in the proportion 100 : 270 the duration of a systole varied in the proportion 136 : 100.

CHAPTER XXVII.

BLOOD PRESSURE.

Blood Pressure in Frog.—Pith the cerebrum of a large frog and plug the hole, and then curarise lightly. Fill the fine glass cannula provided with sodium citrate, 1 per cent. sol., and clip the rubber tube. Pass two ligatures under one of the aortae. Tie the peripheral one as far from the bulbus arteriosus as possible. Pull up the other ligature (placed near the bulbus) so as to constrict the vessel. Between the two ligatures make a V-shaped slit into the artery with *sharp* scissors. Insert the cannula and tie the ligature round it. Do not allow any loss of blood.

Fill the proximal limb and rubber tube of the mercurial manometer with 1 per cent. sol. sodium citrate. Place the frog board so that the arterial cannula is on a level with the mercury meniscus, and connect the tube of the latter with the tube of the manometer. Remove the clip. The mercury rises until it balances the blood pressure, and oscillates with each ventricular systole.

Bring the writing point of the manometer against a slowly moving drum.

Observe the effect of gently compressing the abdomen. This increases both the diastolic filling of the heart and the resistance to systolic outflow. Reflexly inhibit the heart by lightly and frequently striking the abdomen with the handle of a scalpel. The heart distends with blood and arterial pressure falls owing to lessened systolic output.

Inhibition may also be brought about by tetanising the sino-auricular

junction. Gently press on the inferior vena cava, the pressure falls as the diastolic filling is diminished, and so the output is lessened. Expose the sciatic nerve without damaging the blood-vessels. Tie, cut, and tetanise the central end. This will excite the vaso-motor centre and cause reflex constriction and rise of arterial pressure.

CHAPTER XXVIII.

VASO-MOTOR SYSTEM.

Innervation of the Blood-vessels.—Pith the cerebrum of a large frog and plug the hole with a blunt-pointed match to prevent haemorrhage. Curarise the frog lightly, place it on the cork board provided for studying the circulation in the web. Tie out the toes so as to spread the web over the hole in the board. Observe the rate of circulation. Next pass a pin through the occipito-vertebral membrane and destroy the spinal bulb. The circulation will become more rapid owing to dilatation of the arteries. The vaso-motor centre in the rabbit has been localised in the spinal bulb on either side of the middle line extending from a point 1 mm. to a point 4 mm. below the corpora quadrigemina.

After five minutes the flow through the capillaries will be less rapid. The spinal cord contains subsidiary vaso-motor centres, and exerts a tonic action on the arteries after destruction of the chief centre in the bulb.

Expose the cervical cord and tetanise it with a weak current. The flow will be lessened.

Now remove the frog from the board and expose the heart. Suspend the frog in the vertical head-up position. Note that the heart and large vessels are filled with blood. Pass a blanket-pin down the vertebral canal and destroy the spinal cord. The heart and vessels will soon become bloodless owing to the loss of vaso-motor tone. The blood sinks into the dilated abdominal vessels under the influence of gravity. The vaso-constrictor nerves arise from the anterior roots, they have their cell-stations in the sympathetic ganglia, and from thence pass to the limbs along the grey rami to the corresponding spinal nerves. These facts may be demonstrated on the curarised cat by plethysmographing the fore limb, exposing and exciting the anterior roots in the upper thoracic region, and then injecting 10 mgrms. of nicotine to paralyse the cell-stations in the stellate ganglion.

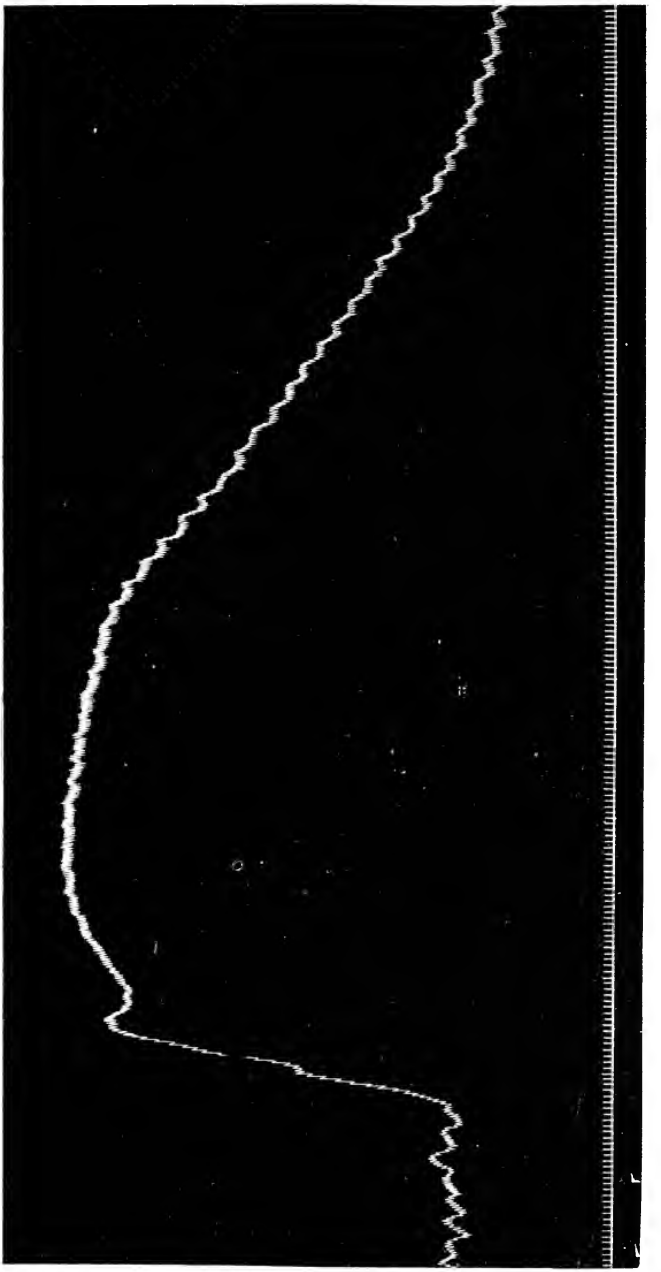


FIG. 235.—Arterial blood-pressure of cat. Effect of injecting supra-renal extract. The time is marked in seconds.

CHAPTER XXIX.

VASO-MOTOR SYSTEM—Continued. PERFUSION OF BLOOD-VESSELS.

Vaso-motor Changes following Excitation of the Sciatic.—Pith the cerebrum of a large frog and plug the opening. Curarise the frog very lightly, but sufficiently to paralyse the muscles. Curare in large doses paralyses the vaso-motor mechanism. Very carefully expose the sciatic nerve in the thigh without damaging the blood-vessels; tie, cut and place it upon electrodes. Observe the capillaries in the web of the foot under the microscope, and note the rate of flow. Tetanise with a current strength just perceptible to the tongue. The blood-flow becomes slower owing to constriction of the arteries. The vaso-constrictor effect is slight and very easily exhausted. If the nerve be excited with single shocks once every five seconds, vaso-dilatation and increased flow may result. Now examine the circulation in the web of the other foot. Note the rate of flow and then tetanise or pinch the skin of the frog. Reflex constriction and consequent slowing of the current may result. The afferent fibres excite the reflex discharge of the vaso motor centre in the spinal bulb and cord. These results, difficult to obtain in the frog, may be easily demonstrated in the cat by the plethysmographic method.

Perfusion of Frog's Blood-vessels.—Destroy the brain and plug the hole in the skull. Expose the heart. Tie one aorta. Place a ligature under the other, snip it with sharp scissors, and allow the blood to escape. Insert a fine-glass cannula into it pointing away from the heart. Fill the cannula with normal saline by means of a capillary pipette. Connect a rubber tube to a glass funnel and clip the tube. Fill the funnel and tube with Ringer's fluid. Connect the tube with the cannula. No air bubbles must be introduced. Snip the sinus venosus and open the clip. Hang the frog in the vertical position. The fluid circulates, runs out of the sinus, and drops from the toes of the frog into a measure glass. Measure the outflow per minute. Circulate Ringer's fluid plus 1 in 1000 sodium nitrate; the outflow is increased owing to vaso-dilatation. Supra-renal extract produces the contrary effect.

CHAPTER XXX.

REACTION TIME.

THE time which elapses between the application of a given stimulus and the prearranged response of the subject to that stimulus is known as the **reaction time.** It is obviously more complex than a reflex action; this will be readily understood from a consideration of the following determination of the reaction time.

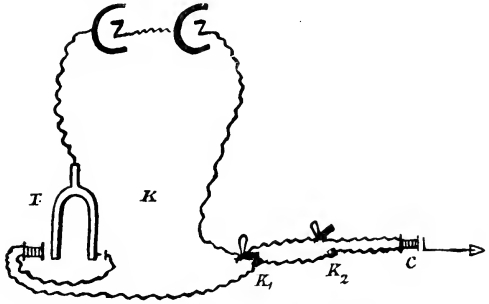


FIG. 236.—Diagram of the apparatus for the determination of reaction time.

The diagram 236 shows W. G. Smith's reaction time apparatus as modified by Colls. The electro-magnetic tuning-fork, T, with 100 vibrations per second, is connected with two Daniell cells and with the chronograph C. By means of either of the two Du Bois keys, K_1 and K_2, the chronograph can be short circuited. The key K_1 is closed and K_2 is open; the tuning-fork is set vibrating, but does not affect the chronograph. The subject, whose reaction time is to be determined, is told to listen for the sound of the opening of the key K_1 and to close the key K_2 directly he hears the sound. When the key K_1 is opened the chronograph vibrates in unison with the tuning-fork and the vibrations are recorded upon a

revolving drum; the closure of the key K_2 by the subject of the experiment brings the chronograph to rest. The number of vibrations recorded upon the drum gives the reaction time for sound in $\frac{1}{100}$ths of a second.

The total reaction time in this experiment is composed of—(1) the time taken by the sound to reach the ear; (2) the time taken for the reception of the stimulus by the sensory endings of the auditory nerve and the transmission of the nervous impulse to the sensory area; (3) the time for the transmission to the higher centres so that volitional impulses may be started in the cerebral motor centres; (4) the time for the propagation of those motor impulses to the nerve cells of the spinal cord; (5) the time required for the generation of impulses in these cells and their passage down the motor nerves to the muscles of the hand; and (6) the latency of the contraction of those muscles.

The reaction time for sound is about 0·150 second, for light 0·195 second, and for touch about 0·145 second.

CHAPTER XXXI.

MÜLLER'S LAW OF THE SPECIFIC ENERGY OF NERVES.

The Law of the Specific Energy of Nerves propounded by Johannes Müller states that each sensory nerve gives rise to its own particular sensation, whatever may be the means whereby it is excited. Thus the retina only gives a sensation of sight, whether it be stimulated by light, a blow or an electrical shock.

This law can be demonstrated by the following experiments.

Sight.—(i) Two clinical electrodes moistened with strong saline solution are connected by means of a key with a Daniell cell; one electrode is placed upon the forehead, the other upon the nape of the neck. On make or break of the constant current the subject will have a sensation of a flash of light.

(ii) The retina can be stimulated mechanically by pressure on the sclerotic. A sensation of light will be experienced (page 109).

Taste.—The end-organs of taste can be stimulated not only by sapid substances, but also by mechanical and electrical means. (i) Gentle tapping of the front of the tongue gives a sensation of a sweet taste.

(ii) When the free ends of two wires connected with a Daniell cell are placed upon the tongue and the current is opened or closed, a sensation of taste is experienced. This experiment can be performed with suitable unpolarisable electrodes, so that the objection, that

electrolysis is produced and the resultant ions are tasted, may be considered negatived. Moreover, weak faradising shocks, which would cause but little electrolytic action, also give rise to sensations of taste.

The anode appears to produce an acid taste, the cathode an alkaline taste.

Smell.—The olfactory nerve-endings give rise to a sensation of smell when they are stimulated with an electric current. The experiment can be performed in the following way. The electric current is sent through the nose by one electrode connected with the nose by filling the nasal cavity with normal saline solution; the other electrode is placed on the forehead. The odour is said to resemble that of phosphorus.

Cutaneous Sensations.—Sensations of touch, cold, warmth, and pain can be evoked by gentle application of the point of a metal rod to the skin of the hand. The areas or spots which on stimulation give rise to the different sensations should be mapped out with ink. Mechanical stimulation with a metal rod warmed to the same temperature as that of the skin of the hand will give rise to sensations of touch, temperature, or pain according to the area stimulated. Müller's law is thus demonstrated in the case of these sensations.

There is some doubt whether there are specific nerves for painful sensations; it may be that excessive stimulation of any sensory nerve causes pain.

The sensibility of different parts of the skin to tactile sensations may be investigated by gently touching the surface with the blunted points of a pair of compasses. The distance between the points is gradually increased, and the blindfolded subject is told to say whether the sensations appear to be those arising from one or two points of contact. The distances between the points of the compasses are measured upon the millimetre scale of an induction coil.

The tips of the fingers will be found to be much more sensitive than the back of the hand, and the latter more sensitive than the forearm.

CHAPTER XXXII.

THE RATE OF DISCHARGE OF NERVOUS IMPULSES FROM THE CENTRAL NERVOUS SYSTEM.

The rate at which nervous impulses can be discharged by the central nervous system can be investigated in the frog by exciting the nerve cells by means of a drug such as strychnine and recording the resulting incomplete tetanus; or in man by the record of the contraction of a

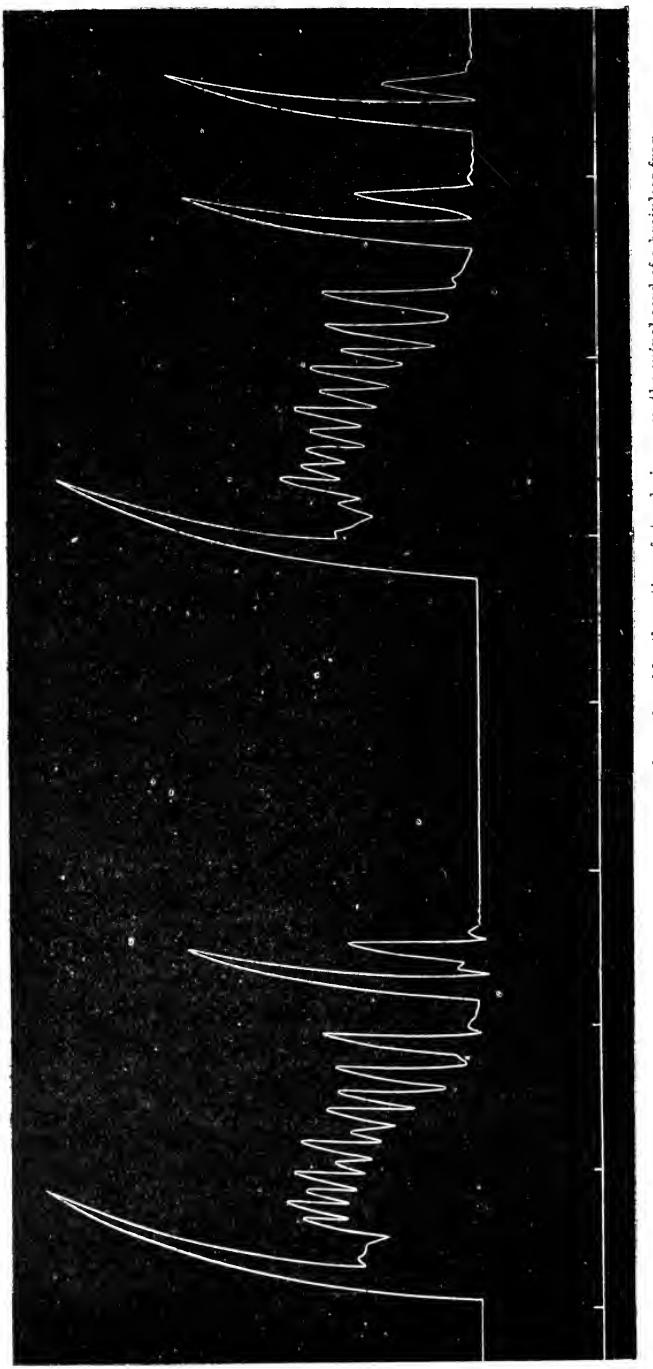


FIG. 237.—Tetanus of the gastrocnemius muscle produced by the action of strychnine upon the spinal cord of a brainless frog. The time is marked in seconds. Temperature of air = 23°. (Pembrey and Phillips.)

muscle thrown into contraction voluntarily, or involuntarily as in shivering.

(a) **The Incomplete Tetanus produced by Strychnine.**—The central hemispheres of a frog are destroyed by compression with a pair of small pliers or Spencer Wells forceps, and then the gastrocnemius muscle is prepared with the circulation intact. A piece of string is placed under the gastrocnemius muscle and is then tightly tied round the upper portion of the tibio-fibula and the remaining muscles; the leg is now cut away below the ligature. In this manner haemorrhage is prevented, the circulation in the muscle is intact, and the muscle is free to move with each contraction. A strong pin is placed through the lower extremity of the femur and is pushed firmly into the cork of the myograph; a piece of moist flannel is pinned down over the body of the frog in order to prevent the contraction of the muscles of the trunk and limbs from disturbing the lever connected with the gastrocnemius muscle.

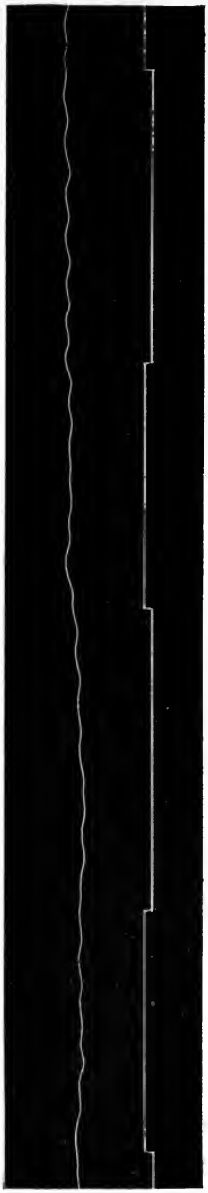


FIG. 238.—Record of a voluntary contraction of the adductor pollicis. The time is marked in seconds.

Strychnine is sparingly soluble in water, 1 in 6700, but a dose of 10-15 minims (0·592 – 0·888 c.c.) of a saturated solution of the drug in normal tap-water saline solution will in a frog produce the characteristic convulsions and death. Such a dose is injected under the skin of the frog's back. Twitches and convulsions soon begin and the contractions of the gastrocnemius muscle are recorded simultaneously with the movements of a signal marking seconds (Fig. 238). The number of contractions is about 8 or 10 per second. This is a measure of the rate of discharge of the nervous impulses from the nerve-cells of the spinal cord. The stage of incomplete *tetanus* is followed by prolonged twitches or *clonus*. If the spinal cord be destroyed by a probe during the stage of tetanus the contractions will cease at once,

showing that the convulsions were due to the action of the drug upon the nerve-cells and dendrites in the spinal end.

Record of a Voluntary Contraction.—If a finger be placed upon a muscle voluntarily thrown into contraction, a series of vibrations can be felt. These can be recorded and their rate determined in the following way.

A receiving tambour, with a button or a piece of cork fixed upon the rubber membrane, is connected with a bellows recorder (Fig. 239),

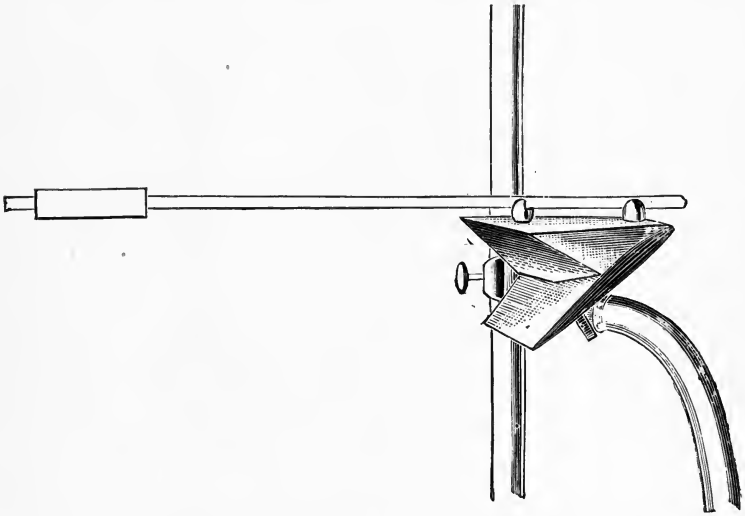


FIG. 239.—Brodie's bellows recorder. The bellows are made of aluminium plates and peritoneal membrane.

which is arranged to write upon a revolving drum. A chronograph is set up for marking the time in seconds. The button of the tambour is placed upon the adductor pollicis, or the masseter muscle of the subject. When the muscle is voluntarily contracted the lever shows a number of vibrations; these are recorded (Fig. 238). The curve obtained resembles an incomplete tetanus with 6 or 8 vibrations per second.

CHAPTER XXXIII.

THE FUNCTIONS OF THE ANTERIOR AND POSTERIOR ROOTS OF THE SPINAL CORD. THE BELL-MAJENDIE LAW.

THE researches of Bell and of Majendie showed that the anterior roots of the spinal cord were motor, and the posterior were sensory; the former nerves are *efferent*, carrying nervous impulses from the spinal

cord to the periphery, the latter are *afferent*, carrying impulses from the periphery to the spinal cord. This law can be proved by experiments upon a brainless frog, but careful dissection and manipulation are necessary.

The following are the several stages in the experiment. A small pair of electrodes is made by passing the bared ends of two pieces of fine insulated wire through a piece of cork, and the induction-coil is arranged for single shocks. The cerebrum of a large frog is destroyed by compression with a pair of Spencer Wells forceps, and then the frog is placed belly-downwards upon a cork board, and is confined to this position by a piece of wet flannel fastened down tightly by pins. A slit is made through the flannel in the line of the vertebral column, and the skin is reflected as far as the end of the urostyle. The ilium is carefully removed on one side, care being taken to avoid cutting any large blood-vessels, for loss of blood would lower the excitability of the spinal cord and obscure the dissection. For a similar reason the medulla oblongata, which contains the vaso-motor centre, was left intact. After the removal of the ilium the nerves of the sacral plexus can be easily found and followed up to the spinal cord. Starting from the top of the urostyle the laminae of the vertebrae are carefully removed by scissors, the points of which should not be plunged deeply inwards, otherwise the spinal cord will be injured. After the removal of several laminae one of the large nerves of the sacral plexus is followed up to its intervertebral foramen, where a black swelling about the size of the head of a pin will be seen. This is the posterior root-ganglion. It is freed from the foramen by careful dissection, and the roots are traced therefrom to the spinal cord. Fine threads are placed under the roots, which are then divided in the middle of their length by clean sharp scissors.

Stimulation of the peripheral end of the motor root will cause a contraction of the muscles of the corresponding leg; stimulation of the central end with a weak induction shock will cause no movement. On the other hand stimulation of the peripheral end of the posterior root produces no movement, but a similar stimulus applied to the central end sets up a sensory impulse which produces reflex movements.

The roots of the spinal nerves are longest in the lower segments of the spinal cord; for this reason the experiment is most readily performed in this region. During development the vertebral column grows more quickly than the spinal cord, and thus the lower posterior root-ganglia in the intervertebral foramina are separated from the spinal cord by a longer length of nerve-roots than in the case of those supplying the upper limb.

THE PHYSIOLOGY OF VISION.

CHAPTER XXXIV.

THE EYE AS AN OPTICAL INSTRUMENT.

Preliminary Consideration of the Mechanism of the Eye.—In order to understand the refraction of the rays proceeding from external objects and forming images on the retina, it is necessary, in the first place, to briefly consider the nature of such an optical system as constitutes the refractive apparatus of the eye.

The simplest form of an optical system consists of two media of different refractive powers separated by a spherical surface (Fig. 240).

If *dpe* be such a surface, separating a less refractive medium *S* from a more strongly refractive medium *R*, *n* is the centre of curvature, and is called the "**nodal point.**" If *p* be the vertex of the curved surface, a line through *p* and *n* will form the **optic axis** *OA*. Rays parallel to *OA* proceeding from *S* will be conveyed to a point F_2 on the optic axis. This point is called the **posterior principal focus.** Rays parallel to *OA* proceeding from *R* will be conveyed to a point F_1, the **principal anterior focus.** *p* is spoken of as the **principal point.** These two foci, the principal point and the nodal point, constitute the **cardinal points** of such a system.

In the actual eye the arrangement is not so simple, as there are several refractive media, and three separate surfaces—the anterior surface of the cornea, the anterior surface of the lens, and the posterior surface of the lens. The arrangement of these is, however, symmetrical, and permits of the reduction to two ideal surfaces for the three actually existing. This brings the number of cardinal points to six, as each of these surfaces will possess its own nodal point and principal point, though the anterior and posterior foci will be identical.

But for practical purposes a further simplification is possible. The two nodal points are not far separated, and the two principal points are

similarly very near, being distant only about ·4 mm. from each other. We therefore take a "mean" nodal point and a "mean" principal point and again reduce the optical conditions to those of a simple

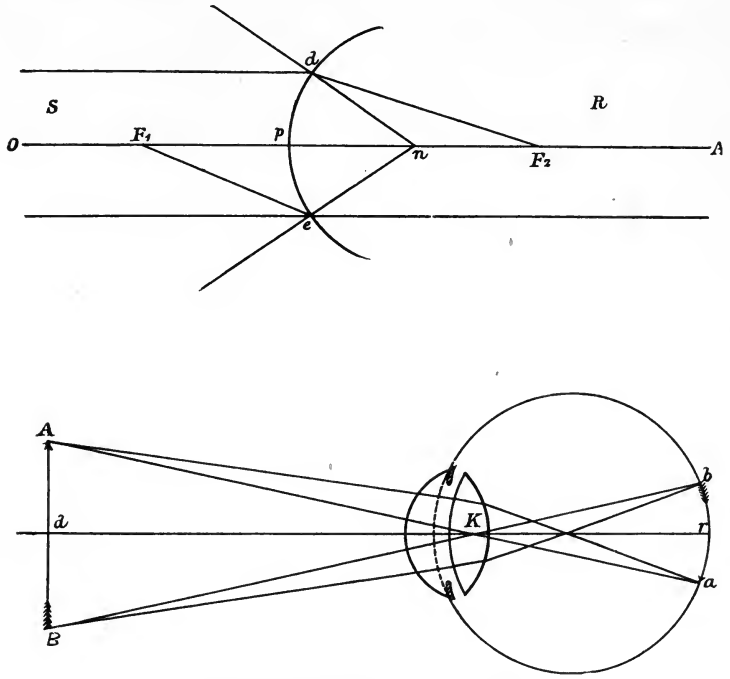


Fig. 240.—Diagrams to illustrate refraction.

optical system, consisting of one (ideal) refractive surface. In such a "**reduced eye**" the cardinal points are as follows:—

Principal point.—2·3448 mm. behind the anterior surface of the cornea, in the aqueous humour.

Nodal point.—·4764 mm. in front of the posterior surface of the lens.

Posterior principal focus.—22·647 mm. behind anterior surface of the cornea.

Anterior principal focus.—12·8326 mm. in front of the anterior surface of cornea.

Radius of curvature of ideal surface, 5·1248 mm.

With these data we are now able to understand the formation of the image on the retina, and are able to calculate the size of the retinal image of an object.

A ray passing through the nodal point K (Fig. 240) will not undergo refraction, and therefore will indicate the position of the image of any external point upon the retina. It follows also that the *size of the actual image* may be calculated if we know AB (the size of the external image), dK its distance from the nodal point. For

$$\frac{ab}{Kr} = \frac{AB}{dK}.$$

But dK = distance of object from cornea + distance of nodal point behind cornea, which latter is equal to 7·44 mm.

Kr is equal to 15·17 mm.

$$\therefore\ ab = \frac{\text{size of external object} \times 15{\cdot}17}{\text{distance of object from cornea} + 7{\cdot}44}$$

If the image be near so as to provoke a considerable effort of accommodation, this equation will not represent the size of the formed image. In this case the anterior surface of the lens will be more curved than in viewing more distant objects, and consequently the constants for the "simple reduced eye" will not hold good. The "reduced eye" of Listing corresponds, strictly speaking, to the lens accommodated for distant objects.

The Ophthalmometer.—This is an instrument by means of which the radius of curvature of the different surfaces of the eye may be measured. The degree of curvature of a reflecting surface will affect the size of the image formed from some external object. If some device be applied for the measurement of the image and the distance of the external object from the reflecting surface be known, then the curvature of the reflecting surface can be calculated.

In Helmholtz's original form of the opthalmometer the measurement of the image was achieved by causing the rays reflected from the cornea to undergo deviation from their direct course by passing through glass plates of a definite thickness. By introducing two glass plates, revolving in a common vertical axis, two images could be obtained, and the degree of overlapping of these images could be adjusted by altering the angle which the two plates made with one another. The distance between corresponding points in the two images could be expressed in terms of the angle representing the degree of tilt of the plates and the refractive index of the glass. The greater the obliquity of the plates the more considerable would be the displacement of the images.

Having obtained a value for the size of the reflected image the curvature of the cornea could be calculated from the equation,

$$\frac{\text{the size of a luminous body } (L)}{\text{size of its reflected image } (I)} = \frac{\text{distance of body from cornea } (d)}{\frac{1}{2} \text{ radius of cornea } (\frac{1}{2}r)},$$

or

$$r = \frac{2d \times I}{L}.$$

A modification of Helmholtz's ophthalmometer was introduced by Javal & Schiötz, in which the double glass plate was replaced by a calc-spar crystal and a similar double image obtained. This was still further improved by Kagenaar, who substituted compound prisms for the crystal, and the instrument so cheapened and improved is generally spoken of as an *astigmometer*. This instrument, which is essentially an ophthalmometer, is represented in Fig. 241.

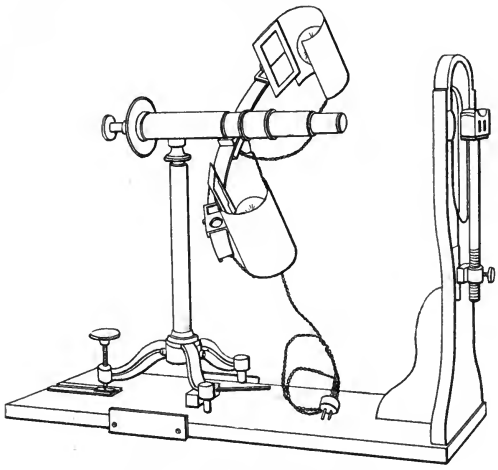


Fig. 241.—The ophthalmometer.

It consists of a telescope, which is directed towards the subject's eye, the head of the subject rests in the frame, opposite the telescope. The eyepiece of the telescope is first adjusted by focusing a thread which lies in the plane of the image formed by reflection from the cornea. This adjustment is carried out by turning the telescope towards a milk glass plate on the left of the subject, and moving the eyepiece till the thread is defined. The telescope is then directed towards the subject's eye, and moved with its stand backwards or forwards towards the observed eye till either of the reflected images of the illuminated areas on the quadrant is clearly defined. In the quadrant is a fixed area opposite a white line corresponding to the number 20 on the scale. Let the quadrant be first placed in a horizontal plane, with the fixed illuminated area to the left. According to the varying position of the right illuminated area two pairs of images will now be seen reflected from the cornea, and attention should be directed to the two middle of these images, which may or may not overlap (Fig. 242). The right moveable area should now be adjusted on the quadrant so that the edge of one image just touches the edge of the other, the 'stepped' image

being to the left and the rectanglar area to the right. A white line on the back of the right illuminated area will now point to some number on the scale; when the images are adjusted as above, this number + the 20 corresponding to the position of the left illuminated area, will express numerically the degree of curvature of the cornea. According to the constants of the instrument if the number 337 be divided by the number expressing the curvature of the cornea as above, the quotient represents the radius of curvature of the cornea in the horizontal meridian examined. The use of the instrument for measuring astigmatism may here be detailed.

EXPERIMENT. **Method of Measuring Astigmatism.**—By the use of the ophthalmometer represented in Fig. 241 the difference of curvature of different portions of the cornea can be easily ascertained.

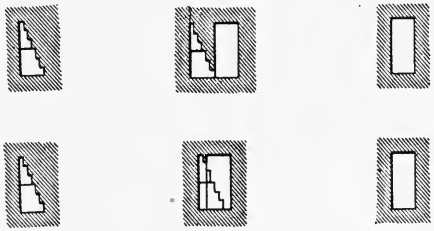


FIG. 242.—The images in the astigmometer.

The apparatus is adjusted as described above, and the horizontal meridian is first observed. If the curvature in this meridian is regular the four figures will be seen to stand on a level base. If this is not the case, the rotating quadrant must be moved till continuity of base line is obtained. The moveable illuminated area is then adjusted till the four reflected images are as in the figure.

The quadrant is then rotated, and as it approaches the vertical the two central images will probably overlap. Note the meridian where the greatest amount of overlap is observed. This will be the most refracting meridian. Each tread of the steps in the illuminated area corresponds to *one dioptre*[1] of curvature. The excess of curvature of the most refracting meridian may thus be read off at once.

[1] A lens in which the focus for parallel rays is at one metre is taken as the standard lens, and its degree of refractive power is represented as one dioptre.

CHAPTER XXXV.

THE OPTICAL DEFECTS OF THE EYE.

1. Myopia and Hypermetropia.—The condition of the refractive media of the eye when either hypermetropia or myopia are present are conveniently tested by what is known as the **shadow test.** If one take a concave mirror (such as that of an ophthalmoscope used for the indirect method), and reflects the light of a lamp at the side of the subject into the pupil of the eye, on looking through the aperture in the mirror the back of the eye is seen to be partially illuminated. If the subject be emmetropic the amount of illumination is small, and on tilting the mirror a little to the right or left a scarcely perceptible movement of the light area may be seen in the opposite direction of the tilt. The image of the lamp formed by the concave mirror is the direct source of illumination of the subject's eye, and this image moves to the right when the mirror is tilted to the right, and in accordance with the inversion of the image on the retina the illuminated area will seem to pass to the left. The general impression that one obtains of the result of tilting the mirror on the emmetropic eye is that the illumination suddenly disappears. With the hypermetropic eye the illuminated area is more distinct, as a large part of it can now be seen, and the passing of this area to the right or left inversely to the tilting of the mirror to the left or right is clearly visible. In the case of myopia the observer must be beyond the far point of the eye and then will see an inverted image of the illuminate area. As the result the apparent illuminated area will be an inversion of the actual area. When therefore the mirror is tilted and the image of the lamp passes across from right to left, the apparent movement will be from left to right, so that the movement of the light on the retina appears to be the same as the tilt of mirror. A small amount of myopia cannot be made out by this method.

EXPERIMENT. If subjects possessing the defects of myopia and hypermetropia cannot be obtained, using the ophthalmoscopic mirror as directed above, observe the movement of the light on the retinal screen in Kühne's artificial eye adapted for these defects. Compare the actual movement of the light on the screen with the apparent movement when observing in front of the eye as above.

2. **Imperfections of the Refracting Media, Entoptic Phenomena.**—(*a*) Certain bright, cloudy appearances may be seen, which disappear after blinking the eyelids. Wavy lines or speckled patches may appear after

rubbing the eyes. These are all due to the condition of the corneal surface, and have been more properly called 'pseudentoptic' phenomena.

(*b*) Dark specks or irregularly stellate figures may be seen, depending upon imperfections in the lens or its capsule.

(*c*) *Muscae Volitantes.* These appear as moniliform threads, clusters of bright or dark circles, and are referable to imperfections in the vitreous.

EXPERIMENT. Place a card which is pierced by a pinhole a little more than a centimetre from the eye (*i.e.* in the position of the principal anterior focus of the 'reduced' eye). Look at an evenly but brightly illuminated surface beyond, as a sheet of thin white paper held in front of a lamp. The rays of light falling on the retina are now approximately parallel, and any shadows that form in consequence of imperfections in the refracting media are rendered more distinct. Notice any of such shadows that may be received by blinking, due to imperfections in the cornea or any comparatively fixed figure due to imperfections in the crystalline lens. These may be practically absent. No difficulty will be experienced in recognising 'muscae volitantes.' These will appear as small particles or threads which appear to move away rapidly when the gaze is directed at them. When the gaze is fixed, as by a mark on the white paper, they are still seen to move slowly downwards. This implies that actually their shadows are moving slowly upwards, and that the objects themselves are similarly slowly ascending in the vitreous.

If, whilst gazing at some distinct cluster of muscae volitantes, the eye move upwards, the cluster will appear to move upwards too. This actually means that the shadow of the cluster is moving downwards on the retina. If the card be moved downwards the same result, as far as the shadows are concerned, will occur. From this it may be inferred that the objects producing the shadow are behind the nodal point (situated in the crystalline lens), and therefore, if the movement of shadow be appreciable, on the vitreous.

Objects in front of the nodal point, such as impurities on the cornea, would appear to move upwards when the gaze is directed downwards, and conversely.

CHAPTER XXXVI.

SENSATIONS OF LIGHT AND COLOUR.

MANY theories have been advanced to explain the phenomena connected with colour vision. The most important of these theories are those connected with the names of Young-Helmholtz and Hering.

The theories are all concerned in referring the multiplicity of colour sensations to fusion of certain simpler sensations, which are described as primary colour sensations. In the Young-Helmholtz theory the primary sensations are those corresponding to red, green, and blue-violet; in the Hering theory they are grouped in pairs, which are the red and green sensations, the yellow and blue sensations, and the white and black sensations. It is necessary to assume the existence of certain photo-chemical substances in the retina, which can be acted upon by the light of the primary colours. The light at the ends of the spectrum would, in accordance with the Young-Helmholtz theory, act upon either the red visual substance or the violet visual substance, in the intermediate part of the spectrum upon all three visual substances to different extents. If all are affected more or less equally, the compound sensation of white is produced.

In the Hering theory there would also be assumed to exist three primary visual substances, but according to the chemical changes in any single substance, whether of the constructive or destructive variety, so a sensation corresponding to one of the complementary colours of the different pairs would be brought about.

A certain classification of colours is necessary. They may be conveniently described as varying in hue, tint, or shade. The **hue** of a colour is its **colour tone**, corresponding to its wave length. The **tint** of a colour depends upon its purity, or whether it is admixed with white—in other words, depends upon its **saturation**. The **shade** of a colour is an expression of its brightness or **intensity**, or, what comes to much the same thing, the degree to which it is admixed with black.

1. **Colour Tone.**—In reviewing the changes of hue that are appreciable in examining the spectrum, it is to be noticed that the changes do not occur at any regular intervals corresponding to wave lengths. Changes of colour tone are most easily appreciated in the yellow, green, and blue green. At the red end and violet ends there appears to be little or no change of hue.

The variations in saturation or tint can be seen by using the red and white discs of a colour mixed in varying proportions and noting the corresponding sensations produced.

2. **Intensity.**—Variations in intensity cause changes in the quality of colours. At their maximum brightness colours tend to give the sensation of white, though they never completely do this. The yellow will the most easily; the blue and violet approach close to it. The red is most distant in producing the sensation of white.

EXPERIMENT I. Looking through a pinhole in a card towards the prism, examine the spectrum formed by direct sunlight or the arc-lamp.

The distinctiveness of the colours is less marked than in the projected spectrum examined on the screen, and the different sensations all approach that of white. The red, however, does not pass beyond a yellow sensation. With much diminished intensity colours become less recognisable. Red in particular is difficult to recognise in a much diminished light.

EXPERIMENT II. Take a small square of red paper and a similar piece of blue paper which in a light of moderate brightness appear of approximately equal intensity. Carry these to an almost dark room and note the dulness or even blackness of the red whilst the blue may still appear bright.

EXPERIMENT III. A similar experiment to Experiment II. may be performed with the material of Experiment I., Section H, of the Milton Bradley Pseudoptics. Taking the red and blue discs it will be noticed that the red is much the brighter colour in ordinary light. But if the two cards be carried to an almost dark room, it will be possible to distinguish the blue disc far more easily than the red.

3. **The Fusion of Distinct Sensations of Black and White. Flicker.**—This fusion depends upon the persistence of the positive after-images each separate stimulus brings about. If separate stimuli follow each other sufficiently rapidly a blending of the different sensations occurs, as is well exemplified in the presentation of the series of rapidly succeeding views in the cinematograph. The phenomena upon which this depends can be shown in a revolving disc divided into rings of sectors of white and black, increasing in number from the centre to the circumference. Such a disc is included in the Petzold series.

EXPERIMENT I. Rotate a disc such as (Fig. 243) slowly, and note that at a certain rate the peripheral ring appears as a uniform grey, a flickering sensation is produced on the neighbouring rings, but the central rings show an alternation of white and black. Increase the rate and note that these can also be caused to blend.

In general it may be stated that when fusion in any way occurs the resulting sensation of grey is the same as if the light reflected intermittently were replaced by the same quantity of light continuously reflected, in other words, as if a uniform grey of a certain shade were substituted for the series of sectors; moreover, if the rate at which the sectors are successively presented to the retina be increased above that necessary for fusion, the intensity of the resulting sensation is not altered. (Talbot-Plateau Law). This may be experimentally confirmed in the following manner.

EXPERIMENT II. Take a lens of short focus and hold it at such

a distance from the disc in the revolving apparatus that no distinct image of the outer rays is obtained, but only a blurred appearance of grey. Then rotate the disc, no alteration in the shade of the grey will result.

The rate necessary for the flickering sensation to pass into complete fusion depends upon the intensity of the light.

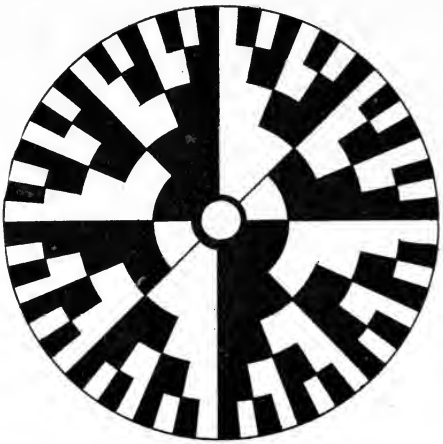


Fig. 243.

Experiment IV. With a metronome, note the rate of revolution necessary to produce complete fusion in the outer ring. Darken the room and observe whether the rate be altered. It will be found that with diminished light a slower rate of revolution brings about fusion. The converse is true up to a certain limit.

The point at which flicker passes into fusion has been used as a means of determining the condition of persistence of visual sensations. It is to be noted that the flicker may be coarse or of a fine tremulous character. The transition of this fine flicker into fusion should be taken as the limiting sensation.

The excitability of any portion of the retina is influenced by the stimulation of that portion of the retina (temporal induction) and changes are simultaneously induced in neighbouring regions of the retina (spatial induction). These factors may be of very considerable influence in determining the point at which flicker passes into fusion. A 'physiological' state is brought about by a certain 'physical' stimulus, and thereby the effect of the stimulus may be increased or diminished. If then a succession of stimuli of say blue and black be presented to the retina at a certain rate flicker will pass into fusion. But if the blue be

intensified by being placed on a black blackground this rate will no longer be sufficient. This may be shown in the following manner.

Experiment V. Take a disc like that shown in Fig. 244 with black and blue semicircular rings, and yellow and black backgrounds. On

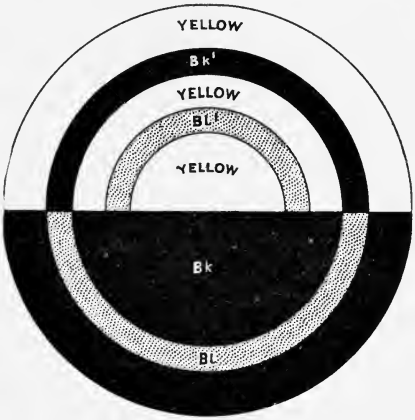


Fig. 244.

rotating this disc it will be observed that the flicker persists much larger in the outer blue and black ring than in the inner blue and black ring.

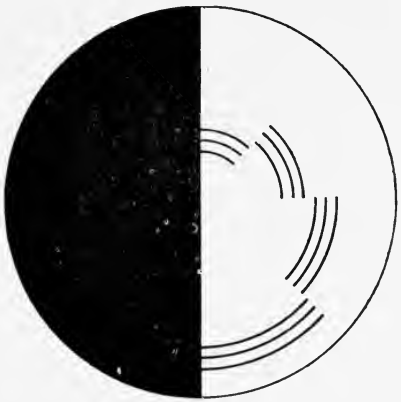


Fig. 245.

Fechner showed that certain colour effects may be produced by slow rotation of discs which consist of black sectors of increasing size on a white ground. They may also be seen in a disc showing black circular lines of different circumferences on a white semicircular area, the other half of the disc being black. Such a disc is shown in Fig. 245.

It has been shown that a bright object on a dark background appears, when suddenly exposed, to be surrounded with a red border lasting a fraction of a second. If the illumination be brighter a blue green effect is visible. These facts in part explain the appearance of colours shown when the discs below are rotated.

EXPERIMENT VI. Rotate the discs (Figs. 245 and 246) and note the coloured fringes or areas appearing according to the direction. The rate must be slow. The simple fusion of sensation corresponding to intermittent stimuli rapidly repeated may be also shown in the Experiments I. and II., Section E of the Milton Bradley Pseudoptics.

FIG. 246.

4. The Fusion of Colour Sensations.—Several methods have been devised with the object of enabling us to fuse separate colour sensations. These depend either upon separate colours forming images on the retina in such rapid succession as to be inseparable, or else upon separate colours forming images in the same portion of the retina so that the sensations are super imposed.

The first method is generally carried out by means of the separate colours being arranged as sectors in a circle, which is rapidly revolved about its centre, the instrument adapted for the purpose being known as a **colour-mixer.** Discs of different colours, such as the Wundt series, are obtainable, and each disc has a radial slit at one point so that these can be arranged upon a common centre and a circle may be made up of sectors of various discs. It is desirable to have discs of two sizes, one about ten inches across, the other four or five inches. It is to be remembered that these discs are not coloured with pure colours of the

spectrum, and the results of their mixture yields various colours which are largely mixed with grey.

EXPERIMENT I. Take two large discs of red and green and two small of black and yellow. Adjust the proportion of the red and green so that rapid revolution produces a yellow. This will be dark in shade and can be matched by the inner discs of yellow and black.

EXPERIMENT II. Take large discs of green and violet and small discs of blue and black. With the large discs a blue can be obtained and matched with the smaller discs.

EXPERIMENT III. Take three large discs of red, green, and violet. To bring about a good result the red should correspond to the red in the spectrum at wave-length 6300, the green to wave-length 5150, and the blue to wave-length 4700. Arrange these so that red constitutes about 118°, green about 146°, and blue about 96°. Arrange also two smaller discs of white and black. As the result of revolution the larger discs will give a grey, which can be matched by about 285° black and 75° degrees white of the smaller discs.

EXPERIMENT IV. Using the three discs of Experiment III., work gradually through the whole spectrum, using different sized sectors of each for the different regions of the spectrum. The sizes of these sectors will roughly correspond to the different degrees in which the three primary colour sensations according to the Helmholtz theory are evoked.

The best method of fusing the colours sensations is to superimpose the various colours of the spectrum by projection of the same on a white screen.[1] By means of lenses the spectrum can be recomposed as white light. By introducing shutters eliminating certain portions of the spectrum the result of fusion of the remaining colours can be examined.

5. **Complementary Colours.**—For every colour in any part of the spectrum there is a colour in another part of the spectrum which, when mixed with it, will yield a white or grey. Such colours are said to be complementary.

EXPERIMENT I. Take from the series of colour discs one of an orange colour. If no disc can be found which in any proportion with the orange disc will give a white or grey, take the blue and green discs and adjust all three so that a grey is obtainable. (This should be estimated by smaller discs of black and white). A certain proportion will exist between the blue and the green. If now the whole circle be

[1] See Abney, *Colour Vision*, p. 18 *et seq.*

divided up into blue and green in this proportion, revolution will give the hue of the colour complementary to the orange originally selected.

It will be found by such experiments as this that orange is complementary to greenish-blue, red to bluish-green, yellow to blue, yellowish-green to violet, and green to purple.

EXPERIMENT II. If a coloured object be viewed on a white surface it may provoke a negative after-image in colour complementary to that of the original object.

In illustration of this perform the experiments Nos. III. and IV. of Section E in the Milton Bradley Pseudoptics series.

6. **Contrast.**—Besides the effect which different colours produce when presented simultaneously, or practically simultaneously, to the retina, as in colour-mixing, other effects also will come about when different colours are presented successively and comparatively slowly to the same portion of the retina, or again, when different colours are presented simultaneously to adjacent areas of the retina.

In the first of these two cases we have the conditions of **Successive Contrast**, in the second we have **Simultaneous Contrast.**

The second experiment in the section on Complementary Colours affords illustration of Successive Contrast. In general the nature of successive contrast may be shown as follows.

EXPERIMENT I. Take a number of small squares of various colours each about 1 cm. in size. Arrange also a series of fields of different colours, as well as one of white; these may be squares of 1 or 2 decimetre side. Taking a small red square, place this in the centre of the large white square and in a good light gaze at it for two or three minutes. Blow the small object away and continue the gaze. An after-image of the object will be obtained of a colour complementary to that of the original. Substitute for the large white square squares of different colour and perform the experiment again. It will be found that the after-image varies in colour according to the ground on which it is viewed. If red be the colour of the original small square, the after-image on white will be green or bluish-green. If projected on violet the after-image will be blue and if on orange a dull brown.

EXPERIMENT II. By projection successive contrast may be easily demonstrated as follows. Two lantern slide glass plates are taken, and on one is marked out, in centre of plate, two concentric circles of about 1·5 and 3 cm. radius, enclosed by black lines of just perceptible thickness and having a central dot of about the same 2 or 3 mm. diameter. On the second glass plate are fixed rings of coloured gelatine of similar size to the two circular rings, the colours

chosen being preferably complementary. The two slides are projected simultaneously and the rings are gazed at (the central dot being used as fixation point), for half a minute. The slide with the coloured rings is then suddenly removed, the gaze remaining on the dot, when the two rings will be seen in colours complementary to the original colours.

Simultaneous contrast may be shown in the following shadow and mirror experiments.

EXPERIMENT III. Arrange two sources of light about six inches apart, and allow each of these to throw a shadow of some opaque upon a screen held about a yard from the source of light. (8 candle-power and 16 candle-power electric incandescent lamps answer very well for the two sources of light.) Interpose a coloured glass plate in front of the weaker light. The shadow corresponding to this will be the same colour as the plate, the other shadow will become coloured complementarily. Observe the variation in intensity of colour according to the proximity of the two shadows. If the object be moved away from the screen the two shadows will separate and the colours will be dull, if the object approach the screen closely the shadows will almost touch and the colours will be extremely vivid.

EXPERIMENT IV. Arrange a mirror horizontally so as to reflect light from a white surface, *e.g.* a white lamp shade. Place a coloured glass plate over the mirror. Interpose an opaque object, as a pencil or the finger, in the course of the white light incident on the mirror. Observe that two reflected images of this are seen, one from the surface of the coloured glass and of the same colour as the glass, the other reflected from the surface of the mirror and complementary in colour. Gently tilt the coloured glass so as to separate the images. It will be found that they are most brilliantly coloured when slightly overlapping.

EXPERIMENT V. Place the dark grey papers of Experiments III. and IV., Section G, of the Milton Bradley Pseudoptics on the different coloured fields and cover with tissue paper. Observe the contrast colour that appears in the grey paper.

EXPERIMENT VI. Arrange on the colour-mixer the discs of Experiment V., Section G, of the Milton Bradley Pseudoptics. On rotating these, the black and white rings will assume a colour in contrast with that of the general field.

EXPERIMENT VII. The Experiments I. and II., of Section G, Milton Bradley Pseudoptics, illustrate the effects of contrast in black and white alone.

The above experiments on Complementary Colour and Contrast depend upon variations in excitability in the retinal area involved or in adjacent retinal areas. The change in excitability that occurs in any retinal area when affected by incident light is spoken of as caused by **temporal induction,** and the change that is brought about in adjacent areas as resulting from **spatial induction.** Successive contrast depends upon temporal induction, simultaneous contrast upon spatial induction. The phenomena connected with the formation of after-images are examples mainly of temporal induction.

With regard to the complementary colour of after-images, this is thought by some to be simply the result of fatigue. Others regard the phenomena as due to initiation of processes, the converse of those brought about by the original stimulus. Hering's theory of colour vision involves an explanation of these processes in accordance with the latter view.

In this connection it will not be out of place to refer to a phenomenon known as **Irradiation.**

EXPERIMENT VIII. Let a black square be inscribed in a white square of three times the side, and conversely, let a white square be inscribed in a black square of three times the side. The side of the inner square will be equal and should be about a centimetre long If the two figures be placed side by side, the inner white square will appear larger than the inner black square. The material for this experiment on a larger scale is also provided in the Milton Bradley Pseudoptics, Section C, Experiment IV. The explanation of this may be due to the dispersive power of the lens, as the appearance is more conspicuous with a large pupil, or it may be due to the chemical processes of a certain kind (katabolic) in the retina tending to encroach on adjacent fields of the retina, the opposite processes (anabolic) apparently not having that tendency.

EXPERIMENT IX. A line passing through the adjacent edges of two rows of black squares, arranged so as to overlap appears oblique. See Milton Bradley Pseudoptics, Section B, Experiment V.

7. **Colour Blindness.**—The inability to distinguish different hues of colours constitutes the condition of colour blindness. It may vary much as regards the failure shown. A person may be red blind and then only appreciates the colour of red objects as far as they show other constituents of white light. Such a person, according to the Helmholtz theory of colour vision, would be entirely lacking in the production of the red sensation. Or a person may lack the green sensation and be green blind, and very rarely violet blindness may exist.

If a red blind person be examined as to his sensations along the range of the spectrum, he sees nothing at the extreme red end of the spectrum at all. A glimmer of what he calls dark green is seen in the position of the red lithium line, and this green gradually becomes more conspicuous to him through the yellow to the proper green. Passing to the blue green he says the colour is grey, being similar to his idea of white admixed with a certain amount of black. Passing further to the blue end he recognises the blue and speaks of the violet as dark blue. Similarly, a green blind person will recognise a grey in the middle of the spectrum, but rather more in the green than the locality thus named by the red blind.

Colour blindness can be conveniently tested by the use of a series of coloured wools of great variety of hue and tint. Such a set of wools are spoken of as Holmgren's wools.

EXPERIMENT. Spread out the wools on white blotting-paper in a good light. Avoid mentioning the names of the colours of any of these wools, but pick out a whitish green, and request the subject to collect all those wools which approximate in hue or tint to the colour presented.

If any errors are made, proceed to test whether he is red blind, green blind, or violet blind. Give him a skein of a magenta hue. If he is red blind he will pick out blue and violet; if green blind he will confuse green and grey.

The matching of colours may be also carried out by rotating the various cards of the colour-mixer, and thus matches of any colour under examination can be obtained. The same result can be obtained by projecting various portions of the spectrum as mentioned in colour mixing.

CHAPTER XXXVII.

BINOCULAR VISION.

THE images formed on the two retinae of an external object amongst its surroundings will not be identical. The lack of identity enables an observer to form a judgment as to its position in space. Such a judgment is more easily formed when the object is comparatively near than when far off, as in this latter case the images are approximately similar. Though the images for objects at a certain distance are not identical, it is necessary that they should be thrown on certain corresponding parts of the retina in order that a single sensation should result.

In order that a single image then should result, it is necessary that

various movements of the eyes should occur, so that the two images should fall on corresponding points.

With reference to the movements of the eyes, it is customary to regard them as taking place about three axes: (*a*) the *sagittal* axis, corresponding nearly to the line of sight; (*b*) the *frontal* axis, extending from right to left in each eye; and (*c*) the *vertical* axis. These axes are regarded as intersecting at one point the *centre of rotation* of the eye. With the head in fixed position the extent of space in which objects can be seen by allowing the maximum of eye movement is called the **Field of Regard.** If the head and body are erect and the eyes are directed towards the distant horizon, the position assumed is spoken of as the **Primary Position.** The point upon which the eyes are fixed is called the **Principal Point of Regard.** A position which the eyes may take up which does not conform to the requirements of the Primary Position is called a **Secondary Position.** If an observer shift his gaze from the principal point of regard to some other point in the field of regard, he may pass directly to this new position, or may pass over a varied number of different points in the field of regard before reaching this final position. The amount of rotation about the different axes of the eye finally involved in adopting this new position will be the same whether the eye pass to it directly or by a number of varied intermediate positions. In other words, only one position is possible when the gaze is shifted to this second point. This is called Donders' law. An extension of the rule is seen in Listing's law, which lays down that in moving from the primary position there is no rotation at all upon the sagittal axis, but merely upon the horizontal and vertical axes.

1. **Binocular Direction.**—In judging of the position of near objects, they are referred not to either eye separately, but to an ideal eye situated midway between the two actual eyes, the so-called Cyclopean eye of Hering. A line drawn through the object to the centre of such an eye is the Binocular Line of Regard.

Experiment. Make a pinhole in a sheet of paper, and starting with the hole well to the right of the right eye, draw the paper across the eye horizontally, so that the pinhole will pass across each eye successively. First one and then a second image of the pinhole will be seen as it passes over each eye, but in either case the hole will be referred to the median plane or the Cyclopean eye, and will seem like a succession of two holes over this eye.

2. **Single and Double Images.**—If the two eyes be directed towards an object about two feet off, and a finger be held up in the binocular

line of regard about a foot from the eyes, a double image of the finger will be seen. In this case the images of the finger will fall upon non-corresponding parts of the retina, and hence the images will not combine to form a single sensation.

EXPERIMENT I. Place a rod vertically about two feet from the eyes. Adjust the vision for a clear image of the rod. Then hold up a finger in the binocular line of regard about twelve inches from the eyes. A double image of the fingers will be seen. Close the left eye, the right image will disappear. Then accommodate for the finger, and a double image of the rod will be seen. Close either eye, and the image on the same side will disappear.

This experiment may also be performed with the material in the Milton Bradley Pseudoptics, Section I., Experiment I.

The double images seen, the above experiment may be crossed or uncrossed. If crossed they are spoken of as heteronymous images, if uncrossed, as homonymous images.

In general, if the optic axes of the two eyes converge towards a certain point, and a circle be described passing through this point and the two centres of rotation of the eyes, then an object outside the circle will produce homonymous images, and an object inside the circle, heteronymous images. With a definite point of regard, then, it will be found that if a circle be described through this point as above, objects lying on this circle will be seen single. Such a circle is called a horopteric circle, and the complete surface (intersected as above by a horizontal plane, forming a circle) is referred to as a **horopter.**

Double images of single lines may be shown in performing the Experiments II. and III., Section I., of the Milton Bradley Pseudoptics.

When double images lie, not symmetrically with regard to the line of regard, but both to one side of that line, that nearer the line of regard is the more distinct, and the other is hardly discernible.

EXPERIMENT II. Fix the eyes on some remote object, and hold a pencil about six inches from the right eye and about two inches to the right of a line passing from that eye to the remote object. The image falling upon the right retina will alone be appreciated. Close the right eye, and the second image will also be observed.

In general the image falling upon the nasal side of one retina will dominate over that falling on the temporal side of the other retina.

3. **Binocular Fusion of Dissimilar Images.**—If two partially dissimilar images, or at any rate not absolutely identical images, fall upon

corresponding points of the two retinae, the sensations corresponding to a single image result.

EXPERIMENT I. Place on a stereoscopic slide, or on a sheet of cardboard, red and green postage stamps at a distance from each other equal to the interocular distance, and similarly arranged. Observe these in the stereoscope, and the sensation of a single image of a black or brown postage stamp will result.

EXPERIMENT II. Perform the experiment in the Milton Bradley Pseudoptics, Section K, Experiment III. The fusion of the two retinal images gives the impression that one is looking through a round hole in the hand.

4. **Binocular Perception of Relief.**—The perception of relief which enables a judgment as to solidity to be formed depends upon the fact that the two pictures presented to the retinae are not identical. The amount of variation in the pictures will depend upon the interocular distance and the propinquity of the objects. The first being constant, it follows that a judgment as to solidity is more easily formed in the case of near objects than distant objects. Similarly, a judgment as to the relative distances of an object from the observer depends upon the difference in position of an object with respect to surrounding objects which exists in the two views presented to the two eyes.

The difficulty in forming a judgment as to the precise position in space of an object when viewed with only one eye may be shown in the following experiment.

EXPERIMENT I. Stick a knife into the wall, and balance on the handle a cork. The height from the ground should be about five or six feet. Close the left eye, and, starting at about ten feet from the wall with the right hand extended forward, walk rapidly to the cork, and by a sweep of the hand attempt to remove the cork. A lack of success will frequently attend the effort.

It is seen from this experiment that it is difficult to locate any object precisely in space when a single ocular view is alone obtained.

On the other hand, if perfectly flat pictures be taken differing from each other to the same degree as actual pictures presented to the two eyes would differ, and if such flat pictures be combined by some form of stereoscope, or by crossing the eyes, the resulting sensations will correspond to a single picture on which the different objects are differently projected into the space embraced by the picture, in which the quality of *depth* is added to the flatness shown by each picture separately.

These effects can perhaps best be shown by examining the Martius-Matzdorff[1] series of diagrams with a stereoscope.

Visual Illusions.—The study of Visual Illusions is somewhat beyond the scope of the present work, but the student may advantageously perform various of the experiments on the Milton Bradley Pseudoptics, which illustrate many of these illusions. The Sections A, B, C, D, and J are specially recommended in this connection.

[1]The diagrams can be obtained from Winckelmann und Söhne, Berlin; Petzoldt, Leipsic; or from Messrs. Baird & Tatlock, Cross Street, Hatton Garden, London. From the latter firm can be obtained any of the instruments mentioned above or the Milton Bradley *Pseudoptics* Series.

HEARING.

CHAPTER XXXVIII

DISSECTION OF THE EAR IN THE SKATE.
AUDITORY SENSATIONS.

Ear of Skate.[1]—1. Pare away the cartilage between the eyes of a skate. When the brain is reached continue the paring laterally, and in the cartilage at the side of the hinder part of the brain there will eventually be exposed one of the semicircular canals. When this is reached remove the upper wall as far as possible. In the hollow formed by the cartilage will be seen the fine **membranous canal**, dilating at one end into an **ampulla**. On continuing the exposure of the membranous canal it will be seen to join a rather large membranous sac, the **utricle**. Separated by a slight constriction is a smaller sac, the **saccule**, and at the anterior end of this is a small membranous projection which represents the **cochlea**.

2. Continue the dissection further so as to completely expose the **three semicircular canals**. Note also a tube leading towards the surface from the utricle, representing the **recessus vestibuli**.

3. Observe that the ampullae are more rigidly adherent to the cartilaginous walls than the length of the membranous canals. Open one such ampulla where comparatively free and note the **crista acustica** running transversely across the tube for about a third of the circumference.

AUDITORY SENSATIONS.

Range of Appreciation of Sound.—EXPERIMENT. In a room as free from noise as possible, let the subject sit with eyes closed and one ear plugged with cotton-wool. Let a watch be held in a line joining the

[1] A dog-fish can be used for this dissection.

two ears, and let it be placed opposite the open ear at such a distance that its ticking is just appreciable. In a quiet room this distance may vary from 2·5 to about 5 meters. Repeat the experiment with the other ear.

2. **Auditory Fatigue.**—The full effect of any sudden sound tends to temporary fatigue, to abolish appreciation of the fainter echoes which succeed it. If the full effect be avoided the fainter echoes may be heard.

Experiment I. Let a sudden intense sound (such as may be made by striking a bench with a hammer) be produced, (*a*) with the ears open, (*b*) with the ears closed for about half a second after the impact. In the first case the intense sound will alone be noticed, in the second case fainter echoes will be noticed in opening the ears.

Experiment II. Strike a tuning fork and place it on the crown of the head with gentle pressure. When the sound is no longer heard, remove it for a few seconds and then replace it again when the sound will be again appreciated.

Experiment III. Using a binaural stethoscope, sound a tuning-fork on a stand, and standing symmetrically with respect to the fork let the opening of the stethoscope be directed towards the fork. Then pinch one tube of the stethoscope, and the sound will be located by means of the patent tube only. When the sound has almost died away reopen the pinched tube, and now the sound will appear differently located and more intense to the ear which has not been fatigued.

3. **Appreciation of Pitch.**—Experiment. With some apparatus which will provide variation in pitch, observe the highest pitch in which tone can still be recognised. Conversely, note the lowest audible pitch in which tone can still be heard.

4. **Recognition of Absolute Pitch.**—By practice a trained musician can name the pitch of different tones. Education is required more for this probably than in naming fine differences of colour.

Experiment. Sit with the back to a piano and name the notes struck at random by the observers. In many cases this experiment may be impracticable.

5. **Beats.**—If two tones of different pitches be produced at the same time they mutually interfere and the resultant sensation is marked by a rhythmic variation in intensity, and is described as characterised by *beats*.

Experiment I. Put two tuning-forks of different pitches into vibration, and frequently the rhythmic beating is easily recognised.

EXPERIMENT II. Take two tuning-forks which produce beats when simultaneously caused to vibrate. Place one at such a distance from the ear that it can scarcely be heard. Bring the other fork gradually closer to the ear and the beats will be recognised.

6. **Compound Tones.**—The tones produced by musical instruments are not simple tones, but blended with other so called **overtones.** The lowest tone of the group gives the **fundamental** tone.

EXPERIMENT. Stretch a violin string between two fixed points. Set this into vibration by pulling it near one end, and immediately touch it in the centre with the finger. The tone will seem to be pitched an octave higher. The fundamental tone of the original group is obliterated, and the lowest tone now is an octave higher, and thus a new fundamental with other less evident overtones give the tone to the group.

7. **Location of Tones.**—EXPERIMENT I. Sound a large tuning-fork and press it against the vertex. The sound will appear to come from inside the head. Then close one ear, and the sound will seem to be localised in the other ear.

EXPERIMENT II. Sound a tuning-fork as above and note the effect of placing it on different parts of the head.

EXPERIMENT III. Sound a tuning-fork and let its foot rest upon the teeth. Close one ear and localise the apparent change in position of the sound.

DEMONSTRATIONS.
CIRCULATION. RESPIRATION. ANIMAL HEAT.

CHAPTER XXXIX.

INTRACARDIAC PRESSURE. BLOOD FLOW.

Intracardiac Pressure.—Owing to inertia the mercurial manometer is unable to respond to the rapid changes of intracardiac pressure. The pulse curves obtained by the mercurial manometer are also distorted by the swings due to the momentum of the mass. To record the changes of intracardiac pressure an instrument must be contrived which is able to follow a change of pressure equal to 1500 mm. Hg per second.

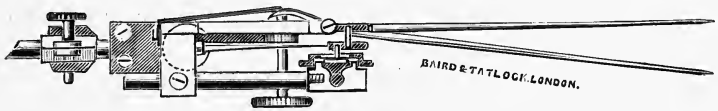


FIG. 247.—Hürthle's spring manometer.

FIG. 248.—Sphygmoscope.

Hürthle's spring manometer consists of a small tambour, 5·5 in diameter, covered with rubber membrane. A button attached to the membrane works against a steel spring. The movement of the spring is magnified by a light lever. Inertia is proportional to the mass and the square of the velocity. By making the tambour very small and the lever very light the errors due to the inertia of the fluid and lever are reduced to a minimum.

The sphygmoscope is an equally good instrument. One end of a rubber finger-stall is drawn over the end of a rubber cork. The cork is inserted into a short piece of wide tube. A glass tube passes through

this cork into the small air-space which is left at the top of the finger-stall. The other end of the wide tube is closed by a rubber cork. A glass tube passes through this cork and is connected with a recording tambour. The finger-stall acts as the spring.

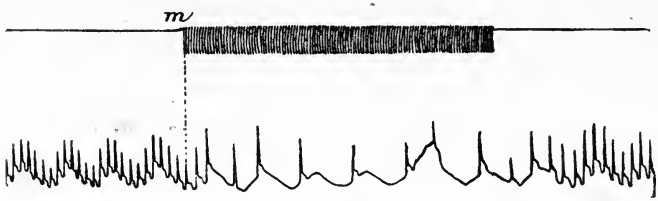


Fig. 249.—Arterial pressure recorded by a spring manometer. Effect of weak excitation of the vagus during the period marked by the signal *m*. (Dubois.)

By means of Hürthle's differential manometer the relations of pressure in any two cavities of the heart may be determined, and the exact moments at which the valves shut and open found. In this instrument

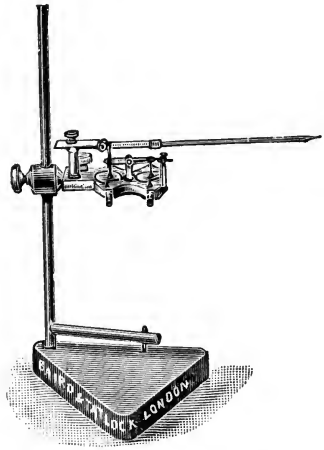


Fig. 250.—Hürthle's differential manometer.

two cannulae are brought into connection with tambours placed on either side of the fulcrum of a lever. The lever works against a spring, which in its turn sets a writing lever in motion. When the pressures are equal the writing point is at zero. It rises or falls according as the pressure in one cavity rises above or falls below that of the other.

Connect by side tubes the mercurial manometer and the Hürthle manometer with the artery in the artificial schema. Take records with each instrument on a moderately fast drum, and compare the results. Connect by side tubes one side of the Hürthle differential manometer

with the chamber of the pump, and the other side with the artery close to the valve. Take a record, and observe how the instrument records the moment when the valve opens and shuts. If a time tracing be

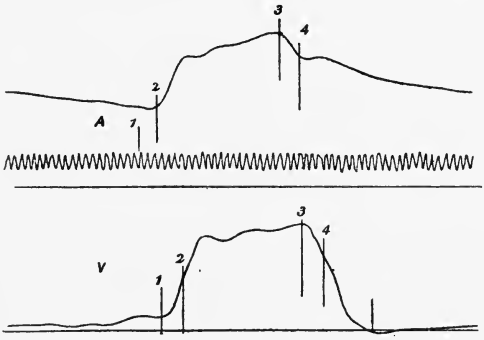


FIG. 251.—Aortic and ventricular pressure curves taken by Hürthle manometers. (Hürthle.)

taken, the time relations of the pump (ventricular contraction) can be exactly determined. The period of ventricular systole is divided into three: (1) the period of rising tension, when all the valves are closed;

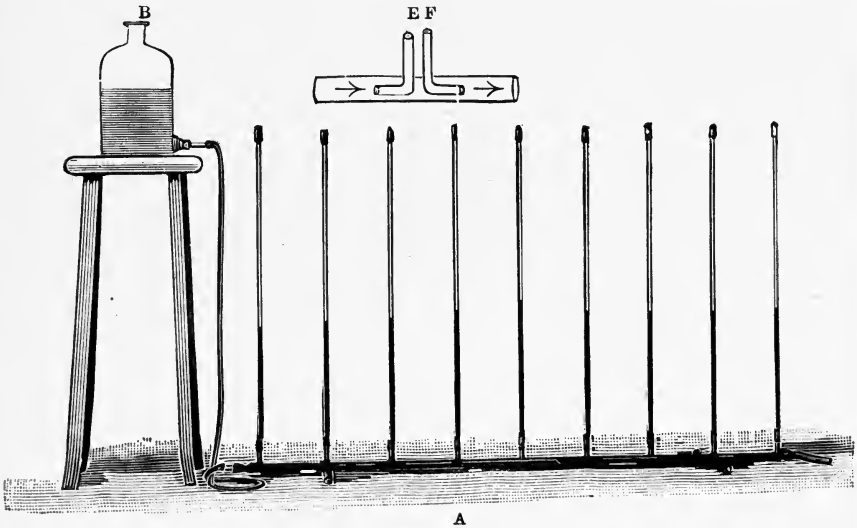


FIG. 252.—Schema to show the velocity and resistance heads. B, Pressure bottle. A, Tube with piezometers. E F, Pitot tubes.

(2) the period of output; (3) the period of relaxation. In simultaneous records of intra-ventricular pressure and aortic pressure the beginning of the aortic rise (2) marks the opening of the aortic valve and beginning of output. The end of output occurs when the semilunar

valves close at the beginning of the dicrotic notch (4). The period of rising tension lasts from the beginning of systole (1) to the opening of the semilunar valves (2).

Velocity of Blood Flow.—Insert the Pitot tubes E and F into a tube A through which water is flowing from a constant head of pressure B (Fig. 253). Note that the water rises to different levels in the tubes. E records the resistance head plus the velocity head. F records the resistance head minus the velocity head. Measure the outflow per minute from the tube A, and note the difference between the heights of the menisci in E and F. Lessen the velocity by partly screwing up the clip on the end A. Measure the outflow per minute, and note that the menisci are nearer together. Close the end of A. The flow ceases, and the menisci in the two tubes reach the same level as that of the head of pressure B. Cybulski makes use of this principle in the construction of the photo-haematochometer, an instrument by which alterations in velocity can be recorded Fig. 253.

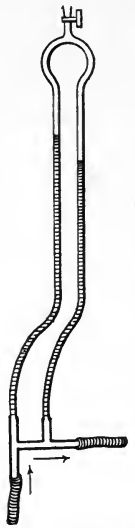


FIG. 253.—Cybulski's photo-haematochometer. A cannula shaped as shown is introduced into the blood-vessel. The oscillations of the mercury-menisci are photographed.

The velocity can also be measured in the artificial schema by injecting 1 c.c. of methylene blue sat. sol. into artery, and noting by means of a stop-watch (or electric signal and drum) the moment of injection, and the moment when the blue fluid reaches the capillary tube.

The Circulation Time.—In the artificial schema measure the circulation time by injecting methylene blue into the vein V, and noting how long the blue takes to reach the venous end of the capillary tube.

CHAPTER XL.

THE WORK OF THE HEART. THE PERICARDIUM.

The Work of the Heart.—To estimate the work of the heart in the artificial schema the mean pressure H, and velocity in the aorta V, and the volume of the systolic output Q, must be obtained.

$$W = QH + \frac{MV_2}{2g}.$$

M = the mass of the output in grammes = Q multiplied by the specific gravity of the blood.

Close the clip on the arteriole tube and start the pump. Note the mean pressure H indicated by the manometer M.

To obtain V inject into the artery, at 1 metre from the capillary tube, 1 c.c. of sat. sol. methylene blue. A side tube is provided for the purpose of making this injection. Note with a stop-watch, or by an electric signal and drum, the time between the injection and the appearance of the blue at the beginning of the capillary tube.

Having obtained V, the output can be reckoned if the sectional area (a) of the aorta be obtained and the time (t) of a cardiac cycle. Measure the diameter of the artery. Half this and obtain the radius.

$$a = \pi r^2.\ *$$

Count the number of pulses per minute, and by dividing the number found by 60 obtain t. Then $Q = avt$.

Now calculate the work of the pump from the data obtained. The work spent in maintaining velocity is almost negligible in comparison with that spent in overcoming resistance.

In man the output may be taken as 110 grms., the average aortic pressure as 120 mm. Hg, the velocity of flow in the aorta as 320 mm. per sec. Mercury 13·5 times heavier than blood.

$$\therefore\ W = 110 \times 120 \times 13{\cdot}5 + \frac{110 \times 320}{2 \times 981}.$$

The right heart is considered as doing one-third of the work of the left heart.

The total work of the human heart is estimated to be about 24,000 kilogramme-metres per day, or 1000 kg.m. per hour. This equals 56·6 kilo-calories (425 kg.m. = 1 kilo-calorie).

In the dog the output can be obtained by estimating the amount of oxygen taken up by the blood from the inspired air in one minute. This can be obtained by Fredericqs' or Zuntz's method (see p. 147). At the same time samples of arterial and venous blood are obtained, and the oxygen difference between the two samples estimated by the blood pump or Haldane's ferricyanide method (see p. 398). The number of heart beats per minute is also counted. Suppose 100 c.c. of oxygen are taken up per minute, the arterial blood contains 5 c.c. per cent. more oxygen than the venous blood, and the heart beats 80 times per minute. Then, as every 100 c.c. of blood carries away 5 c.c. O_2, 2000

* $\pi = \frac{22}{7}$

c.c. of blood must have passed through the heart in the minute. Thus the output

$$= \frac{2000}{80} = 25 \text{ c.c.}$$

The output in mammals is reckoned to be about ·0012 of the body weight per sec.

Stewart's Method of Determining Output of Heart.—In the anaesthetised dog, a cannula is introduced through the jugular vein into the superior vena cava, and connected with a burette containing 1·5 per cent. NaCl. A short cannula provided with a rubber tube and clip is introduced into one femoral artery A. The other femoral, B, is exposed, placed on electrodes with sheet indiarubber beneath. The electrodes are connected with a Wheatstone bridge arrangement, through which weak induction shocks are passed from coil. A telephone is introduced in the bridge and the latter is balanced.

A sample of blood is drawn from A and defibrinated. A measured quantity of the salt solution is then run into the vena cava superior for 10″, the time being noted by a stop-watch; the pulse is counted at the same time. As soon as the salt solution reaches B the resistance alters and the telephone sounds.

The time at which this occurs after the injection gives the jugular to femoral circulation time. While the telephone is sounding a sample is drawn from A. The specific resistance of the two samples is determined by the telephone Wheatsone bridge method (see Lehfeldt's *Physical Chemistry*, p. 62). The samples are placed in small U-tubes immersed in running water, and provided with platinum electrodes. The quantity of 1·5 per cent. salt solution is measured, which must be added to the first sample to make its resistance equal to the second sample. This determination gives us the means of estimating the extent to which the injected solution has been mixed with blood in the heart, and therefore, knowing the quantity of solution run in, we can calculate the output in the given time. Lastly, from the pulse rate, we can calculate the output per second.

Stewart finds the output to be ·0015 to ·003 of body weight per sec.

The Influence of the Pericardium in Preventing Over-distension of the Heart.—A cannula is placed in the superior vena cava of a dead cat, and the inferior vena cava and pulmonary artery are ligatured.

Fluid is run into the superior vena cava from a burette from a height of about 100 mm. The amount of fluid which enters the heart is estimated. After removing the pericardium, twice as much fluid will enter the right heart under the same pressure, and the heart will

become over-distended (Barnard). The pericardium prevents over-distension of the right heart when the abdomen is compressed, or when the body is in the vertical head-down position.

CHAPTER XLI.

PLETHYSMOGRAPHS.

Plethysmographs and Oncographs.—The circulation through the various organs is investigated by placing them in an air-tight box. The box is connected with a piston recorder or tambour. The shape of the box, which may be moulded of gutta-percha, is made to fit the organ investigated. In the case of the spleen, for example, a shallow oblong box is made, with a depression at one side to permit the passage of the splenic vessels. The spleen is placed within it and the depression is packed with cotton-wool and vaseline. A vaselined glass lid is then fastened on by rubber bands. A side tube leads from the box to the recording tambour. Similar boxes are made for the left lobe of the liver, a lobe of the lung, a loop of intestine, etc.

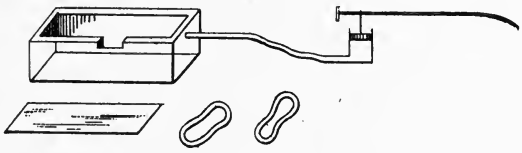


FIG. 254.—Diagram of an oncometer and piston recorder. The rubber bands fasten the glass lid in position.

Oncometery of the Kidney.—A cannula is placed in the external jugular vein of an animal anaesthetised with chloroform and ether. Tracheotomy is performed and the animal curarised. Artificial respiration is established and the air is blown through the anaesthetic bottle. The carotid artery is exposed and connected with a mercurial manometer. The kidney is exposed by a lumbar incision without opening the peritoneum if possible. The kidney is separated from the perinephritic fat and placed in the oncometer. The oncometer is then connected with the oncograph.

To investigate the flow of urine a fine catheter is tied into the ureter, or else a tube is tied into the bladder and the neck of the bladder closed by a ligature. The flow of urine can be measured in a measure glass or recorded by allowing the drops to fall from the collecting tube on to an aluminium disc attached to a lever. The

lever can be made to break a circuit in which an electric signal is set. The signal writes on the kymograph. Thus the arterial pressure, the renal volume, and the secretion of urine are simultaneously recorded.

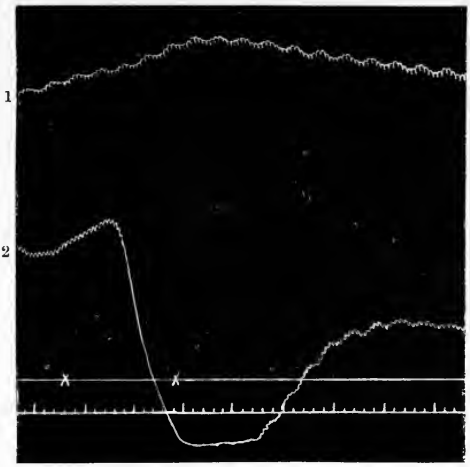


FIG. 255.—Arterial pressure (1) and oncometer tracing (2) of kidney volume. Between the points starred the 10th dorsal root was excited. The time is marked in seconds. (Bradford.)

So long as the venous pressure is constant any increase in renal volume will denote increased blood-pressure in and increased blood-flow through the kidney. The secretion of urine varies as the volume of blood passing through the kidney per minute. (By dividing the renal nerves and exciting the spinal cord or vaso-motor centre the greatest rate of blood-flow through the kidney can be produced.) Ligature of the renal vein stops the secretion of urine. After a temporary obstruction albuminous urine is secreted. Half a grain of citrate of caffeine injected intravenously will produce a fall of arterial pressure and a preliminary contraction of the kidney, followed by great expansion and increased flow of urine.

Plethysmography of the Arm.—The arm is placed in the glass plethysmograph, which is made to fit closely to the upper part of the forearm. The junction is made air-tight by means of a rubber collar or bandage and vaseline. The plethysmograph is connected with a recording tambour, a ⊥-piece being interposed. Record the volume curve on a moderately fast drum. The tracing shows pulse waves and respiratory oscillations.

Note the effect of a deep inspiratory and a forced expiratory effort. The effects are produced by the emptying and congestion of the veins

respectively. If the plethysmograph be connected with the supply tube to a small gas jet the pulse is communicated to the flame and may be photographed on a moving sensitised plate. The plate is run on a traveller behind the slit in the dark room. The curves thus recorded are similar to the velocity curves obtained with Chauveau's haemodromograph, for the change of volume in the limb, if the venous outflow be constant, follows the velocity of the arterial inflow.

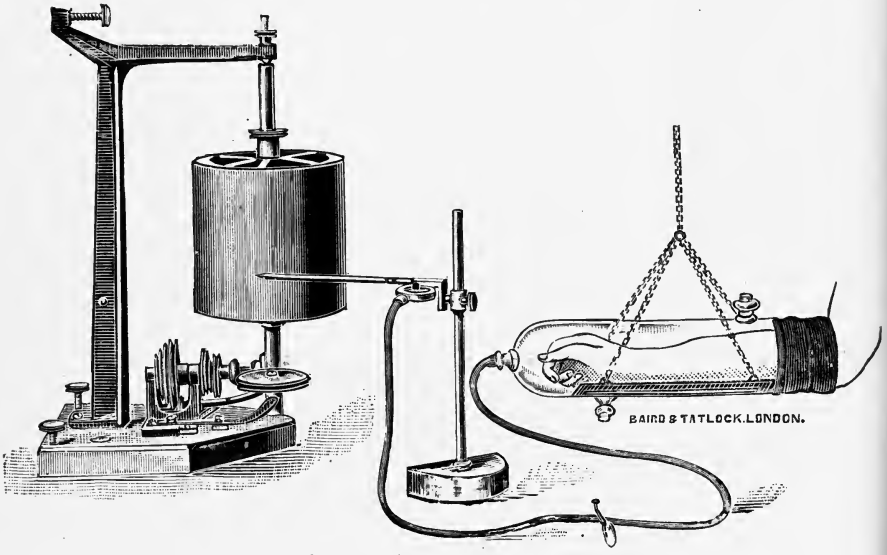


Fig. 256.—Limb plethysmograph.

A Method of Exposing the Spinal Roots for the purpose of Excitation as in investigations of Vaso- Viscero- and Pilo-motor Nerves.—An incision three inches long is made over the spine. The muscles (erector spinae) are rapidly divided on either side of the spinous processes and pulled aside. The haemorrhage is then arrested by pressure with wool pads or artery forceps. The transverse processes and ribs are thus clearly exposed. The spinous processes and neural arches are next removed with the bone forceps and the spinal cord enclosed in the dura mater exposed. Pads of cotton-wool are now packed into the wound and left until all haemorrhage is arrested. The dura mater is then opened and the posterior roots are gently lifted up, ligatured in two places, and divided between the ligature. The ligatured ends are then ready for excitation. The anterior roots are ligatured and divided close to the cord. After division of the posterior root, the entire nerve may be excited outside the dura mater in order to demonstrate the efferent effects. The presence of efferent vaso-

dilator fibres to the limbs have been recently demonstrated by Bayliss in the posterior roots. Excitation, mechanical or electrical, of the 5th-7th lumbar and 1st sacral posterior roots causes prolonged vascular dilatation of the hind limbs after a latent period of 2-8 sec.

CHAPTER XLII.

THE CHEMISTRY OF RESPIRATION.

Experiments to Demonstrate that the Blood Gases are Chemically Combined in the Blood.—Ten c.c. of defibrinated ox blood are placed in a stout flask fitted with a rubber cork. A tube passing through the cork is connected with a Geryk air-pump. The flask is immersed in broken ice. On making a vacuum there occurs no perceptible evolution of gas until the flask is removed to a bath of warm water. The blood then suddenly bubbles and froths and the gases come off. Note the change of colour in the blood when this happens. If the thin film on the side of the flask be examined with the spectroscope, it will give the band of reduced haemoglobin. Further proofs of the chemical combination of O_2 are these: O_2 can be displaced by an equal volume of CO; O_2 in laked blood is displaced by ferricyanide of potassium with the reduction of the latter to ferrocyanide, and the formation of methaemoglobin.

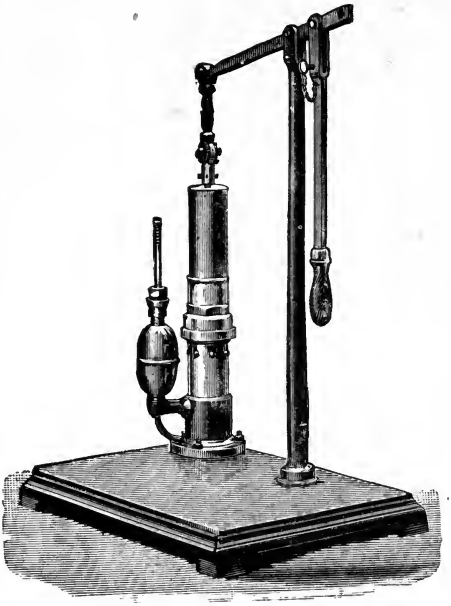


FIG. 257.—Geryk air-pump. The piston is covered with oil and opens a spring valve during its ascent.

The co-efficient of absorption of blood for oxygen is a little less than water, *i.e.* 1 vol. absorbs ·02616 at 30° C. when exposed to an atmosphere of oxygen. Less than ½ vol. % O_2 can therefore be simply absorbed by the blood at body temperature. The amount of oxygen in serum is only 0·26 per cent.; 1 grm. of pure crystalline haemoglobin absorbs 1·25 c.c. O_2 (Haldane). There is about 14 per cent. of haemoglobin in

the blood: $1{\cdot}25 \times 14 = 18{\cdot}5$ O_2 per cent. This agrees with the volume of O_2 that can be extracted with the gas-pump. The co-efficient of absorption of blood, neutralised by tartaric acid, and exposed at body temperature to an atmosphere of CO_2, is about 1. The tension of CO_2 in the tissues is only about 5 per cent. of an atmosphere. One vol. of

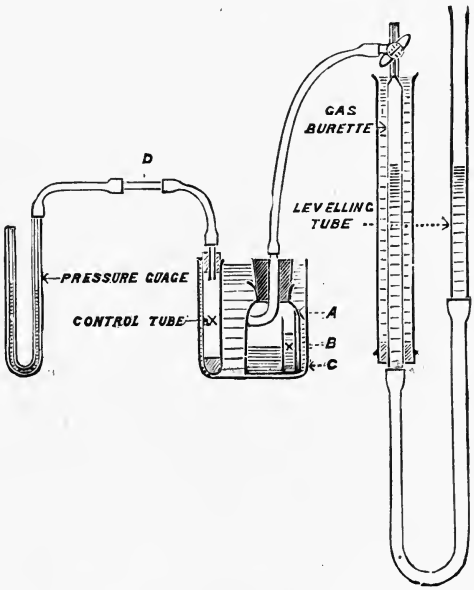


FIG. 258.—Ferricyanide method of estimating the amount of O_2 in blood. 20 c.c. of blood saturated with O_2+30 c.c. of 1/500 ammonia solution are placed in the bottle *C*. 4 c.c. of sat. sol. potassium ferricyanide in the tube *A*. After mixing the gauge is brought back to its previous level by adding cold water to *A*, and then the volume of O_2 is read in the burette.

blood could at this tension take up by simple absortion only $\frac{1}{20}$ of the above, *i.e.* 5 vols. per cent. The CO_2 must therefore be chemically combined. On breathing an atmosphere of CO_2 the blood may take up 150 vols. per cent. owing to the high co-efficient of absorption (100 vols. absorbed and 50 vols. combined).

From blood the whole of the CO_2 is set free in the gas pump, but in the case of serum part of the CO_2 is fixed and is only set free after the addition of an acid. The red corpuscles apparently play the part of an acid. About one-third of the CO_2 is carried by the corpuscles, the rest by the serum. Serum contains a larger quantity of CO_2 than an equal quantity of blood. According to Bunge 1000 grms. of dogs' serum contains 4·341 grms. sodium, of which 3·463 is sufficient to saturate the chloride. The remainder, 0·878 grms., can combine with ·623 grms. CO_2 to form sodium carbonate, and in addition with an equal

quantity to form sodium bicarbonate. 1·246 grms. $CO_2 = 632$ c.c. at 0°, and 760 mm. = 63 vols. per cent. The bases of the blood are shared among the acids—proteids, chlorine, phosphoric acid, and carbonic acid, according to the influence of the mass. No acid in solution is combined with the bases present in that solution to the complete exclusion of other acids also present in the solution. The acids share the bases according to their avidity. The introduction of any salt, acid, or base into the solution upsets the condition of equilibrium, and a new distribution of the acids and bases occurs. In the tissues the mass influence of CO_2 is considerable, while in the lungs it is slight. The kind of change which may occur when the blood passes through the tissues is indicated by the following equation: $Na_2HPO_4 + CO_2H_2O = NaHCO_3 + NaH_2PO_4$. The intra-venous injection of acid in rabbits rapidly lessens the carrying power of the blood for CO_2. In carnivora the injected acid is to a considerable extent neutralised by the salts of ammonia—the precursors of urea.

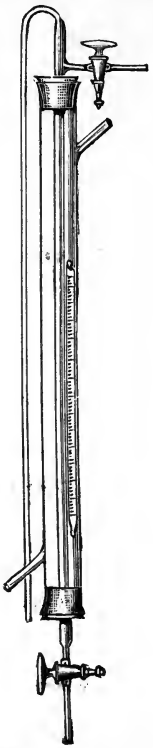


Fig. 259.—Fredericq's Aerotonometer.

Fredericq's Aerotonometer.—Demonstration of Method. The wide tube is filled with a known mixture of gases, say N84 and $O_2$16. It is surrounded with a water jacket through which water at body temperature is circulated. The narrow tube is connected with a cannula in the carotid artery, and the wide tube with the jugular vein. The blood is allowed to flow up the narrow tube and down the wide tube, where it is exposed in a thin film to the atmosphere. The blood is prevented from clotting by a previous injection of leach extract. After the blood has circulated a sufficient time to allow equilibrium between the tension of the gases in the blood and in the wide tube, the circulation is stopped, and the contents of the wide tube analysed. If 14 vols. per cent. of O_2 are now found in place of 16, the tension of O_2 in the blood is taken as 14 per cent. of an atmosphere. The alveolar air is collected by means of a byway cannula introduced into a bronchial tube (Pflüger's lung catheter). One of the tubes terminates in a rubber bag, which is expanded so as to block the bronchial tube. Through the other tube the air in the lobe is withdrawn for analysis. The analysis may be carried out by Haldane's gas analysis apparatus. Fredericq

determined the tensions of CO_2 and O_2 to be as follows in per cent. atmosphere:

	Tissues.		Venous blood.		Alveolar air.		External air.
CO_2	5-9	>	3·81-5·4	>	2·8	>	0·03

	Arterial blood.						
O_2	0	<	14	<	18	<	20·94

Bohr, on the other hand, with another form of aerotonometer, found that the tension of O_2 in the blood is higher than in the bronchial air, and that the tension of CO_2 may be higher in the bronchial air than in the blood. Haldane confirms Bohr as regards O_2.

The exchange of gases, if these observations are correct, must be due to secretory activity of the lungs or more probably of the blood. In the swim-bladder of the fishes taken from great depths (4000 feet) gas has been found containing about 90 per cent. of oxygen. The oxygen in the bladder at this depth must be at a pressure of over 100 atmospheres, while in the sea water the partial pressure of O_2 is only about 0·2 atmosphere. The swim-bladder and the pulmonary epithelium are both developed from the alimentary canal.

CHAPTER XLIII.

THE EFFECTS OF CHANGES IN ATMOSPHERIC PRESSURE.

Decreased Atmospheric Pressure.—A mouse and a frog are placed under the bell glass of the air pump. A side tube is connected with a mercury manometer. The latter must be long enough to indicate the pressure of the atmosphere. On lowering the pressure $\frac{1}{2}$-$\frac{1}{3}$ of the atmospheric pressure the mouse is asphyxiated, while the frog is unaffected. The effect of lessening the atmospheric pressure depends entirely on the partial pressure of oxygen. The normal pressure of O_2 is 20·94 per cent. of an atmosphere. At 10 per cent. of an atmosphere there arises restlessness and dyspnœa, and at about 7 per cent., death. A partial pressure of $O_2 = 7$ per cent. of an atmosphere corresponds to an altitude of 30,000 feet. Death from want of oxygen is common in foul wells, mines, etc., where 'choke-damp' collects.

Increased Atmospheric Pressure.—A curarised frog, with the brain pithed, is placed in the high-pressure chamber, the web of one foot is spread out on a wire ring beneath one of the glass observation discs. The apparatus is screwed up and connected with an oxygen cylinder. The circulation in the web is observed with a microscope using an inch

objective. The pressure is increased to 20-50 atmospheres. The circulation continues unaffected, for the pressure is equally transmitted throughout the fluids of the body. After ten minutes the chamber is decompressed. Emboli, formed of gas bubbles, soon appear in the capillaries, and the circulation ceases. Such gas emboli are the cause of the symptoms (paralysis, etc.) observed in caisson workers and divers.

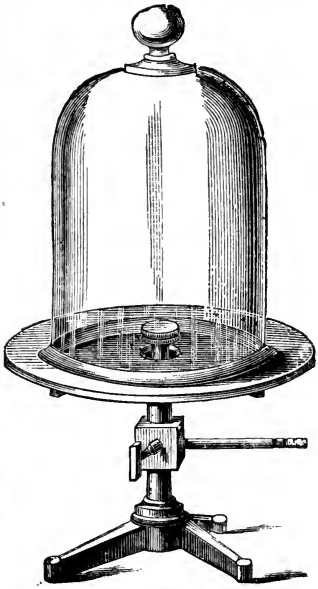


FIG. 260.—Receiver used with Geryk pump in demonstrating the influence of lowering the atmospheric pressure on animals. The apparatus may also be used as a drying chamber.

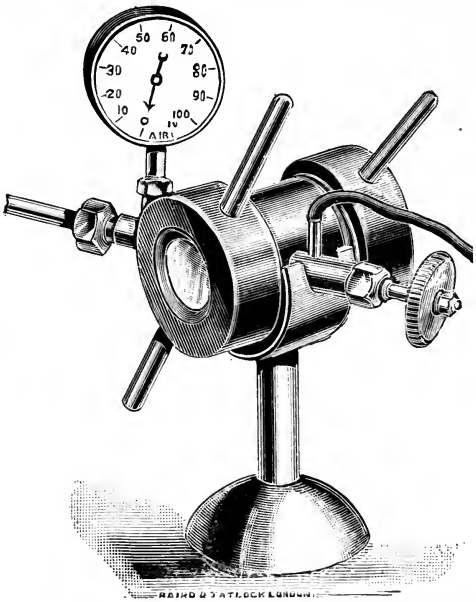


FIG. 261.—Hill's apparatus for studying effects of increased atmospheric pressure. Thick glass discs, provided with leather washers, close the ends of the chamber.

The workers are affected on or after decompression. Four atmospheres is the limit of safety. Every 10 metres in depth of water roughly equals one atmosphere. Re-compression and slow decompression is the rational cure for the symptoms when they appear. Compressed oxygen is also *per se* a poison. It lowers metabolism, diminishing the output of CO_2 and the body temperature. This can be observed in mice placed in a high-pressure chamber. The oxygen is allowed to leak from the chamber through the Haldane-Pembrey absorption tubes. A mouse is affected with dyspnœa in 10 atmospheres of O_2, and soon dies. In 50 atmospheres it is instantly killed.

CHAPTER XLIV.

RESPIRATION IN THE TISSUES.

Cell-respiration.—The processes of oxidation go on in the tissue cells, not in the blood which bathes them. To prove this, Pflüger devised the following experiment:

A large frog is taken and decerebrated. The anterior abdominal vein is exposed, and a cannula introduced into either end. Ringer's solution is injected towards the heart and the blood washed out and the vascular system filled with the solution. The vein is then ligatured. The animal can be put in a measure glass containing O_2 over mercury. After twenty-four hours the animal is withdrawn and the CO_2 absorbed by potash and measured. The animal under these conditions continues for twenty-four hours to put out a normal amount of CO_2.

If a saline solution, saturated with Ehrlich's methylene blue, be injected intravenously or subcutaneously into a living animal the blue is deoxygenated and rendered colourless. After the death of the animal the dying tissues become blue on exposure to air.

Other evidences of cell-respiration are these: (1) Plant-cells, egg-cells, and the lower animals, which have no blood, die in the absence of oxygen. In insects the finest branches of the tracheae run to the single cells of the tissues; (2) In the glow organ of Lampyris Splendidula there are certain cells grouped on the ends of the tracheae, which are stained black with osmic acid. These cells contain, therefore, a substance with a strong affinity for oxygen. The glow organ can be sliced and the slices examined microscopically. The points of light seem to begin in the cells at the ends of the tracheae, and the light goes out when oxygen is withdrawn (Max Schultze); (3) The saliva contains 0·4 per cent. O_2. The presence of O_2 in saliva can be demonstrated by adding it to a solution of reduced haemoglobin, and observing the appearance of the oxyhaemoglobin bands; (4) The reducing substances found in the blood of an asphyxiated animal are in the blood-cells. They do not occur in the plasma or lymph. Owing to the reducing substances in the blood-cells oxygen disappears from shed blood, and the inside of a blood clot becomes black in colour.

CHAPTER XLV.

DETERMINATION OF THE GASES OF THE BLOOD.

A Method of Determining the O_2 and CO_2 in Small Quantities of Blood (Barcroft and Haldane).—The apparatus consists of a small glass vessel attached by pressure tubing of small bore to a pressure gauge also of small bore. The vessel is so arranged that the O_2 of the

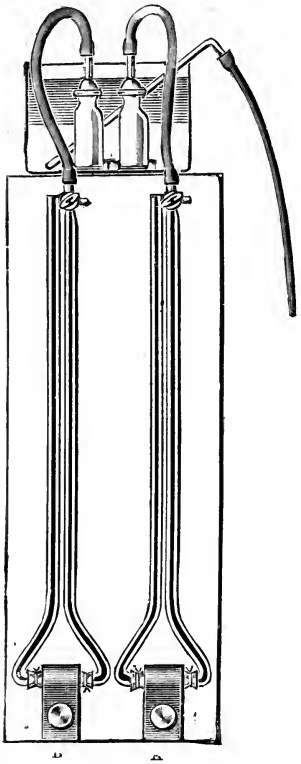


FIG. 262A.—Barcroft-Haldane blood-gas apparatus.

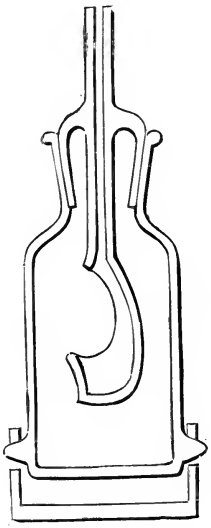


FIG. 262B.—Blood-gas vessel.

blood sample can be liberated within it by ferricyanide, and the increase of pressure measured by means of a gauge. From the increase of pressure the vol. of O_2 can be calculated. By similar manipulations with the use of tartaric acid the CO_2 is subsequently liberated and measured. The apparatus is shown in Fig. 262A. One gauge A is connected with the blood-gas vessel, and the other B with a precisely

similar control vessel. The gauges are graduated in both limbs in millimetres to a height of about 300 mm.

The limbs of each gauge are connected by wide rubber tubing which can be compressed by a screw clamp so as to adjust the levels. The gauges are filled with water, tinged with methylene blue. One limb of each gauge is provided with a three-way tap close to the top. The blood-gas vessel and the control vessel are of similar form, and each has a capacity of about 20 c.c. The glass stopper of each is perforated by a glass tube of narrow bore which widens below into a pocket capable of holding ·3 c.c. The pocket is so arranged that any liquid contained in it can easily be emptied by tilting the vessel.

The two vessels are connected with the top ends of the two gauges by equal lengths of pressure tubing of narrow bore. This tubing is made as short as possible. The control vessel has a few drops of water in it.

Before the sample of blood is collected, 1·5 c.c. of ammonia solution (ammonia sp. gr. 0·88, 5 c.c. in 1000 c.c. water) is measured with a pipette into the blood-gas vessel, and ·25 c.c. of saturated solution of potassium ferricyanide is placed in the glass pocket.

The sample of blood 1 c.c. is collected in a hypodermic syringe from the blood vessel; ·10 c.c. of ammonium oxalate is first drawn into the syringe. In the syringe there is a glass bead, by means of which the oxalate solution and blood can be mixed. The blood is then discharged into the blood-gas vessel beneath the ammonia solution which prevents all contact with air. The blood-gas vessel is then closed and placed in the water bath beside the control vessel. The water is stirred by blowing through it, and the gauges are watched until the pressure, *i.e.* the temperature, in each vessel becomes the same. The gauges are now adjusted at zero by opening the taps for a moment. The blood-gas vessel is then taken out, and the ammonia solution and blood mixed. When the blood is quite laked, and the solution transparent, the vessel is tilted so as to empty out the ferricyanide, and then shaken to liberate all the oxygen. During these manipulations the blood-gas vessel is held in a cloth to prevent warming. It is now replaced in the water bath, and the water is stirred. When the temperature has again become even, the gauges are adjusted so that the levels in the limbs connected with the bottles are at zero. The heights on the other limbs are read off, and if, as is usual, the temperature has risen, so that the level is higher than before in the open limb of the control vessel gauge, the reading of this gauge is deducted from the reading of the other gauge. The temperature of the water bath is now read. The normal barometric pressure equals 10,300 mm. H_2O.

The volume of gas given off equals the volume of air in the blood-gas tube and connections multiplied by the corrected reading of the gauge in millimetres and divided by 10,300.

For example, supposing the reading of the gauge = 100; the total capacity of the blood-gas vessel and connections to the zero of the gauge = 23·35 c.c.; and the capacity minus the volume of liquid within it = 23·35 − 2·75 c.c. = 20·6 c.c.; then the volume of oxygen given off $= 20{\cdot}6 \times \frac{100}{10{,}300} = {\cdot}20$ c.c. If the temperature were 14°, then the volume of gas at standard pressure (760) and temperature (0°) would be $\cdot 20 \times \frac{273}{287} = \cdot 19$ c.c. = 19 c.c. per 100 vols. blood.

The vapour tension of the ammonia used gives an error of about 3 mm. This can be determined by a blank experiment omitting the ferricyanide. The capacity of the blood vessel is ascertained by weighing it empty and full of water. The capacity of the connecting tubing is determined by adjusting the gauge to zero, and then raising the pressure to a certain definite amount, first with the vessel connected, and afterwards with a stopper inserted into the end of the rubber tube in place of the glass tube of the vessel. The capacity of the vessel and connecting tube will be to that of the connecting tube as the first reduction in volume in the right-hand limb of the gauge is to the second.

The stopper of the blood-gas vessel is now removed, and ·25 c.c. of 20 per cent. tartaric acid solution placed in the pocket, the stopper replaced and the gauge adjusted to zero in the same way as before. The acid is then spilt, and the bottle shaken till all the CO_2 is set free. The gauge is again read off and the volume of CO_2 calculated as in the case of oxygen.

As the co-efficient of absorption of the blood mixture for CO_2 is found to be 1 at the temperature of observation, the volume of gas must in this case be calculated from the capacity of the blood-gas vessel without subtracting the volume of the contained liquid.

A slight correction is necessary for the CO_2 in the liquids used. This may be determined by doing a blank experiment, mixing boiled distilled water with the ferricyanide and ammonia solution.

Example of results obtained by this method:

Defibrinated Blood.—Three Samples.

O_2.	CO_2.
18·5	52·4
18·5	52·7
18·8	51·2

CHAPTER XLVI.

RESIDUAL AIR. CARBON MONOXIDE POISONING. TENSION OF OXYGEN IN BLOOD.

Measurement of the Residual Air.—The spirometer is filled with hydrogen generated in a Kipp's apparatus. Make the deepest possible expiration, and then put the mouthpiece in position, open the clip on the tube which connects the mouthpiece with the spirometer, and breathe in and out of the spirometer two or three times, so that the gas in the lungs and in the spirometer reaches the same uniform composition. Clip the tube, and drive a sample of the gas in the spirometer over into the Haldane gas analysis apparatus. Measure the volume of the sample and the amount of O_2 and CO_2 it contains. Suppose the spirometer originally contained 4000 c.c. H, and after the experiment it was found to contain 1000 c.c. of the lung-gases, *i.e.* O_2 and CO_2, then in the spirometer there must be 3000 c.c. H and 1000 c.c. O_2 and CO_2, and in the lungs there must be 1000 c.c. H and the same proportion of O_2 and CO_2, viz. 250 c.c. The residual air is therefore 1250 c.c.

Carbon Monoxide Poisoning.—Blood retains in simple solution (0·5 per cent. volumes O_2 when exposed to air), 2·6 per cent. when exposed to an atmosphere of pure oxygen, 5·2 per cent. when exposed to two atmospheres of pure oxygen. 6-8 per cent. volumes of O_2 are used up in the circulation of the blood. An animal exposed to two atmospheres of O_2 has nearly this amount dissolved in the plasma. A mouse just poisoned with coal gas which contains CO recovers on being placed in two atmospheres of O_2 in the pressure apparatus (Fig. 261). CO poisoning is caused by want of oxygen. The CO combines with the haemoglobin. Otherwise it is, physiologically, an indifferent gas.

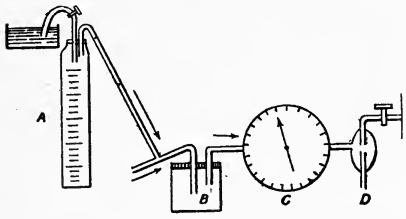


FIG. 263.—Haldane's method for the determination of the tension of oxygen in the blood. A, Measure bottle containing CO. Water drops into this and drives at a measured rate a stream of CO into the air current which passes through the mouse-chamber B; C, metre; D, filter pump aspirator.

Determination of the Tension of Oxygen in the Blood (Haldane's Method).—The maximum amount of CO capable of being absorbed by the blood from air containing a given small percentage of CO depends upon the relative affinities of oxygen and CO for Hb, and the *relative tension* of the two gases in the arterial blood.

A mouse is placed in a bottle through which a current of air

containing a known percentage of carbonic oxide (about ·06 per cent.) is aspirated at a rate of about ·5 to 1 litre per minute. The animal is allowed to breathe the mixture of gases till the haemoglobin of its blood is saturated with CO to the maximum for the percentage of gas present. The bottle is then disconnected and rapidly plunged under water, so that the animal is drowned.

Normal blood when sufficiently diluted gives a yellow, and carbonic oxide blood a pink colour. If blood be diluted 100 times, and a portion of it be saturated with CO, it requires somewhat more than an equal volume of standard carmine solution [1] to bring the unsaturated portion to the same tint and intensity of colour as the saturated portion. The exact relation of the carmine solution to the blood solution is determined by trial.

Narrow test tubes, A, B, C, similar to Gower's haemoglobinometer tubes are employed. 2 c.c. of water is measured from a narrow burette into A. ·02 c.c. of blood is obtained by opening the heart of the mouse immediately after its death. The blood is measured in the pipette of Gower's haemoglobinometer and mixed with water in A. A similarly diluted solution of normal mouse's blood is well shaken with coal gas in a test tube. This is then placed in B, which is filled full and corked.

It is then determined how much carmine must be added to a third sample C of normal diluted blood (1) to make it equal in tint to A, (2) to B. The carmine is added from a narrow burette ·1 c.c. at a time. Suppose ·5 c.c. carmine must be added to C to make its tint equal A, and 2·2 c.c. to produce equality of tint with B, then A is $\frac{\cdot 5}{2\cdot 5} \times \frac{4\cdot 2}{2\cdot 2} \times 100 = 38\%$ saturated.

The calculation of the oxygen tension is made by finding the percentage of CO in air to which the actually observed saturation of the blood corresponds,[2] dividing 20·9 by this, and multiplying by the actual percentage of CO in the air breathed.

[1] *Stock Solution.*—One grm. of pure carmine is mixed in a mortar with a few drops of ammonia, and dissolved in 100 c.c. of glycerin. *Standard Solution.*—5 c.c. of the stock solution is added to 500 c.c. of water. This must be prepared fresh. The amount required to render normal diluted blood equal to the sample of diluted blood saturated with CO must be found at each determination, for the carmine tint varies slightly with daylight.

[2] See the curve of dissociation of COHb in air which has been constructed by Haldane and L. Smith, *Journal of Physiology*, xxii., p. 233. The following are some of the data from which the curve was constructed:

Ox-blood 1 % solution	% CO.	Temp.	% Saturation of Hb with CO.
was shaken with	·195	37° C.	74·6
air containing	·125		64·3
	·086		54·8
	·069		48·5
	·037		36·4

For example, suppose the blood was found to be 46% saturated (corresponding to ·06% CO in air), and the actual percentage of CO breathed was ·08, then the oxygen tension of the blood would be $20{\cdot}9 \times \frac{{\cdot}8}{{\cdot}6} = 27{\cdot}9$. As the alveolar air contains about 6% of aqueous vapour this result has to be reduced to 26·2.

In man the determination can be made by substituting a mouthpiece and inspiratory and expiratory valves (Fig. 264) in place of the bottle B.

The finger is pricked and a sample of blood obtained, from which the CO determination is made. Haldane finds the oxygen tension in human blood to be higher than in the atmosphere. Lowering the body temperature or pneumonia abolishes the O_2 absorptive power of the lungs, while diminishing the amount of O_2 in the air breathed excites it.

CHAPTER XLVII.

OXYGEN CAPACITY AND MASS OF THE BLOOD.

Total Oxygen Capacity and Mass of the Blood in Man (Haldane's Method).—A known volume of CO* is administered until completely absorbed. Then, by the carmine method, the percentage to which the Hb has become saturated with CO is determined. We thus estimate the volume of CO (or O_2) capable of being taken up by the whole of the blood. Also, we determine at the same time the volume of CO (or O_2) capable of being taken up by 100 grms. of blood. We can then, knowing the total volume taken up, calculate the total mass of blood in the body.

The subject breathes through a mouthpiece into a rubber bag 1-2 litres in capacity. Between the bag and mouthpiece there is interposed a cylinder made of tinned iron, and filled with soda lime to absorb the expired CO_2. The cylinder is made of two tins which slide over one another, the junction being made air-tight with wax. The soda lime is kept in position by a piece of wire gauze at either end of the cylinder. Oxygen is supplied to the bag from an oxygen bottle. The gas is bubbled through a test tube containing a little water to roughly gauge the rate of supply.

A measure bottle filled with CO is arranged as in Fig. 264 and connected with the bag. The temperature and barometer are noted.

*To ensure correctness the CO in the cylinder should be analysed, for it always contains a trace of air.

The subject then begins to breathe from the bag, oxygen being supplied as required. The required volume of CO (about 130 to 150 c.c.) is driven into the bag 30 c.c. every two minutes. After the last of the CO has been washed into the bag the subject continues to breathe in and out of it for about 3′. A sample of his blood is then taken.

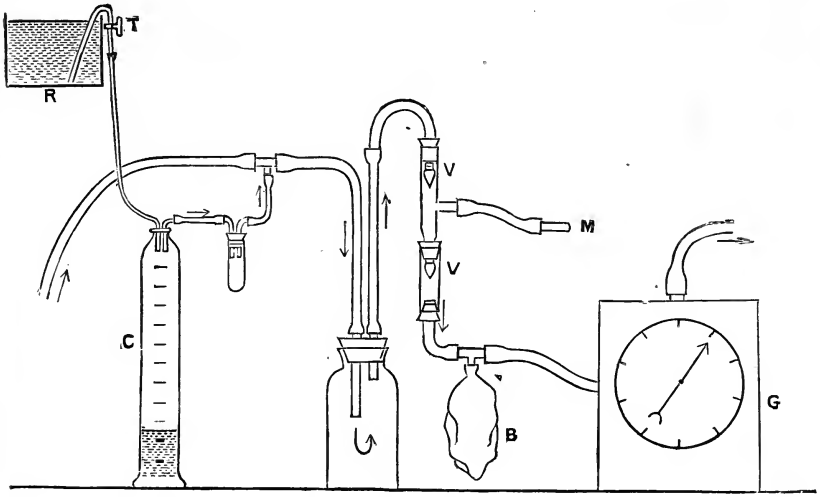


FIG. 264.—Haldane's apparatus for determining O_2 tension in human blood. R, T, C, apparatus for delivering CO at measured rate; M, mouthpiece; V, valves made of pieces of intestine; B, air-bag for controlling pressure during expiration; G, metre.

The volume of dry CO, reduced to 0° and 760 mm., is then calculated. From this volume and the percentage saturation of the blood the volume of CO or of O_2 capable of being taken up by the whole blood is calculated.

Example: Supposing the vol. of CO taken up is 150 c.c., and the saturation of the blood sample with CO equals 25%, then the CO (or oxygen) capacity of the total blood is $150 \times \frac{100}{25} = 600$ c.c.

From the total and percentage O_2 capacities of the blood the total volume is calculated.

Supposing the percentage O_2 capacity is 20, then $600 \times \frac{100}{20} = 3000$ c.c. To obtain the mass × by 1·05 (the specific gravity of blood) = 3165 grms.

The average weight of blood in man was found to be $\frac{1}{20{\cdot}5}$ of the body weight. In a fat man it was $\frac{1}{30}$.

In chlorosis, the *total* amount of haemoglobin in the body is not

decreased, but the blood is more watery. In pernicious anaemia, on the other hand, the total amount of haemoglobin is diminished.

Ventilation.—The ventilation per hour of a room may be determined by burning one or more candles in it and estimating with Haldane's gas analysis apparatus the percentage of CO_2 in the air of the room. The amount of CO_2 produced per hour by the candle must be determined beforehand. To determine this the candle may be placed in a large air-tight bottle, through which a current of air is aspirated. The air is analysed by the Haldane-Pembrey apparatus. The air in a room of 1000 cubic feet is changed at least once an hour even when the doors and windows are shut. The ventilation takes place through the walls. The wall area in proportion to the cubic content of a room varies inversely as the square of the diameter. Thus the larger the room, the worse is the ventilation.

CHAPTER XLVIII.

PRODUCTION AND LOSS OF HEAT.

Methods of Investigating the Seats of Heat Production.—The temperature of the organs of the body can be investigated by either delicate thermometers calibrated in $\frac{1}{100}$th of a degree between 35° and 40° C., or by thermo-electric junctions.

The thermometers are made with long slender bulbs. One is passed up the femoral artery into the blood stream of the aorta. The other is used to measure the temperature of the organ during states of rest and activity.

(1) In the case of the salivary gland a ⊥-tube is connected with the duct-cannula and with a reservoir full of warm water. The thermometer is inserted in one branch of the ⊥-tube, and the latter is filled with the warm water. When the temperature of the water has reached the same level as the aorta, the chorda tympani is stimulated. The saliva is found to be *not* perceptibly hotter than the aortic blood (Hill and Bayliss). The circulating blood carries away the small amount of heat formed in the gland. Reid failed to demonstrate heat-production in the liver on stimulating the hepatic nerves.

(2) In the case of the muscles a thermometer is buried in the thigh muscles of each leg. The circulation is then temporarily arrested by compression of the aorta and the sciatic nerve excited on one side. The tetanised muscles show an increase in temperature.

(3) One thermometer is inserted at the junction of the subclavian and jugular veins, and pushed down into the vena cava inferior. This thermometer will register a temperature slightly higher than that in the aorta. On the contrary, the blood in the superficial veins, for example, in the femoral vein, is cooler than the arterial blood.

FIG. 265A.—Thermometer for determining temperature of blood.

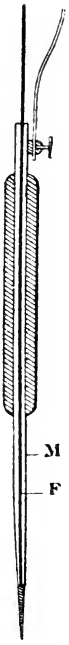


FIG. 265B.—Thermo-electric needle.

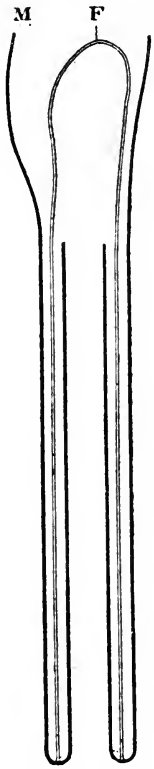


FIG. 265C.—Thermo-electric catheter.

Mosso asserts that the brain is an active seat of heat-production. It is questionable whether he measured the true aortic temperature. His results can be attributed to the varying amount of blood which circulated through the brain during the experiments. The estimations of the exchange of blood gases in the brain made by Hill and Nabarro do not favour Mosso's conclusions. The muscles are the only organs in which the production of heat has been certainly demonstrated by thermometric methods. Thermo-electric junctions are made by soldering the ends of an iron and a German-silver wire together. An alloy

called constantine and copper make the most delicate junction. The wires can be threaded down a fine gum-elastic catheter, and the soldered end exposed in the eye of the catheter. The catheter can then be passed up the femoral artery into the aorta. A similar thermo-electric junction is buried in the substance of the organ under investigation. The two junctions are put in circuit with a very sensitive low resistance galvanometer. The needle of the galvanometer is provided with a mirror. The instrument can be calibrated by placing two junctions in water baths of different temperatures, and noting the swing of the galvanometer on the scale. The temperature of the water baths is determined by a delicate mercurial thermometer.

For the investigation of frog's muscle Blix' apparatus is convenient. The muscles are clamped above and fastened to recording levers below. They lie in contact with constantine-copper junctions, which are connected with the galvanometer. The galvanometer, junctions, etc., make one compact piece of apparatus. The muscles are enclosed by a cylinder of felt to prevent loss of heat. The muscle on one side is excited, and the swing of the galvanometer measured on the scale. At the same time the work done by the muscle can be measured. Under the most favourable conditions, a quarter of the energy of the muscle appears as work and three-quarters as heat.

The Loss of Heat in Man (Waller's Method).—With the flat-bulbed surface thermometer determine the surface temperature of the body at various parts, *e.g.* the upper arm, thigh, face, abdomen. The deep temperature of these parts[1] is also determined, and the temperature of the air. The evaporation from the skin is determined by a hygrometer. This consists of a capsule containing calcium chloride. The capsule is weighed and then applied to the skin. After 10 minutes it is weighed again. The increase in weight gives the amount of sweat evaporated. The operation is repeated at different parts of the body. The area covered by the capsule is known. The total area of the skin of the body can be calculated. The surface of an animal is roughly proportional to $\sqrt[3]{}^2$ of its volume. $S = k \sqrt[3]{}^2 v$. The value of the constant k is 11·2 for mammals (Rübner). Assuming the sp. gr. does not vary in different animals weight can be substituted for volume in this formula. Thus $S = 11{\cdot}2 \sqrt[3]{}^2$ weight of body. Thus the total loss of water (and of heat) by evaporation can be calculated. The rate of loss of heat from the skin can be inferred if the temperature indicated by the surface thermometer be found when the instrument is applied to a vessel of warm water which is losing heat at a known rate.

[1] Axilla, groin, mouth, rectum.

CHAPTER XLIX

SWEAT.

Sweating.—Rabbits and rats are said not to sweat at all; the cat sweats on the hairless pads of the feet; the horse sweats, like man, on all parts of his skin.

Sweat Nerves.—DEMONSTRATION. A cat is anaesthetised with ether and chloroform. The sciatic nerve is exposed and divided. On exciting the peripheral end beads of sweat appear on the pads of the feet. The same result is obtained after occlusion of the aorta or femoral artery, or even in an animal which has been just killed.

The sudorific fibres for the hind-limb issue by the white rami of the last two thoracic and first three or four lumbar nerves. Their cell stations are in the sixth and seventh lumbar and first and second sacral ganglia of the sympathetic chain. The fibres leave by the grey rami of these ganglia, and enter the corresponding anterior roots and so the sciatic nerve.

Sudorific fibres supply the fore-limb from the fourth to the ninth thoracic nerves. The cell station of these fibres is the stellate ganglion. The grey rami of this ganglion reach the brachial plexus and so the median and ulnar nerves.

Sudorific fibres for the face leave the cord by the second, third and fourth anterior roots, and run up the cervical sympathetic nerve to the cavernous plexus, thence to the infra-orbital branch of the fifth nerve. After the intra-venous injection of 3 mgrms. of atropine sulphate the excitation of the sciatic is ineffective.

Subsequent injection of 10 mgrms. of pilocarpine will cause sweating, while the sciatic nerve still remains inexcitable. The atropine paralyses the secretory nerve endings, while the gland cells are directly excited by the pilocarpine. If the foot of a cat is enclosed in a glass plethysmograph and the junction is made tight by means of a rubber collar so that the pressure in the plethysmograph can be raised above the arterial pressure sweating will still take place on excitation of the sciatic nerve. Sweating can be provoked in the cat's feet by asphyxia or by warming the blood, and even after the cord has been divided in the lower thoracic region. In man sweating does not occur below the level of the lesion after section of the spinal cord, not even after the injection of pilocarpine. The level of the lesion may be determined by this method.

Sweating in Man.—The palm of the well-washed hand is pressed on to paper sensitised with silver nitrate. Spots of silver chloride, formed at the mouths of the sweat glands, become visible on the paper. A pad soaked in atropine solution 1 per cent. is fixed with collodion on a small piece of the palm. No sweat spots will be obtained from this piece next day. The local subcutaneous injection of pilocarpine provokes sweating.

On a warm day one hand is held for 10′ in water at 45° C. and the other in water at 20° C. Exercise is then taken. The hand which was in the warm water will not sweat for some time. The terminal sweat apparatus is depressed by either excessive cold or heat.

Measurement of Cutaneous Excretion (Barratt's Method).—A glass plethysmograph provided with entrance and exit tubes and a rubber collar is fitted on to the fore-arm.

The exit tube is connected with weighed Haldane-Pembrey absorption tubes: (A) Sulphuric acid, (B) soda-lime—sulphuric acid. The entrance tube passes through soda-lime bottles and pumice-sulphuric acid. Air is drawn through the apparatus by a filter pump and a meter is interposed. A rate of 1 litre per minute is sufficient. After a half hour the increase of weight in A and in B is determined. The increase in A gives the amount of water and in B the amount of of CO_2 excreted. The excretion of water is diminished by painting the skin with 20 per cent. carbolic acid and producing dry dermatitis.

PART IV.

ADVANCED PHYSIOLOGICAL CHEMISTRY

NOTE.—In the chapters on advanced physiological chemistry, many elementary details of methods of analysis have been omitted. In many cases references are given to those portions of the elementary course of physiological chemistry, where the student will find the necessary details. The index to the chemical portions, Parts II. and IV., will also enable the student to rapidly find the elementary portion of the subject at which he is working.

PART IV.

PHYSIOLOGICAL CHEMISTRY (ADVANCED)

CHAPTER I.

CARBOHYDRATES.

Chemical Relationships.—Although the '**hexoses**' are the sugars of greatest physiological interest, it must be remembered that '**pentoses**' (wood sugars) do occur in the animal organism. They have been separated from a nucleo-proteid obtained from the pancreas by Hammarsten, and from yeast by Kossel. They differ from the hexoses in that they *do not ferment with yeast.* When given by the mouth they are excreted unchanged by the urine, indicating that they are not assimilated by the organism.

The Chemical Constitution of Sugars has recently been determined by Emil Fischer, chiefly by studying the compounds formed with phenyl-hydrazine.

Place 0·1 gr. of dextrose in a test-tube, dissolve in water, and add 0·1 gr. phenyl-hydrazine hydrochloride and 0·2 gr. sodium acetate crystals, warm gently till everything is dissolved, and then place for half an hour in a boiling water bath. Allow to cool gradually, when a yellow precipitate of osazone will separate out. Examine this under the microscope, and notice that the precipitate is composed of needle-shaped crystals arranged in rosettes or sheaves (Fig. 266).

The chemical reaction takes place in two stages.

Firstly, the 'O' of the $-CHO$ group of the sugar reacts with the 'H_2' of the 'NH_2' group of the phenyl-hydrazine to form H_2O, the sugar and phenyl-hydrazine combining to form *Hydrazone.*

$$CH_2OH(CHOH)_3\,CHOH - CH\boxed{O + H_2}N - NH(C_6H_5).$$

Sugar. *Phenyl-hydrazine.*

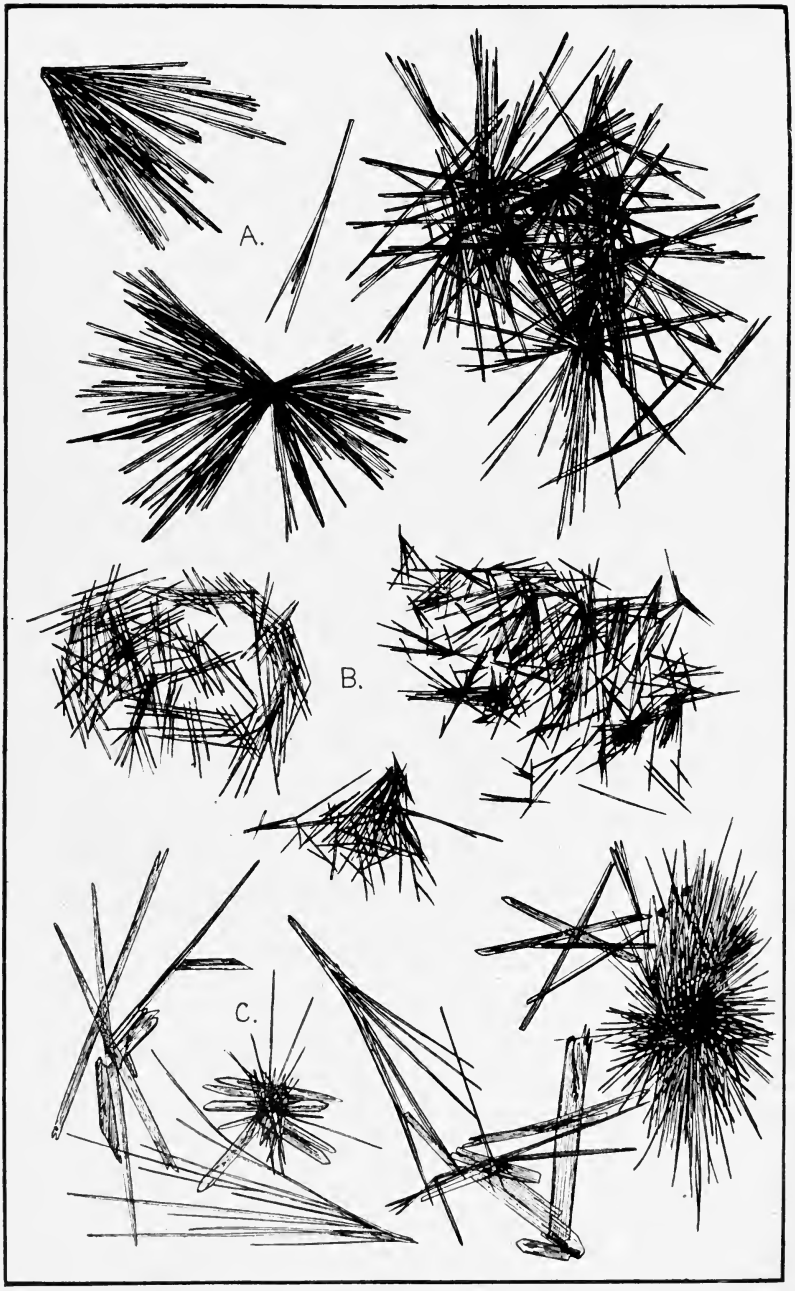


FIG. 266.—Osazone crystals. × 400.
A, Phenyl-glucosazone; *B*, Phenyl-maltasazone; *C*, Phenyl-lactasazone.

Secondly, another molecule of phenyl-hydraxine reacts on the last 'CHOH' group of the hydrazone, expelling water from it and itself losing the H_2 of its NH_2 group, forming an osazone thus:

$$CH_2OH(CHOH)_3\,C\boxed{HOH} - C - H$$
$$\qquad\qquad\qquad\qquad\quad \| \qquad\quad \| N - NH(C_6H_5)$$
$$+\boxed{H_2}N \qquad NH(C_6H_5)$$

The liberated '*H*' goes to split up more phenyl-hydrazine into aniline and ammonia. If now an osazone be hydrolysed by treating with fuming HCl it breaks up, phenyl-hydrazine being set free, and a body called an *osone* resulting. This latter has the formula $CH_2OH-(CHOH)_3-CO-COH$ from which it is seen that it contains both an aldehyde and a ketone group. The former of these groups can be converted into the CH_2OH group of sugar by treating with a reducing agent.

$$CH_2OH-(CHOH)_3-CO-COH+H_2=CH_2OH-(CHOH)_3-CO-CH_2(OH)$$

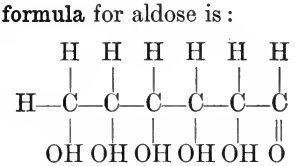
which is the formula for laevulose (a ketose).

The aldoses can thus be changed into the ketoses, and if the aldose obtained by condensation of HCHO (formaldehyde) be used as the starting-point an interesting synthesis from a simple aldehyde to a more complex one and then to a ketose is illustrated.

The various sugars have also been artificially prepared by careful oxidation of the corresponding alcohols and by reduction of the corresponding acids. There are three hexatomic alcohols differing from one another in their constitutional formulae. From each of these a different aldose or ketose can be produced by oxidation, and these latter can be further oxidised to form three different mono-basic acids, or further still, to form three di-basic acids, thus:

Alcohol.	*Aldose.*	*Ketose.*	*Mono-basic*[1] *acids.*	*Di-basic*[2] *acids.*
Sorbite.	Dextrose.	—	Gluconic.	Saccharic.
Mannite.	Mannose.	Laevulose.	Mannonic.	Manosaccharic
Dulcite.	Galactose.	—	Galactonic.	Mucic.

The **constitutional formula** for aldose is:



It is seen from this formula that there are four *C.* atoms on to which four *different* groups or substitutes are attached. These are, therefore,

[1] Monobasic acids have formula $CH_2OH-(CHOH)_4-COOH$.
[2] Dibasic ,, ,, $COOH-(CHOH)_4-COOH$.

called asymmetrical C-atoms because their substitutes might have different *spacial* arrangements, and still the empirical formula remain the same.

This variable arrangement of the substitutes causes these slight differences in the chemical behaviour of the body. It also manifests itself, however, by differences in the stereochemical behaviour of the bodies, and this is ascertained by examining the rotatory power on a beam of polarised light.

Polarisation of Light.—When two slices of tourmaline, a semi-transparent mineral, are cut parallel to the axis of the crystal and laid over one another, it will be noticed that the amount of light which passes through the combination varies according to the relative positions of the two slices to one another. If the slices be at right angles to one another no light passes through, and in intermediate positions only a certain amount, so that an opaque combination is obtained. A ray of ordinary light contains vibrations in all planes passing through the ray; but, when the light passes through a tourmaline plate, it vibrates in one plane only. Ordinary light may, therefore, be likened to a wheel, the axle representing the ray of light and the spokes the planes along which it vibrates. On passing through the tourmaline plate, however, the light is capable of vibrating in one plane only, which would correspond, in our example, to two opposite spokes. The light which vibrates in one plane is called plane-polarised light, and cannot be distinguished by the naked eye from ordinary light. By placing a second, similarly cut, tourmaline plate in its course, however, it can be detected, for it will pass through this only if its axis corresponds to the axis of the first plate. The first plate is called the *polariser* and the second plate the *analyser*. The mechanism of this action of the analyser and polariser can be easily illustrated by a piece of string stretched between two posts; it can vibrate in all planes. If a comb be placed in the course of the string the vibrations can only take place along one plane corresponding to the direction of the teeth of the comb. This comb represents the polariser. If now, a second comb be placed along the string it will permit the vibration of the string or stop it, according to the position of its teeth; if these be in the same direction as those of the first comb the string will go on vibrating, but if they be placed at right angles the string will cease to vibrate. Polarisation of light by tourmaline illustrates the principle of the polariser, but in this instrument itself it is found more convenient to use a polariser and analyser made of a *Nicol's prism*. A Nicol's prism consists of a crystal of Iceland spar. Such a crystal has the power of splitting light into two rays, one of which, the *ordinary ray*, passes through it as it would through glass, and the other one, the *extraordinary ray*, is more refracted. Consequently, on looking at a dot on a sheet of paper through a piece of Iceland spar laid flat on the paper, a double image of the dot is obtained, and if the crystal be rotated, one of the dots—the extraordinary ray—will be seen to move round the other—the ordinary ray—which remains stationary. Now both these rays are polarised, but in different planes. If the crystal be cut across along a diagonal line and the two surfaces re-cemented by means of Canada balsam, the ordinary ray, when it meets the balsam, will be totally reflected and pass out at the side of the crystal, whereas the extraordinary ray will be transmitted through the balsam, and will finally emerge at the end of the prism, parallel to its original

direction; but, of course, plane polarised. To detect the polarisation a similarly constructed prism, or analyser, must be used.

Certain other bodies, *e.g.* a quartz plate, a solution of sugar or albumin, have the power of *rotating the plane of polarised light*. Thus, supposing that the plane polarised light vibrates along a vertical plane, one of the bodies may twist it into an oblique plane. If the analyser be so placed that none of the plane polarised light can pass through it (*i.e.* the field is black), and if a piece of quartz be inserted between the polariser and analyser, it will be found that now a certain amount of light passes through the analyser (*i.e.* the field becomes opaque), and, in order to obtain darkness again, it is necessary to rotate the analyser in the direction of the hands of a watch, as seen by the observer. Consequently, rotation has taken place to the right, *i.e.* dextro rotation is said to have occurred. If a solution of albumin or laevulose be employed the rotation of the analyser must be to the left, *i.e.* against the hands of the watch. When the plane of white light passes through the quartz plate, however, the various colours of the spectrum are rotated to a different degree, so that, instead of having a mere opacity (as is the case with intermediate positions of two 'tourmaline' plates) different colours are obtained according to the amount of rotation. There are also samples of quartz which rotate the plane of light to the left.

Dextrose and a quartz plate produce the same amount of rotation, and therefore it is possible to determine the rotatory power of a solution of the former by compensating its rotation by means of a quartz plate of known rotatory power.

We are now in a position to understand the construction of a **polarimeter** or **saccharimeter.** It consists of the following parts:

(1) A Nicol's prism, called the *polariser.* This polarises light in a vertical plane.

(2) A biquartz, or other device for rotating, in opposite directions, the two halves of a polarised beam. A biquartz consists of a disc of quartz made of two semicircular halves of equal thickness, but of opposite rotatory powers. Each half is of such a thickness that it rotates the plane polarised light to 90° in opposite directions so that, on emerging from the disc the plane of light is now horizontal. Instead of a biquartz many instruments contain a semicircular plate of quartz.

(3) A tubular **liquid holder** to hold 10 c.c. of the liquid to be examined. If the length of this tube be 188·6 mm. the amount of rotation in angular degrees will correspond to percentage of dextrose in the fluid (*e.g.* urine) examined.

(4) **A Compensator.**—This shows how much rotation has been produced by the solution. It is connected with a scale representing angular degrees, and the pointer carries a vernier, so that tenths of a degree can be read off. In some instances the compensator consists of two wedge-shaped pieces of quartz, so arranged on one another that the total thickness of quartz interposed in the path of the polarised beam can be varied by means of a screw. In other instruments the quartz

plates are dispensed with, the amount of rotation being measured by rotating the next part of the instrument, namely the

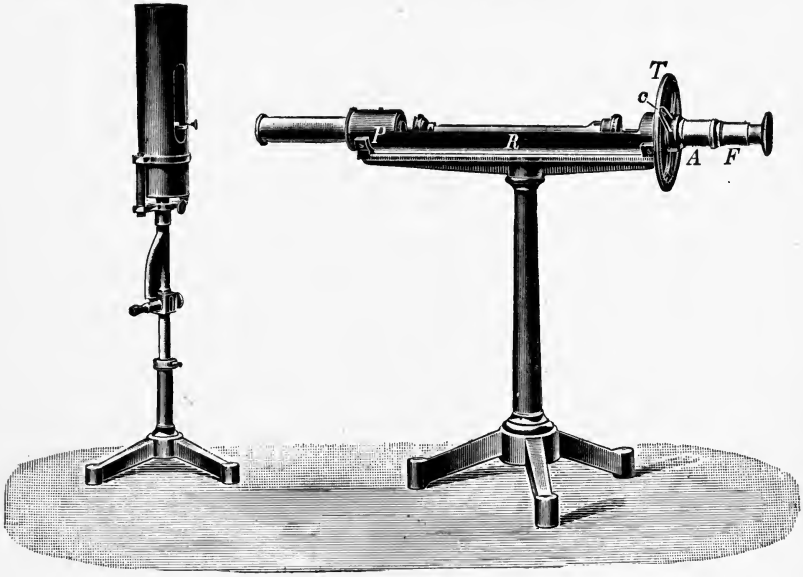


FIG. 267.—Polarimeter of Mischerlich with Laurent's polariser. *P*, polariser and device for obtaining half shadow; *R*, fluid container; *T*, scale with vernier *c* attached to pointer; *A*, compensator and analyser; *F*, lens.

(5) **Analyser,** so as to obtain uniformity of tint in the two halves of field.

(6) **A Lens.**

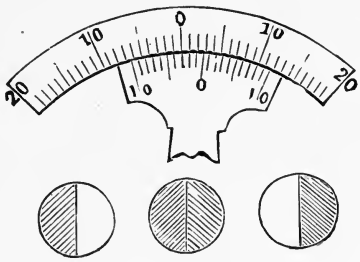


FIG. 268.—Diagram of scale and field of vision of polarimeter. Above is represented the scale for measuring the compensation necessary. In the position represented in the diagram the reading is 2·7 dextro rotation. The lower part of the diagram shows the three appearances of the field of the polarimeter, the central one representing the appearance at zero, *i.e.* when there is no rotation.

When the tube (3) is filled with water or an optically inactive fluid, and the compensator or analyser rotated until a violet colour of uniform tint fills the field, the indicator will be seen to stand at zero (if not so, the error must be noted). If now, an optically active fluid be

placed in the tube the two halves of the field will become of different tints, *i.e.* rotation of the plane of polarised light has occurred. In order to measure the amount of this rotation, we must move the screw or pointer connected with the compensator or analyser until the uniform tint is again obtained. The amount of 'compensation' necessary is read off on the scale and, if the holder be not 188·6 mm. long, the necessary calculation is made in order to ascertain the strength of the solution (for formula see below).

To estimate the percentage of sugar in urine the chief precautions are, (1) to see that it is *perfectly clear*, and (2) to see that it contains no proteid.

In order to obtain a specific or comparative number (*i.e.* a result always obtained under the same conditions) it is necessary to adopt a standard. This consists of the rotation, in degrees of a circle, produced by 1 gr. of the substance dissolved in 1 c.cm. of fluid and contained in a tube 1 dcm. long. This is called the *specific rotatory power* and is represented by $(\alpha)D$.[1] It is determined by the following formula:

$$(\alpha)D = \pm\frac{a}{p \times l},$$

where a = the observed rotation,

l = the length, in decimeters, of the tube in which the solution is placed,

p = the weight in grammes of the substance contained in 1 c.c. solvent.

The rotation produced by a substance depends upon its concentration in a solution; if, therefore, the index $(\alpha)D$ of any substance be known, and its rotation be ascertained, its percentage P in any fluid can be ascertained by the formula.

$$P = \frac{100a}{sl}$$

where $s = (\alpha)D$.

For rapidly and accurately determining the percentage of sugar in any fluid (*e.g.* urine) the polarimeter—and especially that form of it in which the scale reads percentages of sugar—is a very valuable instrument. It is much used for this purpose in the continental clinics.

The Specific Rotatory Power of certain of the sugars is as follows:

Monosaccharides: Dextrose: + 52·6°.

Galactose: + 83°.

Laevulose: rotates a variable distance to left.

Disaccharides.—The $(\alpha)D$ of these carbohydrates changes when they

[1] The 'D' indicates that sodium light is used.

are hydrolysed. On standing, also, the rotatory power may increase (half rotation) or diminish (bi-rotation).

Cane Sugar; $+66{,}5°$ – after hydrolysis becomes laevorotatory.

Maltose; $+137°$ – after hydrolysis becomes less—shows bi-rotation.

Lactose; $+52{,}5°$.

Fermentation of Sugars with Yeast.—Shake up a 1 per cent. solution of dextrose, which has been previously boiled to expel air, with a piece of yeast the size of a split pea. Place the opalescent solution thus obtained in a Southall's ureometer (p. 274) so that it completely fills the vertical tube. Now place the tube in the incubator over night when it will be found that a certain amount of gas (CO_2) has collected at the top of the tube. As a control a tube should be filled with water and yeast.

Repeat this experiment with similar solutions of the various sugars, and note that after 24 hours lactose and cane sugar have scarcely undergone any fermentation. If they be left longer, however, a considerable amount of gas will collect since hydrolysis is gradually produced by another ferment (invert ferment) in the yeast, on the products of which the alcoholic ferment then acts.

CHAPTER II.

GLYCOGEN.

THE following chapter includes an accurate method for the estimation of glycogen in organs or tissues, and shows how the *post-mortem* transformation of glycogen into sugar may be demonstrated. A dog or a rabbit is killed by an overdose of chloroform, the abdomen quickly opened and a cannula placed in the portal vein. The liver is excised. The cannula is connected, by means of indiarubber tubing, with a pressure bottle containing iced water, which is perfused through the hepatic blood-vessels till the liver becomes quite pale and the washings colourless. The iced water also cools down the liver and hinders the *post-mortem* transformation of glycogen with sugar.

The liver is then divided into two, and each half quickly weighed.

One half **A** is placed in an incubator. (An incubator may be improvised by placing a large beaker on a water bath, the flame under which is kept low so that the temperature of the beaker does not rise above 36° C.)

In the other half **B** the glycogen and sugar are determined. 1. For *the estimation of glycogen* Pflüger's method is employed. This is as follows: 100 gr. liver are quickly chopped up into small pieces and

mixed in a flask with 100 c.cm. 60 per cent. KOH (Pflüger recommends 1a Merck). After vigorous shaking for a few minutes the flask is placed on a boiling water bath for two hours. By heating with this strength of KOH (viz., 30 per cent.) all the glycogen—both that which is free and that which is combined with proteid—is extracted, and, on the other hand, there is no hydrolysis of glycogen, because strong KOH attracts water and thus prevents its being added to the glycogen molecule. Another advantage of strong KOH is that it destroys proteid to such an extent that this no longer is precipitated by alcohol. The use of proteid precipitants at a later stage is thus obviated.

(While this is heating the estimation of sugar as described below (B II.) should be proceeded with.)

The brown opaque contents of the flask—after heating for two hours—are made up to 400 c.cm. by the addition of water, and filtered through asbestos or glass wool. (Such a filter can be made by placing a small cone of wire gauze in the apex of the funnel and then the asbestos.) If the filtrate comes through opaque refilter it till it is clear; 100 c.cm. of the filtrate are then mixed in a beaker with 100 c.cm. 96 per cent. alcohol, and the mixture briskly stirred. By this means the glycogen is precipitated. The precipitate, after standing a few minutes, is collected on a small filter paper (15 cm. diam.). As filtration at this stage is slow the filter paper may be folded as described in the Appendix (Fig. 278). After all the fluid has drained away the precipitate is washed twice with alcoholic potash of the following strength: 1 vol. 15 per cent. KOH + 2 vol. 96 per cent. alcohol, then twice with 96 per cent. alcohol and once with ether. A pure glycogen precipitate is thus obtained. To ascertain its amount it is hydrolysed into sugar, which is then estimated as described on p. 274. The technique of the method is as follows: the stem of the filter funnel is closed by a piece of indiarubber tubing and a clip, and the funnel is filled with distilled water. The glycogen soon dissolves, and then the clip is removed and the solution run into a large flask (500 c.cm.). This process is repeated till the last trace of glycogen has disappeared. A piece of litmus paper is then thrown into the glycogen solution and HCl (con.) added, drop by drop, to neutralisation. The volume of the solution is then made up to 500 c.cm. and 25 c.cm. HCl (con.) added to it. This gives a solution containing 2·2 per cent. HCl, which is the most suitable strength for producing hydrolysis. The flask is now placed on a boiling water bath for 4 hours, after which it is cooled, neutralised with KOH and the sugar estimated by titration, etc. (p. 274).

B II. For *the estimation of sugar*, 10—20 gr. of chopped-up liver are pounded in a mortar with boiling water, faintly acidified with acetic acid, and sand; boiled for a few minutes then filtered, neutralised, and the sugar estimated as on p. 274.

A. The glycogen and sugar in the incubated liver are determined by the same methods.

It will be found that by standing at 36° C. much of the glycogen disappears from the liver and that sugar increases. The liver possesses the power of hydrolysing glycogen even after death. It probably has a similar power during life. Immediately after death this hydrolysis of glycogen proceeds very rapidly, but can be much retarded by perfusing with iced water.

CHAPTER III.

PROTEIDS.

Chemical Nature.—In order to construct the structural formula of proteids it is necessary, first of all, to determine the exact nature of their decomposition products. This has been done by decomposing them, either by means of acids or alkalies, or by means of the ferment trypsin.

The isolation of the chief amido acids has already been given on p. 227. The following method may be used for *the separation of the hexone bases* (Kossel's method) and of *glutaminic acid.*

A quantity of dried proteid is treated with three times its weight of concentrated hydrochloric acid, to which some stannous chloride is added to prevent oxidation. It is boiled with this for three days, the mouth of the flask being connected with a Liebig's condenser so arranged that the condensed vapour runs back into the flask. After this time the contents of the flask, which are deep brown in colour, are removed, treated with H_2S to remove zinc, filtered, and evaporated to a syrup. This is placed on ice and in the course of some hours large clear crystals of *Glutaminic acid hydrochloride* separate out. This is a di-basic amido acid having the formula:

$$CH<\begin{matrix}NH_2\\COOH\end{matrix}$$
$$CH<\begin{matrix}COOH\\CH_3\end{matrix}$$

and is closely allied to Aspartic acid (see p. 230). These crystals are separated by filtration. The filtrate is then diluted with water and treated with a watery solution of Phospho-tungstic acid. The resulting *precipitate*, which contains the *hexone bases*, is then dissolved in boiling water, and treated with powdered baryta in the heat till most of the ammonia gas has been evolved and all the phospho-tungstic acid precipitated. The preparation is then filtered and CO_2 bubbled through the filtrate to precipitate the barium (as $BaCO_3$). The Ba-free solution is again saturated with CO_2, and mixed with a watery solution of mercuric chloride till the reaction is acid. This treatment precipitates the

Histidin as a mercury salt. The *precipitate* (1) is collected on a filter paper, suspended in water and treated with H_2S gas to separate the Hg (as HgS) which is filtered off. The mercury-free filtrate is evaporated to small bulk at a low temperature, and allowed to stand, when histidin chloride crystallises out.

The *filtrate* (1) is rendered free of Hg as above described and treated in warm solution with powdered silver sulphate. This latter is added in small portions at a time until a sample of the solution, when placed in a watch glass, gives a brown precipitate with baryta-water (and not, as at first, a white precipitate). This test shows when an excess of silver comes to be present in the solution. The silver sulphate also removes all traces of HCl from the solution. By now adding baryta-water, the excess of silver combines with **Arginin** to form a *precipitate* (2) which is collected on a filter paper, washed with, and then suspended in, water and the silver removed by means of H_2S. The silver-free filtrate, after being carefully neutralised with nitric acid, is evaporated to a syrup, which on standing yields crystals of Arginin nitrate.

The *filtrate* (2) contains **Lysin.** After removing silver and barium (by H_2S and H_2SO_4) and evaporating the filtrate from these to small bulk an alcoholic solution of picric acid is added. This precipitates lysin as a picrate, which is then shaken in a separating funnel with dilute HCl and ether. The HCl displaces the picric acid from its combination, and chloride of lysin is produced, which goes into solution in the ether. The latter is separated, and on evaporation yields lysin chloride.

To obtain large yields of the hexone bases it is desirable not to prolong the heating of the proteid with HCl for more than 7-8 hours. For glutaminic acid 3 days are necessary.

The above method is only qualitative. A quantitative method has been worked out by Kossel and Kutscher,[1] but is too complicated for description here.

Various attempts have been made to build up artificial proteids from their decomposition products, but these attempts have not as yet been successful. A substance very like proteid has, however, been prepared by Grimaux, who by heating together certain amido bodies with an aromatic radicle obtained a substance which he called Colloid, and which gave most of the ordinary tests for proteids. It also caused intravascular clotting of the blood when injected into the circulation of an animal, thus resembling nucleo-proteids. Proteids have also the power of combining with the Halogen elements to form definite compounds.

The Physical Properties of Proteids. Method of obtaining Crystals.—The whites of several eggs are mixed with an exactly equal amount of a fully saturated solution of ammonium sulphate. This precipitates the globulins. The ammonium sulphate solution must be exactly neutral in reaction, and should be added to the egg-white in small

[1] *Zeitschr. f. phys. Chem.*, Bd. 38, S. 165.

quantities at a time, the mixture being briskly stirred between each addition. The precipitated globulin is filtered off and the filtrate, which reacts alkaline to litmus, is treated with the ammonium sulphate solution drop by drop till a faint haze of precipitated albumin is obtained. A drop of water is added so that the haze just disappears. The solution is now treated with 10 per cent. acetic acid, cautiously added drop by drop, until a precipitate of albumin just forms. The flash is laid aside, and in twenty hours it will be found that the originally amorphous precipitate has developed a large number of needle-shaped crystals (see Fig. 143).

The Colour Reactions of Proteids.—Besides the three colour reactions already described (viz., Millon, xanthoproteic, and the biuret) there yet remains to be described *Adamkiewicz's reaction*. The reaction is due to the presence in the proteid molecule of tryptophane (see p. 452).

Mix in a test-tube two parts of glacial acetic acid, and one part of concentrated sulphuric acid; now add a few drops of a solution of egg albumin, when a violet red colour will be produced. Hopkins and Cole have shown that the reaction is due to the presence of glyoxylic acid, which is a common impurity of glacial acetic acid. A pure solution of glyoxylic acid gives a very distinct reaction. The purest forms of acetic acid do not give the reaction since they do not contain glyoxylic acid.

The Precipitants of Proteids.—Sodium sulphate possesses at 30° C. the same proteid precipitating powers as ammonium sulphate. It is of great advantage when it is desired to estimate the amount of proteid contained in any fluid. By precipitating with sodium sulphate, the amount of proteid can be determined by estimating the amount of nitrogen contained in the precipitate (determined by Kjeldahl's method) and multiplying by 6·25. When ammonium sulphate is employed, it is obvious that this method cannot be used.

Coagulation of Proteids by Heat.—Native proteids are coagulated by heat in the presence of neutral salts. A solution of albumin in distilled water, however, does not coagulate even on boiling. If blood serum be weakly acidified with acetic acid, and very gradually heated in a warm bath, it will be found that a dense coagulum is obtained at 75-77° C. and that, if this be filtered off, the filtrate will give another, but fainter coagulum, at about 84° C. By this method —called fractional heat coagulation—it has been attempted to identify several varieties of proteid in blood serum, but, since it is only in their heat coagulation points that the fractions differ from one another, it is highly improbable that they are really different varieties of proteid.

Classification of Proteids. I. Protamines.—These differ from all

other proteids in that they do not yield mon-amido acids as decomposition products, but only the hexone bases Arginin, Histidin, and Lysin (see p. 170). They are very basic in nature, a solution of them in water reacting alkaline to litmus. They accordingly combine readily with acids to form salts, and it is as a salt with nucleic acid that they exist in the spermatozoa of certain fishes. The protamin can be displaced from its combination with nucleic acid by means of sulphuric or picric acids, and the resulting salt is used for the isolation of protamin. These artificial salts of protamin are precipitated by phospho-tungstic acid, this being the precipitant used to separate them from other bodies.

If a solution of protamin in water be treated with an ammoniacal solution of albumin, a precipitate is obtained which is said to be the same as *histon* (see below). Protamines are considered by Kossel as the nucleus of construction of all other proteids. Several varieties have been isolated.

II. **Albuminoids.**—**Collagen,** the precursor of gelatin, forms the chief constituent of white fibrous tissue and of the organic substance of bone. It also exists in cartilage, where, however, it is mixed with several other bodies (see p. 429).

Preparation of Collagen.—A piece of tendon is macerated overnight in 1 per cent. caustic alkali to remove proteid and mucin, and then washed with water till alkali free. The resulting mass is collagen. Place a piece of this in a flask and boil it for ten minutes with water which is rendered faintly acid with acetic acid. By this treatment, the collagen is transformed into gelatin and, on cooling the solution, it gelatinises.

Gelatin.—This is really the hydride of collagen, the boiling with acidulated water in the above experiment having caused the collagen to take up a molecule of water. Conversely, the gelatin can be reconverted into collagen by heating it to 130° C., whereby it loses water.

Divide a solution of gelatin in lukewarm water into four portions, to which apply the following tests: (1) the biuret reaction: a violet colour is produced. (2) the xantho-proteic reaction: only a slight colouration is produced. (3) the Millon's test: only a slight reddening of the precipitate occurs on boiling. (4) the glyoxylic test: absent or very faint.

The reason why the last two tests are not very distinct, is because gelatin is an albuminoid, and consequently does not yield aromatic bodies on decomposition, and both these tests depend on the presence of aromatic bodies. Some varieties of gelatin give these reactions more distinctly than others, and absolutely pure gelatin is said not

to give them at all, so that their presence is held to depend on native proteid in the gelatin.

The other albuminoids are unimportant. They are **Keratin**, which occurs in the skin appendages and in the medullary sheaths of nerves, and which is remarkable for the large percentage of sulphur which it contains; **Elastin** found in elastic fibres, and which contains a very small percentage of sulphur, and a considerable amount of aromatic bodies.

All these albuminoids except keratin yield glycin as their chief decomposition product. They also yield the hexone bases, since protamin forms part of their molecule.

III. **True Proteids.** (1) **Native Proteids** (p. 176). (2) **Albuminates** (p. 177). (3) **Proteoses and Peptones** (p. 177).

IV. **Compound Proteids.** (*a*) **Gluco-Proteids.**—It has recently been discovered that a carbohydrate can be split off from most proteids. Thus, if egg albumin be decomposed by boiling with acid, the resulting product is capable of reducing Fehling's solution although it does *not* ferment with yeast. These reactions would point to its being a pentose (see p. 417). A similar sugar is also obtained from the nucleo-proteid of the pancreas and from several other proteids, so that the gluco proteids probably form a very extensive class. Besides mucin (see p. 178), this class also includes the **Mucinoids** and the **Chondro Proteids.** The mucinoids are distinguished from the mucins in that they are not stringy in nature, and that they are not so easily precipitated by acetic acid, and the precipitate is very readily soluble in excess of the acid. They are represented by pseudo-mucin, which occurs in ovarian cysts, and by ovo-mucoid in the white of egg. The chondro proteids occur along with collagen in cartilage. On decomposition with an acid they yield proteid and a reducing body called chondroitin-sulphuric acid, which can be further decomposed to yield a body called chondrosin, which is even more strongly reducing than dextrose, and which contains nitrogen. By still further decomposing this latter body glucosamin is obtained, which is also the chief decomposition product of chitin, an important constituent of the carapace of arthropods.

(*b*) **Nucleins.**—The general relationships of the different bodies in this most important group of proteids have already been given in the elementary part (p. 179). All that remains to be done here is to describe how the different bodies are prepared.

Nucleo-Albumin and Nuclein.—A pancreas or thymus gland is chopped into small pieces, and is macerated for several hours with about four times its bulk of water containing 0·5 per cent. ammonia, the mixture being frequently stirred. After this time, the extract is strained through muslin, and the residue again extracted. The extracts

are then mixed together, and 5 per cent. acetic acid added to the combined extract till it becomes faintly acid in reaction. A dirty white precipitate is produced, which is *nucleo-albumin.* In order to purify it, the precipitate is collected on a filter paper and redissolved in ammonia water, the nucleo-albumin being again precipitated by the addition of acetic acid. In order to prepare *nuclein* from this, the precipitate is transferred to a small beaker or flask, and to it is added about ten times its bulk of 0·2 per cent. hydrochloric acid, and about 10 c.c. liquor pepticus. The mixture is placed in the incubator at 37° C. for about 48 hours, after which time it will be noticed that a considerable amount of the precipitate has dissolved, and that a brownish sediment has fallen to the bottom of the vessel. This sediment is nuclein, the albumin having gone into solution as peptone. The nuclein is collected on a filter paper, and washed free of impurity by means of 0·2 per cent. hydrochloric acid. In order to further purify it, the precipitate is dissolved in 0·5 per cent. ammonia water, and the nuclein reprecipitated by the addition of 5 per cent. acetic acid.

From the above method of preparation it will be noticed that both nucleo-albumin and nuclein are soluble in alkali, and insoluble in acid. In this respect they agree with mucin, and frequently they are confused with this body. They are distinguished from mucin, however, by the fact that they contain phosphorus. This can be detected by fusing some nuclein in a silver basin with potassium hydrate and potassium nitrate. By so doing the organic matter is burnt off as carbonic acid and ammonia, and the liberated phosphorus unites with the alkali to form a salt. After cooling, the mass is dissolved in water, transferred to a beaker, and made acid with nitric acid.

The technique of the method is as follows:

A weighed quantity of the organic substance (about 1 gr. nuclein) is placed in a silver basin and about 10-12 times its weight of caustic soda (NaOH) and a minute pinch of nitre (KNO_3) added to it. A small amount (2-3 c.cm.) of water is then added, and the silver basin placed on a boiling water bath until a solid cake has formed. The basin is then caught hold of by means of two pairs of crucible tongs and held over a free flame till the cake melts, the basin being meanwhile gently rotated so as to keep its contents in motion and thereby prevent spirting. The heating is continued, a pinch of nitre being added about every minute, until all the carbon, which soon separates out as black specks, has been oxidised. Great care must be taken that the oxidation is not too rapid (*i.e.* the nitre must be cautiously added and the flame kept low), and the basin must be continuously rotated to prevent spirting.

When all the carbon has disappeared and the 'fuse' has become colourless, the basin is cooled, water added, and its contents carefully washed into a beaker and the *P* determined by the usual gravimetric method. When burning a sub-

stance to liberate its sulphur, NaOH prepared from metallic sodium must be used, because ordinary NaOH contains *S* as an impurity.

The phosphorus is precipitated by adding about a fourth its bulk of a solution of ammonia molybdate containing nitric acid, and a fourth its bulk of a saturated solution of ammonium nitrate. The mixture is placed on the water bath at about 50° C., when a yellow precipitate of molybdenum phosphate will develop. If it be desired to quantitatively determine the amount of phosphorus present, this precipitate is collected on a filter paper, washed with a weak solution of ammonium nitrate, and then dissolved in a 3 per cent. solution of ammonia. The phosphorus is now precipitated from this solution by the addition of magnesia mixture, the resulting precipitate being allowed to stand overnight, and then collected on an ash-free filter paper, and incinerated in a crucible. The ash, which consists of $Mg_2P_2O_7$, is weighed, and from it the amount of phosphorus calculated.

Nucleic Acid.—If nuclein be decomposed by means of alkali, the albumin is split off and the nucleic acid combines with the alkali to form a salt, which can then be decomposed by the addition of acid alcohol, when the nucleic acid will separate out as a precipitate.

Mix 100 grammes of baker's yeast with 300 c.c. of a 3 per cent. sodium hydrate solution, and thoroughly stir the mixture for about 5 minutes. Now add weak hydrochloric acid till the solution is neutral, and then acetic acid till no more precipitate of the proteid results. Filter off the proteid and add water to the filtrate to make its volume equal to 300 c.c. Then add 5 c.c. of a 20 per cent. solution of hydrochloric acid so as to obtain, approximately, a mixture containing 0·4 per cent. HCl. Now add an equal bulk of spirit containing 0·4 per cent. HCl, when the nucleic acid will be precipitated. It is collected on a filter paper,—and if it be desired to still further purify it—is dissolved in weak ammonia water and again separated from proteid, as above described.

Chemically, nucleic acid consists of a compound of phosphoric acid, and alloxuric or nuclein bases (for the chemistry of these see p. 179). The phosphoric acid can be detected by the method described under nuclein. *In order to isolate the nuclein bases* the nucleic acid, or organ containing it, is boiled with 0·5 per cent. sulphuric acid for four hours; filtered; the filtrate treated with acetate of lead to separate the proteid; the lead removed by means of sulphuretted hydrogen, and the bases precipitated in the lead-free filtrate by means of an ammoniacal solution of silver nitrate.

The nucleic acids separated from different organs and tissues yield different bases, and they are sometimes classified according to the nature

of these.[1] Thus, the nucleic acid obtained from the thymus yields mainly adenin, whereas that obtained from the pancreas almost entirely guanin. Besides these alloxuric bodies, other decomposition products have been obtained, such as a body called *Thymin*, so called because it was first obtained from the thymus nucleic acid. It has since been obtained from several other varieties of nucleic acid.

Several nucleic acids also contain a carbohydrate in their molecule, thus pancreatic nucleic acid yields a pentose, and yeast nucleic acid a hexose and a pentose. On the other hand, thymus nucleic acid does not yield any carbohydrate.

CHAPTER IV.

FATS AND ALLIED BODIES.

Method of Extracting an Organ or Tissue with Ether.—The simplest method is by means of Soxhlet's apparatus (Fig. 269). This consists of an extracting chamber into which opens, near the top, a side tube, connected below with a flask, in which is placed the ether. This flask is placed on a water bath, and the ether passes into the chamber, and then into a Liebig's condenser, where it is condensed and trickles back into the extracting chamber, in which it gradually accumulates till it reaches the level of the bend in the side tube when syphon action is established, and the whole of the ether drains into the distilling flask. The tissue or organ to be extracted is placed in the extracting chamber, being wrapped up in a piece of filter paper. The lukewarm condensed ether as it accumulates in the chamber dissolves out the fat, and carries it into the distilling flask. The process should be allowed to proceed for several hours. The contents of the distilling flask are then removed to a flat dish, and the ether allowed to evaporate.

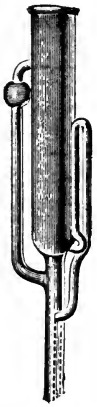


Fig. 269.—Soxhlet's apparatus.

Method for Separating Neutral Fat and Fatty Acid.—Fatty acid combines with sodium carbonate to form a soap, whereas neutral fat does not. In order to separate the one from

[1] More usually, however, they are named according to the organ from which they are separated.

the other, therefore, the method is to heat the substance containing these two bodies on a water bath with a half-saturated solution of sodium carbonate, until the mass has become nearly dry. The soap, formed by the sodium carbonate uniting with the free fatty acid, is separated from the unchanged neutral fat by dissolving the residue in water, and shaking the resulting solution in a separating funnel with ether. The ether takes up the neutral fat, the water takes up the soap, and the two fluids can easily be separated from one another by allowing the mixture to stand, when the ether rises to the top and the underlying water can be run off by opening the tap. The soap solution can then be decomposed by pouring it into a warm solution of 20 per cent. sulphuric acid, when the *fatty acid* rises to the top as an oily layer and can be removed with a glass rod after cooling. The *neutral fat* is obtained by evaporating away the ether.

Tests for Fatty Acid.—Apply the following reactions to some fatty acid prepared as above.

(1) Place some fatty acid on a piece of ordinary paper, and hold the paper near a flame. The fatty acid will melt and produce a greasy stain on the paper.

(2) Dissolve a small piece of fatty acid in ether, and add to the solution two drops of a saturated solution of phenol-phthaleine[1] in absolute alcohol, and then, drop by drop, a very dilute solution of sodium hydrate until the solution becomes distinctly red. Repeat this experiment with a solution of neutral fat in ether, and note that, in this case, the red colour develops with much less alkali.

(3) Place a small piece in a half-saturated solution of sodium carbonate, warm and shake, when the fatty acid will dissolve, a solution of soap being formed, which gives a lather on shaking.

Divide the *soap solution* into two parts, *a* and *b*.

To a add a few drops of a solution of calcium chloride—a white precipitate of calcium soap falls down.

To b add some lead acetate solution—a white precipitate of the lead soap falls down (lead plaster).

(4) Apply the acrolein reaction (see p. 182). It is negative.

The fatty acids prepared by the above method usually consist of a mixture of *Palmitic*, *Stearic*, and *Oleic*. These differ from one another mainly in two points, viz., in their melting points and in the case with which they can be precipitated by lead acetate. Those reactions are taken advantage of in separating them from one another.

[1] An indicator which is red with alkali and colourless with acids, and which is specially sensitive towards fatty acids.

To Separate the Solid (*i.e.* palmitic and stearic) from the Fluid Fatty Acids (*i.e.* oleic).—Melt the fatty acids in a beaker, and add to the resulting fluid about four times its bulk of 70 per cent. alcohol. Place the beaker on the boiling water bath for a few minutes, and then filter quickly through a folded filter. Allow the filtrate to cool, when the solid acids will separate out as a crystalline mass, whereas the oleic acid will remain in solution. The two can then be separated by filtration. The further separation of stearic from palmitic acid is a laborious process, and consists of the addition of an alcoholic solution of lead acetate in small quantities at a time to a solution of the acids in alcohol. Each addition produces a precipitate which is filtered off and treated with dilute hydrochloric acid and ether. The acid decomposes the lead salt and the liberated fatty acid goes into solution in the ether. This process is called fractional precipitation, and the higher the melting point of the acid the more easily is it precipitated by the lead acetate.

To Estimate the Melting Point of a Fatty Acid or other Body.—The substance is placed in a capillary tube of such a width that a pin can easily be pushed into it. This tube is tied on to the bulb of a thermometer so that the substance is opposite the centre of the bulb. The thermometer is then placed in a test-tube, which is suspended in a combustion flask containing concentrated sulphuric acid, which is very gradually heated over a thick asbestos plate. The substance is very carefully observed as the temperature rises, and the exact temperature at which it begins to melt is noted.

Lecithin. *Preparation.*—The principle of the method depends on the fact that lecithin is not so soluble in cold ether as the other fatty bodies are, but it is easily soluble in alcohol at 60° C. The yolks of two eggs are shaken with *cold* ether until the ether is no longer pigmented. The residue is then extracted with methylated spirit at a temperature of about 60° C. The alcoholic extract is evaporated to dryness, and the residue frequently washed with cold ether. The purified residue is dissolved in as little absolute alcohol as possible, filtered, and allowed to evaporate slowly, when the lecithin remains as a gummy mass. (The lecithin can be obtained as small round crystals by cooling the alcoholic solution to 5° C.)

Reactions and Tests for Lecithin.—These depend entirely on its chemical constitution, and consist in the recognition of the various decomposition products. It will be remembered that lecithin consists of a central molecule of glycerin, two of the hydroxyl radicles of which are combined with a fatty acid, and the third one with phosphoric acid, and that this latter is combined with a body called cholin. By boiling with baryta water the fatty acid separates as an insoluble

soap, the cholin is liberated and goes into solution, and the glycerin and phosphoric acid remain combined with one another as glycerin-phosphoric acid.

In order to obtain these decomposition products, it is not necessary to prepare pure lecithin as described above. All that is necessary is to extract the yolks of several eggs with warm ether in a Soxhlet's apparatus. The ether is then evaporated off and the residue, which will contain lecithin, boiled in an evaporating dish with baryta water, prepared of such a strength that, for each yolk used, 5 grammes of solid barium hydrate and 50 c.c. of water are employed. The boiling should proceed for about one and a half hours, water being meanwhile added to replace that which is lost by evaporation. The solution is then filtered to remove the insoluble soaps which have formed, and the filtrate is freed from barium by passing a stream of carbon dioxide gas through it. The barium-free filtrate is then divided into two parts *A* and *B*.

A is slowly evaporated to dryness, and the residue melted in the silver basin with nitre and caustic potash, the melt dissolved in water, and the phosphorus is precipitated. The presence of phosphorus shows that the extract contained glycerin-phosphoric acid, as any free phosphates, which may have been contained in the ethereal extract of yolk, would have combined with the baryta to form an insoluble salt.

B is also evaporated to dryness, and the residue extracted with absolute alcohol and filtered. The alcoholic extract is treated with an alcoholic solution of platinic chloride (1 in 10). After standing an hour or so, the precipitate of the double salt of cholin and platinic chloride, which separates out, is filtered off, washed with alcohol, and then dissolved in water. The watery solution is placed in a desiccator over sulphuric acid, when the cholin salt crystallises out as six-sided orange-coloured plates.

Lecithin forms the chief constituent of the medullary sheaths of nerves, and consequently can be obtained in great abundance by extracting the white matter of the brain with warm alcohol. Such an extract on cooling deposits crystals of a body called *Protagon*, which on boiling with baryta water yields, besides the decomposition products of lecithin, a body called *Cerebrin*, which contains nitrogen, and yields on hydrolysis with an acid a carbohydrate which has been identified as galactose.

It is said that when nerve degeneration is taking place, as in inflammatory affections of the nervous system, cholin is detectable in the cerebro-spinal fluid and in the blood (Halliburton and Mott).

Cholin is a strongly basic body, and may be considered as ammonium hydrate, in which three of the hydrogen atoms are replaced by three methyl radicles and the third by oxy-ethyl:

$$N\begin{cases}(CH_3)_3\\CH_2—CH_2OH\\OH.\end{cases}$$

Lecithin sometimes exists in combination with proteid, the compound being called *Lecithin-albumin.*

Cholesterin.—The chemical relationships of this body are not well known, and consequently the reactions and tests are entirely empirical. Besides those described in the elementary portion, the most important is **Salkowski's reaction.**

A few crystals of cholesterin are placed in a thoroughly dry test-tube and are dissolved in a few c.c. of chloroform. An equal bulk of concentrated sulphuric acid is now added, and the mixture is well shaken for about a minute and then allowed to stand. The chloroform swims to the top and is coloured, at first red and afterwards purple, and the acid sinks to the bottom and takes on a green fluorescence. If some of the chloroform solution be removed to another test-tube, and shaken with a drop of water it becomes colourless, to be again coloured by adding more sulphuric acid.

CHAPTER V.

MILK.

THE conversion of caseinogen into casein takes place in two stages. The first action of the rennet is to change the caseinogen into a body called soluble casein; this then combines with calcium salts to form an insoluble compound called caseinate of calcium. In order to study the conditions necessary for the clotting of milk a solution of caseinogen should be prepared by the following method (Ringer's):

300 c.c. of milk are mixed with an equal bulk of water, and 10 per cent. acetic acid is added till all the caseinogen has been precipitated. The precipitate is filtered off and thoroughly washed with distilled water until the washings are no longer acid in reaction. It is then removed from the filter paper, and ground up in a mortar with solid calcium carbonate. The resulting paste is thrown into 500 c.c. of water placed in a tall vessel, and the solution is allowed to stand for several hours. The fat, which was contained in the precipitate, rises to the surface, the calcium carbonate sinks to the bottom, and the

intervening fluid contains the caseinogen in combination with calcium as calcium caseinogenate, which is soluble in water (Osborne).

Three samples of the opalescent solution are removed by means of a pipette, and placed in three test-tubes labelled *A*, *B*, and *C*. To *A* are added a few drops of rennin; to *B* a few drops of a 0·5 per cent. phosphoric acid and some rennin; to *C* a few drops of a 0·2 per cent. solution of calcium chloride and some rennin.

The three test-tubes are placed in the water bath at 40° C., when it will be noticed that coagulation occurs only in *B* and *C*, in which, besides the ferment, soluble calcium salts are present.[1] In *A*, although no visible change has taken place, the caseinogen has been converted into the so-called *soluble casein*, and all that is necessary for the production of clotting is the presence of calcium in solution. That this is so can be demonstrated by boiling the solution *A* so as to destroy the ferment, then cooling and adding a few drops of a 2 per cent. solution of calcium chloride, when a clot will at once form.

The Chemical Nature of Caseinogen.—Caseinogen is of the nature of a weak acid. It is insoluble in water, but, if a few drops of a weak alkali be added, it at once dissolves because it forms a salt which is soluble. Shake up some pure caseinogen (prepared as described on p. 186) in a test-tube with distilled water; it does not dissolve. Add some powdered calcium carbonate and shake. The caseinogen, being acid in nature, expels a certain amount of the carbonic acid, and takes its place, forming caseinogenate of calcium, which is soluble. That partial solution of the casein has taken place can be proved by filtering. The filtrate is opalescent, and gives a precipitate of caseinogen on adding a weak acid.

In another test-tube shake up some caseinogen with a weak alkali, such as lime-water: the caseinogen dissolves, and an opalescent solution is produced. If all the caseinogen has not dissolved, filter. Now add to the opalescent solution sufficient litmus solution to colour it faint blue, and then neutralise carefully with a 0·5 per cent. solution of phosphoric acid. This combines with the excess of calcium hydrate to form calcium phosphate, and the caseinogen becomes swollen up so that a solution looking like skimmed milk is obtained, especially on slightly warming the solution. Besides these reactions caseinogen salts are precipitated by the addition of weak acids or by neutral salts, used in the same strengths as for globulins (p. 176). Their solutions are *not* coagulated by boiling.

Chemically caseinogen is of the nature of a *Pseudo-nuclein* (p. 179), and

[1] The phosphoric acid added to *B* brings some of the Ca salts suspended in the opalescent fluid into solution.

therefore, can be shown to contain phosphorus. If it be digested with pepsin solution of the usual strength a certain amount of the proteid is split off as peptone, and the sediment which settles down will be found to contain a much higher percentage of phosphorus than the original casein (for detection of this see p. 179). If, on the other hand, a strong pepsin solution be employed, and the digestion be allowed to proceed for several days, all the proteid will become changed into peptone, the phosphorus partly separating as phosphates, and partly remaining in organic combination with the albumoses and peptones which are simultaneously formed.

The Quantitative Determination of the various Bodies in Milk.—The methods here described can be employed for other fluids besides milk.

(1) **The Percentage of Water.**—A weighed quantity of milk is mixed with a weighed quantity of fine quartz sand, which has been previously heated to redness and then cooled in a desiccator. The weight of the mixture is accurately determined, and it is then placed in a hot air bath heated to 100° C. until all the water has been driven off and the weight is constant. The amount of weight lost corresponds to the amount of water which the sample of milk contains.

(2) **The Percentage of Proteid.**—Three grs. of milk are diluted with four times its volume of distilled water, a few c.c. of a solution of sodium chloride are added, and then a solution of tannic acid until all the proteid has been precipitated. The precipitate is filtered off through an ash-free filter paper, and thoroughly washed with distilled water. The filter paper with the precipitate is removed to a Kjeldahl's combustion flask, and the nitrogen estimated as described on p. 242. The result multiplied by 6·37 gives the total amount of proteid contained in the sample of milk.

(3) **The Percentage of Fat.**—The dietetic value of a milk depends to a large extent on the amount of fat it contains. There are, therefore, numerous methods employed for the quantitative estimation of this, some of which are only approximate. The following method (Adam's) will be found very simple and sufficiently accurate for most purposes:

Measure 5 c.c. milk and drop it on to a strip of Adam's fat-free porous paper;[1] allow this to dry in the air bath at 60° C., then roll it up and place it in the extractor of Soxhlet's apparatus (see p. 432). The weight of the distilling flask is ascertained before beginning the extraction, and then again after the extraction has been allowed to proceed for about one hour and the ether has been distilled off; the increase of weight gives the amount of fat in 5 c.c. of milk. Sufficient

[1] The paper can be obtained from any of the dealers.

ether should be used to fill the Soxhlet one and a half times, and it should be made to siphon over at least twelve times.

(4) **The Percentage of Sugar.**—Ten c.c. milk are mixed with twice that amount of alcohol (meth. spt.) so as to precipitate all the proteid, which is then filtered off. The precipitate is thoroughly washed with alcohol, and the washings are added to the filtrate. Filtrate and washings are then placed on the water-bath till all the alcohol has evaporated. The contents of the evaporating basin are then carefully washed into a 100 c.c. measuring cylinder, and the volume made up to 100 c.c. This is then placed in a burette and titrated with boiling Fehling's solution as described on p. 274. Ten c.c. Fehling's solution correspond to 0·0676 g. lactose, therefore the number of c.c.'s of the diluted extract required contains 0·0676 gr. lactose. In order to calculate the percentage it must be remembered, that each c.c. of the solution in the burette corresponds to 0·1 c.c. of the original milk.

(5) **The Percentage of Ash.**—A weighed quantity of milk is evaporated to dryness on a water bath in a weighed crucible. The crucible is carefully heated over a free flame until a perfectly dry and black ash has been obtained. The flame is now strengthened and the ash is heated until it becomes white. The crucible is then allowed to cool in a desiccator, after which it is weighed.

CHAPTER VI.

MUSCLE.

The Extractives of Muscle.—The most important of these are **Creatin, Hypoxanthin** and **Xanthin** ("alloxuric bodies"), and **sarcolactic acid.** They can be separated from the same muscle extract by the following procedure:

500 grammes of meat, from which as much fat and tendon as possible have been removed, are passed through a mincing machine; the mince is stirred up with 500 c.c. water in a large evaporating dish or enamelled basin, and heated on a water bath to about 55° C., for about half an hour. The extract is strained through muslin, and the residue extracted several times in a similar manner, the extracts being mixed together. The proteid in this extract is then coagulated by boiling and, after cooling, the coagulum is removed by filtration. So far the preparation of the extract should be carried out

by the demonstrator, or a similar extract may be prepared by dissolving some commercial meat extract in water.

To remove the phosphates and the last traces of proteids from this extract a saturated solution of subacetate of lead is added to it until no more precipitate is produced. (Care should be taken that an excess of the subacetate solution is not added. This may be ascertained by filtering samples of the extract and seeing if these yield further precipitates with the subacetate solution.)[1] The precipitate thus obtained is removed by filtration.

The excess of lead is precipitated from the filtrate by passing a current of sulphuretted hydrogen through it. The precipitate of lead sulphide is removed by filtration. The filtrate is then evaporated to small bulk (any sulphur which may separate out being removed by filtration) and allowed to stand on ice for two or three days, when a large number of crystals of *creatin* will have separated out. These are collected on a filter (and for this purpose a suction pump will be found necessary) and are thoroughly washed with alcohol until no more pigment is removed. The filtrate is preserved for the isolation of the other extractives.

The Chemistry of Creatin.—If the oxygen which is attached to the carbon atom of a urea molecule be displaced by an amido group, the body called *guanidin*

$$NH=C<\begin{matrix}NH_2\\NH_2\end{matrix}$$

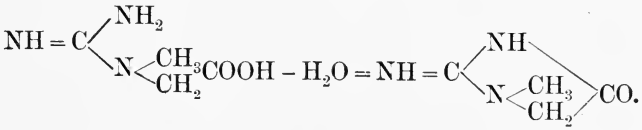
is obtained. If the two hydrogen atoms attached to one of the side amido groups be displaced, the one by a methyl radicle and the other by acetic acid, we obtain methyl-guanidin-acetic acid, which is *creatin*. It is prepared synthetically by the union of cyanamide $CN.NH_2$ and sarcosin (methyl amido acetic acid $CH_2.NH(CH_3).COOH$). When cyanamide is hydrolysed by boiling it with baryta water it yields urea, and, consequently, when creatin is similarly treated it yields sarcosin and urea. Thus:

$$\underset{\text{(Creatin)}}{NH=C<\begin{matrix}NH_2\\N<\begin{matrix}CH_3\\CH_2\end{matrix}COOH\end{matrix}} + \underset{\text{(Water)}}{H_2O} = \underset{\text{(Urea)}}{O=C<\begin{matrix}NH_2\\NH_2\end{matrix}} + \underset{\text{(Sarcosin)}}{CH_2-NH<\begin{matrix}CH_3\\COOH\end{matrix}}.$$

On account of this close chemical relationship it has been supposed that

[1] The following amounts are suitable for this preparation: Ten gr. bovril are dissolved in 200 c.c. water, and to this is slowly added 60 c.c. of a saturated solution of subacetate of lead. After the precipitate has settled down a sample of the supernatant fluid is removed by a pipette to a test-tube and tested with the subacetate solution to be certain that no more precipitate is produced.

creatin is the chief precursor of urea in the tissues (see p. 253). Creatin crystallises in oblique rhombic prisms (Fig. 150). It is insoluble in alcohol but soluble in water, especially in the heat. There are no tests by which it may be recognised, but it is very easily changed into creatinin by boiling it with a mineral acid, the reaction being that it loses a molecule of water. Thus:



(Creatin) – (Water) = (Creatinin)

Dissolve a few crystals of creatin in dilute hydrochloric acid, and place the test-tube in a boiling water bath. After about twenty minutes, cool the test-tube and apply the tests for creatinin.

This process of dehydration takes place during the excretion of creatin into the acid urine. It takes place with great ease and this must be borne in mind when examining any organ or tissue for the relative amounts of creatin and creatinin.

The Alloxuric Bodies.—The creatin-free filtrate is made strongly alkaline with ammonia, and is then mixed with ammoniacal solution of silver nitrate. The alloxuric bodies are thus precipitated. The precipitate is collected on a filter paper and thoroughly washed with dilute ammonia, and the hypoxanthin and xanthin, which are the alloxuric bodies represented in muscle, are separated from it by the following method: the precipitate is removed from the filter paper and dissolved in boiling nitric acid (spec. grav. 1·1), a few crystals of urea being added to the solution so as to destroy any nitrous acid which may be present, and which would decompose the alloxuric bodies. When all the precipitate has dissolved the solution is quickly filtered hot, and the filtrate is allowed to stand over night, when it will be found that a precipitate consisting of fine needle-shaped crystals (Fig. 270) has separated out. This consists of hypoxanthin silver nitrate combined with nitric acid; to remove the nitric acid wash it with distilled water, transfer it from the filter to a small beaker and boil it with ammonia until the crystals break up and become amorphous, and then, to remove the silver, pass in H_2S, filter off the silver sulphide, and evaporate the filtrate slowly to dryness, when a white chalk-like mass of *hypoxanthin* will be obtained. In order to obtain the xanthin silver salt the filtrate from hypoxanthin should be treated with ammonia, when a few yellow flakes of the salt

will be obtained. To separate the *xanthin* this precipitate is treated in exactly the same way as for hypoxanthin.

Test for Hypoxanthin.—Place a piece of hypoxanthin in a small evaporating dish with a few drops of concentrated pure nitric acid and evaporate slowly to dryness : a brilliant yellow residue is obtained.

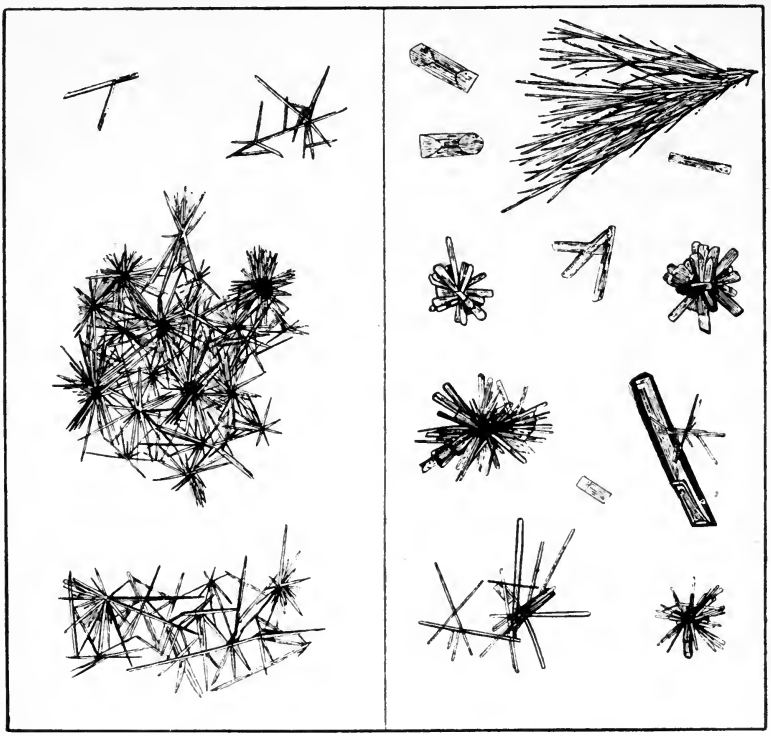


FIG. 270. FIG. 271.

FIG. 270.—Hypoxanthin silver nitrate. × 300.
FIG. 271.—Zinc sarcolactate. × 300.

Cool, and then add a drop of sodium hydrate solution, when the residue will change to orange. If the residue be dissolved in water and the solution again evaporated to dryness the orange colour persists, thus differing from the murexide stain which, when similarly treated, loses its colour (see p. 258).

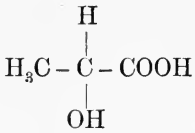
Test for Xanthin.—Repeat the same test as for hypoxanthin and note that the sodium hydrate produces in this case a deep red colour, which persists on dissolving in water and evaporating.

Sarcolactic Acid.—The ammoniacal filtrate, from which the alloxuric bodies have been separated, is treated with sulphuretted hydrogen gas

so as to remove the silver which it contains: the silver sulphide is filtered off, and the filtrate evaporated till all the ammonia has been expelled. It is then made strongly acid with phosphoric acid, and the lactic acid, which is hereby liberated, is dissolved out by shaking it in a separating funnel with ether (the method employed is the same as that described for separating fats and soaps on p. 182).

After extracting three or four times, the ethereal extracts are combined and the ether evaporated away by placing on a water bath heated to about 60° C., the flame underneath which has been extinguished. An acid syrup remains behind; this is impure lactic acid. In order to purify it, dilute three times with water, bring the resulting solution to the boil, and then carefully add powdered zinc carbonate until the reaction is neutral. Filter. Evaporate the filtrate to small bulk, and add an equal bulk of spirit and allow to stand, when *zinc sarcolactate* will crystallise out (Fig. 271). The zinc salt is filtered off, washed several times with spirit, dissolved in water,[1] and the zinc separated by passing a stream of sulphuretted hydrogen through the solution. The zinc-free filtrate is then freed of water by evaporation, when the *lactic acid* is obtained as a syrup.

Chemical Reactions and Tests.—The third member of the fatty acid series is called Propionic acid, and has the formula CH_3CH_2COOH. If one of the hydrogen atoms of the central methyl group of this be replaced by a hydroxyl radicle, we obtain lactic acid, which has, therefore, the formula $CH_3—CH(OH).COOH$. By examining this formula more closely it will be seen to contain an asymmetric carbon atom; that is to say, an atom of carbon whose affinities are occupied by four dissimilar radicles. Thus:



Now, it will be remembered when studying carbohydrates that, when such a condition as this exists, the substance has stereochemical properties, *i.e.* that it rotates the plane of polarised light, and, moreover, that it presupposes the existence of two bodies with the same structural formulae, and consequently possessing the same chemical reactions, but differing from one another in their stereochemical behaviour, one rotating to the right, the other to the left. It also presupposes that there may exist a body which is optically inactive, being composed of

[1] The watery solution should be evaporated until the crystals of zinc sarcolactate begin to appear, this being ascertained by examining a drop under the microscope.

an active and an inactive molecule. There are accordingly three lactic acids, and two of these, viz., the inactive and dextrorotatory occur in the body. The former of these is produced by the fermentation of lactose (see Milk), and the latter is sarcolactic acid.

There are two important chemical reactions for both forms of lactic acid. The one is *Uffelmann's reaction* (see p. 188). The other consists of the fact that their *zinc and calcium salts* crystallise in small four-sided prisms, and their calcium salts in fine needles arranged in clusters (Fig. 271).

The amount of lactic acid increases very much during the death of the muscle, and it is also said to be increased in amount by muscular activity. It must be remarked, however, that in order to demonstrate a distinct increase after muscular contraction, it is necessary to subject the muscle to prolonged stimulation, and that, after this, the death of the muscle is accelerated. It is probably from the proteid, and not, as was at one time supposed, from the glycogen that the lactic acid is derived.

Carnic Acid.—If a weak solution of ferric chloride be added to a muscle extract (from which the proteids have been removed by boiling and the phosphates by the addition of calcium chloride and ammonia) a brown precipitate is obtained. This is called *Carniferrin*, and consists of the iron salt of a body called phospho-carnic acid. If, further, the phosphoric acid and iron be split off from this we obtain carnic acid, and this, curiously enough, has the same formula as, and gives nearly all the reactions of, anti-peptone.

CHAPTER VII.

DIGESTION.

Gastric. The Acidity of the Gastric Juice.—Besides Günzberg's test for free mineral acid, there remains another very delicate one known as the **Tropaeolin test.**

Place a drop of a saturated solution of Tropaeolin—OO in 94 per cent. methylated spirit on a white slab, and dry it with moderate heat (40° C.). To the still warm, dry, yellow stain which remains apply a drop of the filtered gastric juice. A blue violet colour is produced if free hydrochloric acid be present. The reaction is obtained with other mineral acids besides hydrochloric, but only if these be present in considerable amount.

It must be noted that a negative result with either Günzberg's

or the Tropaeolin test does not exclude the presence of a trace of hydrochloric acid, as peptone, etc., may mask the reaction. If the reactions be positive, however, the presence of free hydrochloric acid may be assumed.

Tests for Organic Acids (lactic, acetic, and butyric).—As explained above, these acids become developed when there is a deficiency of hydrochloric acid in the gastric juice, for then micro-organisms grow in the gastric contents, and, by their action on the food-stuffs, lead to the production of organic acids.

In order to test for them, it is best to extract the gastric contents (vomit) with ether. For this purpose shake up about 20 c.c. of the fluid with about five times its bulk of ether in a separating funnel as described on p. 492. Remove the ethereal extract, and divide it into two portions *A* and *B*; place each portion in a porcelain basin. Allow *B* to stand exposed to the air, and place *A* on a warmed water bath. After the ether has evaporated in *A*, dissolve the residue in water and apply Uffelmann's reaction (see p. 188)—a positive result indicates *lactic acid.* When the ether has evaporated from *B*, dissolve the residue in water and divide the resulting solution into two parts. Neutralise one of these with sodium carbonate, and add a drop of a very dilute solution of ferric chloride—the red colour indicates the presence of *acetic acid.* To the other part add a small fragment of calcium chloride. If oily drops appear on the surface *butyric acid* is present. Since both these acids in *B* are volatile, they would be driven off unless the ether had been evaporated at low temperature.

Estimation of Total Acidity of Gastric Contents.—A measured quantity (10 c.c.) of filtered gastric contents is mixed in an Erlenmeyer's flask with ten times its bulk of distilled water. Two or three drops of a solution of phenol-phthalein are added, and the solution is titrated with *n*/10 sodium hydrate solution (see page 245) until a permanent pink colour is just obtained. The number of c.c. of soda required is read off, and the result expressed as the amount of *n*/10 alkali required to neutralise the acids in 100 c.c. filtered gastric contents. Thus, an acidity of 40 would mean that 40 c.c. of *n*/10 sodium hydrate had been required to neutralise the acids of 100 c.c. gastric contents. The result may also be expressed in terms of HCl, and this is the method most useful in physiology. 1 c.cm. *n*/10 alkali equals 0·00365 gr. HCl (see p. 245). If, for example, 100 c.cm. of gastric juice require 50 c.cm. *n*/10 alkali to neutralise it, the acidity in terms of HCl will be 0·1825. In other words the *percentage of HCl* will be 0·1825.

Estimation of Hydrochloric Acid present (Method of Mörner and Sjöqvist).—10 c.c. filtered gastric contents are placed in a silver basin (although a good porcelain evaporating dish may do), and mixed with pure powdered barium carbonate. The barium combines with the hydrochloric acid to form barium chloride; and with a lactic acid to form barium lactate. The whole is carefully evaporated to dryness on the water bath, and the residue is incinerated over a free flame. By this treatment the barium lactate is oxidized to barium carbonate, whereas the chloride remains unchanged. After cooling, the residue is treated with boiling water and filtered. The filtrate contains the barium chloride. It is acidified with sulphuric acid and boiled, whereby the barium is precipitated as barium sulphate. When the precipitate has settled it is collected on an ash-free filter, dried, incinerated in a platinum crucible, and the barium sulphate thus obtained weighed. One molecule $BaSO_4$ equals two molecules HCl, or, expressed gravimetrically, 233 parts $BaSO_4$ correspond to 73 parts hydrochloric acid.

As was mentioned on p. 222 the chief function of HCl in the gastric juice is to assist the action of pepsin. In doing so, the acid combines with the proteid. The avidity of proteoses and peptones for the acid is much greater than is that of native proteids; consequently, as artificial digestion proceeds, and proteoses gradually accumulate in the digest, the free acid gets less and less in amount. If the gastric contents be removed, by means of a stomach tube, during the first two hours after a proteid meal, however, no free HCl can be detected in them, but gradually makes its appearance later. This is because the gastric glands continue to secrete HCl till an excess is present, whereas in an artificial digest no such addition is made.

The usual method for quantitatively estimating the free acid is to titrate it with a decinormal alkali using Gunzberg's reagent as indicator. A much more accurate method,[1] however, is to compare the amount of inversion—measured by the polariscope—of a cane sugar solution which a measured quantity of filtered juice can produce with the inversion which a known quantity of acid produces on a similar cane sugar solution (*i.e.*, of the same original dextro-rotatory power). If the solutions be of the same volume, and both be incubated for the same length of time, the calculation of the free acid in the unknown solution is by simple proportion.

Inversion in known sol. : Invers. in unknown sol. :: HCl in known sol. : X the amount of acid in unknown sol.

[1] This method depends on the fact that only free acid can exercise this catalytic action which causes the inversion of cane sugar. The method was originally worked out by Hofmann on Ostwald's suggestion. Details of its simpler application are given in the *Lancet*, Vol. CLXV., p. 313.

Method of Preparation of Pure Gastric Juice.—The most satisfactory method for obtaining pure gastric juice, unmixed with semi-digested food, is *Pawlow's*. This consists in removing the secretion from an isolated pouch of stomach so prepared that the nervous and blood connections are the same as those of the stomach itself (see Part I., Chapter xliv., p. 156).

The methods employed for obtaining the ferment from the gastric mucosa after death, always yield an impure product on account of the ferment adhering to the proteoses, which are always present.

To Prepare the Extract the thoroughly washed stomach of the pig is taken, and the mucosa is scraped off with a knife. The scrapings are mixed with a large excess (100 times their bulk) of 0·4 per cent. hydrochloric acid, and the mixture is digested for several hours in the incubator. The extract is then filtered through muslin, and may be employed for general work without further purification. In order to separate the pepsin from the excess of proteoses which this infusion contains, the digestion should be allowed to proceed for several days, so that the proteoses may become changed into peptones. The product is saturated with ammonium sulphate crystals—the resulting precipitate of undigested proteoses, which carries down the pepsin with it, is pressed free of fluid and again incubated for several days with 0·5 per cent. hydrochloric acid, after which the digest is again saturated with ammonium sulphate, the resulting precipitate being approximately pure pepsin. The ammonium sulphate can be removed from the preparation by dialysis through parchment.

The scrapings, after being treated with weak acid to convert the pepsinogen into pepsin, can also be extracted with glycerin. This is the method which is most used commercially.

Method of Separation of Products of Gastric Digestion.—Fibrin is boiled first with tap water, and then with 0·1 per cent. hydrochloric acid to purify it. It is placed for 1-2 hours in an incubator, along with five times its volume of artificial gastric juice prepared as above, or with five times its bulk of 0·2 per cent. hydrochloric acid and a sixth its bulk of commercial peptic extract.

The products of digestion can be separated from this digest by the following process:

(1) Boil the solution in a beaker or basin, cool and separate the coagulated **native proteid** by filtration.

(2) Carefully neutralise the filtrate with 1 per cent. sodium carbonate solution; the **acid albumin**, which is precipitated, is separated by filtration.

(3) The resulting filtrate is now saturated in the cold, with ammo-

nium sulphate crystals. This precipitates most of the *proteoses*. Filter and wash the precipitate with saturated ammonium sulphate solution. Preserve the filtrate and washings which contain peptone and traces of secondary proteoses (label it *B*), and proceed to examine *the precipitate* (label it *A*).

(4) Dissolve the proteose precipitate *A* in distilled water. Remove samples from the resulting solution, and apply the tests for proteoses (nitric acid, salicyl sulphonic acid, biuret, etc. (see p. 223). Place the remainder in a large evaporating dish, and boil with powdered barium carbonate till no more ammonia gas is evolved. This removes ammonium sulphate from the solution, the barium uniting with the sulphuric acid to form insoluble barium sulphate, and the liberated carbonic acid and ammonia being driven off by the heat. Filter. The filtrate contains the proteoses. Saturate it with sodium chloride. A precipitate of **primary proteoses** results (label it *C*). Filter. The filtrate contains the **secondary proteose** which has not been precipitated by sodium chloride (label it *D*). The precipitate of primary proteoses (*C*) is washed with a saturated solution of sodium chloride, and dissolved in distilled water. The resulting weak saline solution is placed in a dialyser against running water for several days, and then against distilled water. The precipitate, which forms in the dialyser, is **hetero-proteose** as this is insoluble in distilled water. Collect it on a filter paper, wash and dry. The filtrate contains **proto-proteose**, which may be precipitated by the addition of alcohol, and the precipitate filtered and dried. The filtrate (*D*) containing **secondary or deutero-proteose** is acidified with acetic acid, whereby the proteose is precipitated. It is washed with alcohol and dried.

The Impure Peptone Solution (*B*) is now saturated with ammonium sulphate crystals at boiling temperature, first in acid reaction, and then in alkaline reaction. It is then cooled and filtered. By this means all traces of proteose are removed. The filtrate is then boiled with solid barium carbonate till no more ammonia is given off, cooled, and filtered. The peptone in the filtrate may be precipitated by adding alcohol. The peptone, thus obtained, is of two kinds, **anti-** and **hemi-peptone.** These may be separated from one another by subjecting the powder to tryptic digestion, whereby the hemi-peptone is further digested, the anti-peptone remaining unchanged (see Tryptic Digestion, p. 226). Anti-peptone, prepared by this method, has recently been shown to be impure containing both basic and acid organic substances. By another method of preparation (precipitation with iron salts) Siegfried has however shown that such a body does actually exist and appears to be identical with carnic acid (see p. 445).

The various reactions described on page 224 should be applied to each of the separated products.

Method of Estimating Activity of Pepsin Solutions. (1) **Grutzner's Method.**—Fibrin, purified as above described, is stained with carmine solution,[1] and washed free of adherent stain. Equal weighed quantities are then placed in two test-tubes, and 10 c.c. of 0·2 per cent. hydrochloric acid are added to each. Equal quantities of the pepsin solutions which it is desired to test are added, and the tubes placed in the incubator. As the fibrin becomes digested the carmine is liberated, and stains the solution. The more deeply stained solution, therefore, contains the stronger ferment. The exact amount of carmine liberated may be determined by comparing the digests with an artificial scale consisting of ten solutions of carmine of different known strengths.

(2) **Mett's Method.**—A narrow glass tube, 1 to 2 mm. in diameter, is filled with egg white, and is then heated so that a column of coagulated albumin is obtained. It is then cut into segments of equal length and two of these are placed in a test-tube which contains the pepsin solution acidified with 0·2 per cent. hydrochloric acid. Two similar tubes are placed in another test-tube with the other pepsin solution. Both are placed in the incubator for several (10) hours. The length of dissolved proteid column is then measured in both cases, and the desired result is obtained by squaring this distance.

Thus if in one test-tube the length were 2, and in the other 3, the strength of the two pepsin solutions has the ratio of 4 to 9.[2]

CHAPTER VIII.

PANCREATIC DIGESTION.

The Products of Digestion of Proteids by Trypsin.—The method of preparing the digest, and the chemical nature of the products of it, have already been described on page 227. All that remains to describe here is the method for separating the more important of these products (Tyrosin, Leucin, and Antipepton). If it be desired to isolate the hexone bases, the method described on page 426 may be employed.

[1] Dissolve 1 gr. carmine in 1 c.c. ammonia and mix with 400 c.c. distilled water. Place in a loosely stoppered bottle till the smell of ammonia has become faint, and then cork tightly.

[2] This law is only approximately correct.

Separation and Properties of the Chief Products of Tryptic Digestion of Proteids.—200-250 gr. fibrin, or the whites of four eggs, are digested in alkaline reaction for 48 hours with a minced pancreas, several crystals of thymol being added to prevent excessive bacterial growth. The digest is made very faintly acid, and boiled to remove any native proteid or alkali albumin which it may contain. A sample of the filtrate is removed and tested for proteose. A negative result is usually obtained, since *no primary proteoses* are developed during tryptic digestion.

1. **Separation of Tyrosin.**—The remainder is evaporated on the water-bath to a thin syrup. This is allowed to stand on ice or in a cold place for several days. White flocculi of *tyrosin* separate out. These are filtered through fine muslin, and removed to a beaker by means of a jet of cold distilled water and washed several times with distilled water by decantation. They are then dissolved by boiling with water made alkaline by the addition of a few drops of ammonia, and the resulting solution is quickly filtered hot. The filtrate is heated till all the ammonia is expelled; it is then cooled, when the tyrosin separates out as a white precipitate. This is collected on a filter paper, washed, and dried. The following *reactions* may be applied to the resulting powder:

(1) Tyrosin is insoluble in cold water, slightly soluble in hot water, and very soluble in dilute alkali.

(2) A solution in hot water gives a red colour on the addition of Millon's reagent. This is because tyrosin contains an aromatic radicle (p. 172).

(3) *Piria's Test.*—Place some of the powder in a dried test-tube, add about 2 c.c. concentrated sulphuric acid, and place the test-tube in a boiling water-bath for half an hour. Now cool and dilute with water, transfer to an evaporating basin, and remove the sulphuric acid by adding powdered barium carbonate; filter off the barium sulphate, evaporate the filtrate to small bulk, and add a drop or two of very weak ferric chloride solution. A violet colour results. This reaction is due to the formation of tyrosin-sulphuric acid.

2. **Separation of Leucin.**—The tyrosin-free filtrate is evaporated till a skin of leucin forms on the surface. It is then mixed with several times its bulk of alcohol, whereby the **antipepton** (see p. 449) which it contains is precipitated, the leucin remaining in solution. The precipitate is removed by filtration, and the filtrate evaporated to dryness: the residue is mainly *Leucin.*

Reactions of Leucin.—(1) It is much more soluble in water than is tyrosin; it is soluble also in alcohol.

(2) When heated in a piece of dry glass tubing, a sublimate forms on the cool parts of the tube.

(3) Like other amido acids, it gives off ammonia gas when heated in a test-tube with a piece of solid caustic potash and a few drops of water. If the melt be cooled, dissolved in water, and then acidified with sulphuric acid, it gives a smell of valerianic acid on heating.

(4) *Scherer's Test.*—Heat some leucin with a drop of nitric acid on a piece of platinum foil, add to the dry residue some caustic potash, when a yellow stain results. Heat still further, and the stain rises up into a globule which runs off the platinum.

(5) Examine a solution of leucin with the polariscope (p. 421). It is *dextrorotatory* ($(\alpha)_D = 17{\cdot}5$). The leucin which is obtained by boiling proteid with baryta, or that obtained synthetically (by the action of ammonia on α-bromocaproic acid) is *optically inactive*, and the *laevorotatory* form may be obtained from this by allowing penicillium glaucum (a fungus) to grow on a solution of it. The fungus destroys the dextrorotatory part, but leaves the laevorotatory untouched.

The Diastatic Action of Pancreatic Juice.—This can best be studied with an infusion of minced pancreas prepared at 40° C. The method is the same as that for ptyalin (p. 218).

The Fat-splitting Ferment (the method of demonstrating this is described on page 231).

Steapsin is not soluble in glycerine, so that a glycerine extract of pancreas cannot be employed for this experiment.

Tryptophane.—If bromine water be cautiously added to a tryptic digest of several days' standing a deep violet-red colour will result, and if the mixture be shaken with amylic alcohol, this latter will take up the colour. The glyoxylic reaction will also be very distinct in the digest even after the biuret reaction has disappeared (*i.e.* after the proteid molecule has been quite destroyed). Both these reactions are, therefore, probably common to some decomposition product of proteid. Hopkins and Cole have recently shown this substance to be tryptophane, which is closely related in its chemical structure to certain of the aromatic substances which are produced by the bacterial digestion of proteids (see p. 238).

Tryptophane may be prepared by the following method: A large amount (500 gr.) of commercial casein[1] (plasmon or protene) is mixed with liq. pancreaticus (200 c.c. Benger) and 0·8 per cent. Na_2CO_3 and placed in the incubator for about a week. The ferment should be added half at the beginning and the rest three or four days later. Antiseptics should be added.

[1] The digest employed for the separation of leucin and tyrosin may also be used for the separation of tryptophane. It is better, however, to employ casein for this purpose.

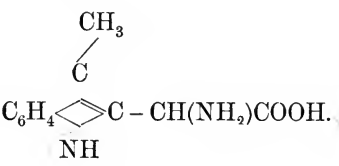
Digestion is allowed to proceed until the bromine water reaction is maximal. The digest is then boiled, cooled and filtered, and H_2SO_4 added to the filtrate, so as to bring the amount of H_2SO_4 in the latter to 5-6 per cent. If any precipitate is hereby formed it should be filtered off. The clear filtrate is then mixed with an excess of an acid solution of mercuric sulphate (10 per cent. mercuric sulphate dissolved in 10 per cent. H_2SO_4 and filtered). This reagent may precipitate besides tryptophane some tyrosin and cystin.

From tyrosin the precipitate is freed by washing it with 5-6 per cent. H_2SO_4, the mercury compound of tyrosin being very soluble in this. From cystin (which is scanty in a digest of casein) the tryptophane is separated by reprecipitation. For this purpose the washed mercury precipitate is suspended in water and decomposed with H_2S gas. To complete this reaction the suspension must be saturated with the gas, then warmed and saturated again. The HgS precipitate is filtered off, the filtrate warmed to rid it of H_2S, then acidified to 5-6 per cent. H_2SO_4, and the mercuric sulphate reagent added to it until a small permanent precipitate is produced. This is mainly cystin, and is filtered off. The tryptophane in the filtrate is then completely precipitated by mercuric sulphate, and the resulting precipitate treated exactly like the first one.

In this way a solution of tryptophane in 5-6 per cent. H_2SO_4 is obtained. The H_2SO_4 is now precipitated by adding $Ba(OH)_2$ water in the heat and filtering. Great care should be taken that the filtrate contains no excess either of H_2SO_4 or of $Ba(OH)_2$. The watery solution of tryptophane is then mixed with half its bulk of alcohol and evaporated on a water bath. During evaporation small quantities of alcohol are added from time to time to prevent the browning which occurs if watery solutions of tryptophane are heated alone. Evaporation proceeds till crystallisation commences, when the basin is removed and allowed to stand. The crystals (glistening plates) are collected on a filter, and, to purify them, may be recrystallised.

A solution of the crystals gives the bromine and the glyoxylic reactions very distinctly, and if the crystals be heated in a dry test-tube indol and skatol (see p. 238) are evolved.

Hopkins and Cole have shown the constitution of tryptophane to be skatol-amido-acetic acid, its formula, therefore, is :



By allowing anaërobic bacteria to grow in a nutritive solution containing tryptophane, the amido group of the side chain is split off as ammonia and skatol-acetic acid is formed. If aërobic bacteria are now introduced they oxidise the skatol-acetic acid, first of all into skatol-carbonic acid, then into skatol, then into indol (see p. 238).

BACTERIAL DIGESTION.

This is studied by allowing the artificial pancreatic digest to proceed *without* the addition of thymol. To obtain the **aromatic bodies** (see p. 227) which are produced, the following method is employed:

The digest is placed in a large flask connected with a Liebig's condenser and distilled, the distillate being received in a smaller flask. After distilling for about an hour, a sample is removed from the distillate, to which the following reactions for aromatic bodies may be applied:

(1) Note the penetrating faecal odour.

(2) **Legal's Test.**—Place about 5 c.c. of the distillate in a test-tube and add a drop or two of a weak watery solution of sodium nitroprusside. A red colour results, which is very much intensified by the addition of a drop of 20 per cent. caustic potash. Now acidify with acetic acid, when the red colour will be replaced by blue. These are the colours obtained when *indol* is in excess; if *skatol* be in excess the red is replaced by a yellow, and the blue by a violet colour.

Nitroso-indol Reaction.—Fuming nitric acid gives, with indol, a deep red colour, or the reaction may be still better performed by mixing the distillate with a drop of pure nitric acid and then carefully adding a 2 per cent. solution of potassium nitrite. Skatol does not give this so-called nitroso-indol reaction.

The remainder of the distillate is shaken with ether in a separating funnel. The ether dissolves out the aromatic bodies along with volatile fatty acid. The ethereal extract is, accordingly, shaken with weak caustic alkali which removes the fatty acid, and the purified ethereal extract is slowly evaporated to dryness, the residue consisting of a mixture of **indol** and **skatol**.

CHAPTER IX

BILE.

In connection with the composition of bile, it is of importance to note that the compound proteid which it contains is, in some animals—*e.g.* the ox—**nucleo-albumin**, whereas in others—*e.g.* man—it is **mucin**. In all cases it is probable that this proteid does not really come from the

liver cells, but is only added to the bile as it passes along the bile ducts. So far as can at present be ascertained, the amount of pigment and of bile salts do not bear a quantitative relationship to one another, so that it is improbable that they are both derived from the same source. Quantitative estimations of these two bodies in bile, obtained from a biliary fistule are, however, far from numerous, not only on account of the rarity of suitable cases, but because there is no accurate method for quantitatively determining the pigment.

Separation of Bile Salts. To Separate all the Bile Salts.—Thoroughly mix 50 gr. pure animal charcoal with 200 c.c. of ox-bile in an evaporating dish, and evaporate the mixture to dryness on a water bath. During the drying the mixture should be frequently stirred. The black powder thus obtained can be kept a considerable time. To extract the bile salts from it, mix it with absolute alcohol in a flask and place on the boiling water bath for about a quarter of an hour, cool, filter into a dry beaker, and add ether to the filtrate till a permanent haze is produced. Now cover the beaker with a ground-glass plate and allow it to stand in a cool place till next day, when it will be found that a crystalline mass of bile salts has separated out (**Plattner's crystalline bile**). The crystals can now be collected on a filter paper and allowed to dry in the air.

A 1 per cent. solution of the crystals should now be made, and Pettenkofer's reaction (see p. 233) applied to it by the following method:

Dissolve a few grains of cane sugar in the solution, and run concentrated sulphuric acid down the side of the tube so as to form a layer underneath the watery solution. A violet ring is formed where the two fluids meet. Now place the test-tube in a beaker of cold water, and shake gently so as to mix the two fluids. A violet solution is thus obtained. By cooling the test-tube in water too great a rise of temperature is avoided. Divide the violet solution into two parts, *A* and *B*. Add *A* to some ether and examine by means of the spectroscope—a distinct band is seen in the green. Add *B* to some absolute alcohol and note that, although the spectrum is at first the same as in *A*, a band gradually develops in the blue, and that, along with the development of this, the tint of the solution changes from green to brown.

To Prepare Pure Glycocholic Acid.—In certain districts of Germany and America it has been observed that the glycocholic acid can be separated from the bile by a very simple process, and, so far as it has as yet been tried, the bile obtained from oxen reared in this country appears to be suitable for the process.

The method is as follows:

Some ox bile is placed in a stoppered cylindrical vessel, and mixed with ether and hydrochloric acid in the proportion of ten parts of the former and four parts of the latter, for every hundred parts of bile. A few crystals of glycocholic acid are added to the mixture so as to start the crystallisation, the vessel is stoppered, vigorously shaken, and then allowed to stand in a cool place. After some time the mass will be found to be 'solid' with crystals. These are collected in a filter paper, and washed with cold distilled water till no more pigment can be removed. They are then removed to a flask and dissolved in boiling water; the solution is filtered hot, and the filtrate, on cooling, deposits numerous acicular crystals of the acid. These may now be collected, washed with distilled water, and dried (for Chemistry and Reactions see p. 233).

The other amido acid is **Taurin**, and is peculiar in that it contains sulphur.

Preparation of Taurin.—Bile from a carnivorous animal—cat or dog—is heated on a sand bath with one-third its bulk of concentrated hydrochloric acid until a resinous-like mass of the anhydride of cholalic acid (called *Dyslysin*) has formed. This can be drawn out into brittle threads by means of a glass rod. The dyslysin is filtered off and the filtrate is evaporated to small bulk, the sodium chloride, which crystallises out during the evaporation, being removed by filtration. The thin syrup is then poured into fifteen times its bulk of alcohol, and left standing twenty-four hours, when the *taurin* will have crystallised out. It can be purified by collecting the crystals on a filter paper, washing with cold water, redissolving in boiling water, and allowing the solution to cool.

Properties of Taurin.—It crystallises in long glancing needles. It is insoluble in alcohol, but soluble in boiling water. If heated on a piece of platinum foil it turns black, and gives off fumes of sulphur dioxide. If fused with solid sodium carbonate on a piece of platinum foil, and the melt dissolved in water, a solution is obtained which evolves sulphuretted hydrogen on the addition of a mineral acid. The sulphuretted hydrogen can be detected by means of a piece of filter paper soaked in lead acetate solution. Its chemical constitution is demonstrated by the following reactions:

If ethylic alcohol be treated with concentrated sulphuric acid, one of the hydrogen atoms is replaced by the radicle SO_3H, and isethionic acid is formed.

$$C_2H_4\begin{matrix}OH\\H\end{matrix} + OH - SO_3 - H = C_2H_4\begin{matrix}OH\\SO_3H\end{matrix} + H_2O.$$

Ethylic alcohol. Sulphuric acid. Isethionic acid.

If, now, the isethionic acid be treated successively with phosphorus pentachloride and ammonia it yields, first chlor-ethyl-sulphonic acid, and then the *Cl* of this becomes replaced by an amido group to form amido-isethionic acid which is taurine.

I. (*a*) $C_2H_4\begin{matrix}OH\\SO_3OH\end{matrix} + 2\,PCl_5 = C_2H_4\begin{matrix}Cl\\SO_2Cl\end{matrix} + 2\,POCl_3 + 2\,HCl.$

Isethionic acid. Chlor-ethyl-sulphon-chloride.

(*b*) $C_2H_4\begin{matrix}Cl\\SO_2Cl\end{matrix} + H_2O = C_2H_4\begin{matrix}Cl\\SO_2OH\end{matrix} + HCl.$

Chlor-ethyl-sulphonic acid.

II. $C_2H_4\begin{matrix}Cl\\SO_2OH\end{matrix} + 2\,NH_3 = C_2H_4\begin{matrix}NH_2\\SO_2OH\end{matrix} + NH_4Cl.$

Taurine.

CHAPTER X.

URINE.

In the following chapter the various headings will be taken up in exactly the same order as in the elementary course, and, since the theoretical points in connection with the chemistry have already been fully discussed there, it will be necessary to deal here only with certain technical details in connection with the separation and quantitative estimation of the various urinary constituents.

Kjeldahl's Method for the Estimation of Nitrogen.—*Incineration.*—This process may be materially quickened by adding to the contents of the combustion flask some metallic oxide. The oxides most commonly employed are those of mercury and copper, more especially those of copper, since it has been shown that mercury combines with a certain amount of the liberated ammonia to form a compound from which it is not expelled by the subsequent distillation with caustic alkali. The oxide itself is not added, but the sulphate and the amount of this required for 5 c.c. of urine is 0·5 gr.

As a rule, about 3 gr. of potassium sulphate are also added. This becomes changed into potassium pyrosulphate in the heated acid, and this has the property of very materially assisting the oxidation.

The addition of these two bodies is especially necessary, where bodies rich in carbon are being oxidised. They are, therefore, invariably added when it is necessary to burn filter paper, or where the nitrogen in milk, muscle, or any other food-stuff is being determined.

Distillation.—In order to keep the end of the distilling tube just touching the surface of the fluid in the receiving flask, the latter should be placed on a platform which can be raised or lowered by a screw (see Fig. 150).

Instead of employing litmus to ascertain when all the ammonia has distilled over, a solution of *alizarin* may be used. This is yellow in acid solution, and pink to purple in alkali. A drop is allowed to run down the distilling tube, (the end of which is meanwhile removed from the surface of the distillate) so that the alizarin mixes with the hanging drop of the distillate. If this latter still contain ammonia a purple colour is developed, if there be no ammonia the orange colour remains unchanged.

Titration. Method for standardising a deci-normal solution.—It is necessary that a stock solution of very accurately standardised acid be kept, with which other standard solutions may be compared. The best acid to employ for this purpose is, I think, sulphuric; it can easily be obtained pure, it keeps well, and it can be used for all the acidimetric processes necessary in medical chemistry. To standardise it, anhydrous sodium carbonate is employed. About 5 grammes of pure sodium carbonate are heated to dull redness in a crucible for ten minutes, cooled in an exsiccator, the exact weight taken, some transferred to an Erlenmeyer's flask, the weight again taken, and the amount removed thus estimated. Several accurately weighed samples are removed in this manner, and each is dissolved in distilled water, the solutions being then coloured yellow by methyl orange solution. The acid, the strength of which it is desired to determine, is now run into the sodium carbonate solutions until the neutral point is attained, the exact amount necessary in each case being noted. The data for making the calculation are now at hand.

FIG. 272.—Flask for accurately measuring fluids.

100 c.c. of a normal acid should exactly neutralise 5·3 grm. sodium carbonate, *i.e.* that 100 c.c. of a $\frac{n}{10}$ acid should neutralise 0·53 grm.

Suppose 0·246 grm. sodic carbonate required 41·5 c.c. of the acid, then

$$0{\cdot}246 : 0{\cdot}53 :: 41{\cdot}5 : x = 89{\cdot}4$$

Again suppose that another sample containing 0·2153 gm. required 36·32 c.c. of the acid, then

$$0{\cdot}2153 : 0{\cdot}53 :: 36{\cdot}32 : x = 89{\cdot}4.$$

To correct the acid, therefore, place 894 c.c. of the acid in a litre flask (Fig. 272) and fill up to 1000 c.c. with distilled water, when a $\frac{n}{10}$ solution will be obtained, 100 c.c. of which will exactly neutralise 0·53 grm. sodic carbonate.

PREPARATION OF UREA.

1. **From Urine.**—To about 400 c.c. urine add barium mixture (1 vol. saturated barium nitrate solution mixed with 2 vol. baryta water) until there is no further precipitate of sulphates and phosphates. Filter and evaporate the filtrate,—at first over a free flame, afterwards on a water bath—to a thin syrup. Now mix this syrup with about 100 c.c. methylated spirit and, after allowing the mixture to stand for about half an hour so that the precipitate of inorganic salts may settle, filter the alcoholic extract into an evaporating dish and evaporate it nearly to dryness on a water bath. Allow the residue to cool, and then add to it about double its volume of concentrated pure nitric acid, meanwhile placing the basin in a dish of cold water, and stirring the contents with a glass rod so as to accelerate the formation of the urea nitrate. After about half an hour the crystals of urea nitrate are filtered off by means of a suction filter, sucked as dry as possible, and then placed between several thicknesses of filter paper between which they are pressed so as to dry them. In order to convert the nitrate into urea, the crystals are placed in an evaporating dish and dissolved in as little water as possible; the basin is then placed on a heated water-bath, and powdered barium carbonate added with a penknife in small quantities until the fluid reacts neutral. By this treatment the urea nitrate is decomposed, the nitric acid combining with barium to form barium nitrate, and the urea being thereby liberated. The mixture is now filtered, the filtrate evaporated to dryness and the urea taken up from the residue by extracting with absolute alcohol, which does not dissolve the barium nitrate. The alcoholic solution of urea is now evaporated to dryness, when a mass of urea crystals is obtained.

The above process may be considerably curtailed by omitting the preliminary precipitation of phosphates, etc. with barium mixture, the evaporated urine being simply mixed in a test tube with nitric acid, which is kept cool by submersing it in a beaker of water. The crystals of urea nitrate are then filtered off, dried between filter paper and treated with barium carbonate as above described.

2. **Separation of Urea from Blood, Serous Fluids or Watery Extracts of Tissues.**—About 100 c.c. of the fluid are mixed with four times its volume of methylated spirit, vigorously shaken and allowed to stand over night. By this treatment the proteids are coagulated, whereas the spirit dissolves the urea. The coagulum is now filtered off, washed with spirit, and the washings are combined with the filtrate, the whole being then evaporated to dryness on a water-bath. The residue is

extracted with absolute alcohol, the extract filtered, again evaporated to dryness and re-extracted with absolute alcohol, this process being repeated until the evaporated residue is entirely dissolved in the alcohol. The purified residue is now cooled by placing the dish containing it on ice, and is mixed with one or two drops of pure nitric acid, the mixture being allowed to stand on ice till next day when it is examined for crystals of urea nitrate.

The alcoholic extracts usually contain a considerable amount of fatty acid which may mask the separation of urea nitrate. To remove this, the first alcoholic extract should be mixed with a few drops of a solution of basic lead acetate till no more precipitate is produced, after which a few drops of a solution of ammonium carbonate are added to cause the suspended precipitate of lead soaps to settle down. The solution is then filtered, and the lead removed from the filtrate by passing a stream of H_2S gas through it.

Estimation of Urea. Mörner and Sjöqvist's Method.—*Principle.*—By the addition of certain reagents to a measured quantity of urine, all the nitrogenous bodies except urea and ammonia are precipitated. The precipitate is removed by filtration, and, after expelling the ammonia by heat, the nitrogen of the filtrate is determined. This, multiplied by 2·143, gives the amount of urea present.

Solutions necessary.—

1. A saturated solution of chloride of barium containing 5 per cent. baryta.
2. A mixture of 1 vol. ether and 2 vol. absolute alcohol.
3. Apparatus, etc. for Kjeldahl's nitrogen determination.

Determination.—5 c.c. urine are mixed in a small stoppered flask with 5 c.c. of barium mixture and 100 c.c. alcohol-ether mixture, whereby a copious precipitate falls down. The flask is corked and left standing over night. The contents are then filtered through a small filter paper (10 c.m. in diameter), the filtrate being collected in a Kjeldahl combustion flask. When all the solution has passed through, the precipitate is washed at least three times with alcohol-ether mixture, and then the flask is placed on the water-bath heated to 60°C., a pinch of magnesium oxide being added to the contents so as to drive off the last traces of ammonia from the solution.[1] When the volume of fluid in the flask has reached about 10 c.c. concentrated sulphuric acid, cupric sulphate and potassium sulphate are added to it, and the nitrogen determined by Kjeldahl's process.

In order to accelerate filtration the suction pump may be used, a

[1] This stage of the process can be much accelerated by carefully transferring the contents of the flask to an evaporating dish which is then placed on a water bath at 60° C.

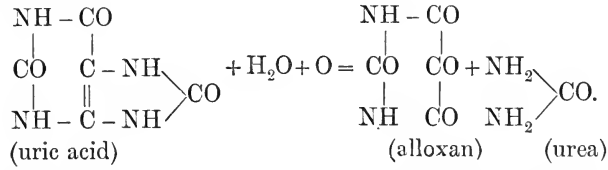
cork being fitted into the mouth of the flask and being doubly bored, one hole for the filter funnel, the other for a tube connected with a suction pump. By this means, also, a large amount of the alcohol and ether is removed through the pump.

Hippuric Acid.—The quantitative determination of this body is exceedingly difficult, and consists of extracting it by means of alcohol and ether from urine made alkaline with sodium carbonate.

CHAPTER XI.

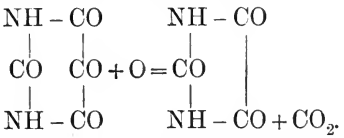
URINE—Continued.

Chemical Reactions which show the Relationship of Uric Acid to Urea.—*By Analysis.*—If uric acid be carefully oxidised in acid solution (*i.e.* by treating it with cold concentrated nitric acid) it decomposes into alloxan and urea:



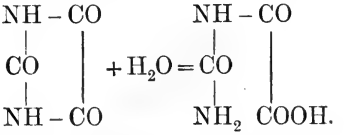
(uric acid) (alloxan) (urea)

If the oxidation be more energetic (as by using warm nitric acid) the alloxan is further split up into parabanic acid and carbonic acid gas:



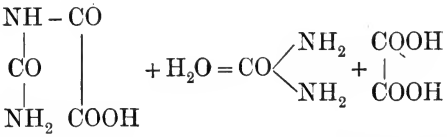
(alloxan) (parabanic acid)

If now parabanic acid be caused to take up a molecule of water (by heating it with alkalies) it changes into oxaluric acid



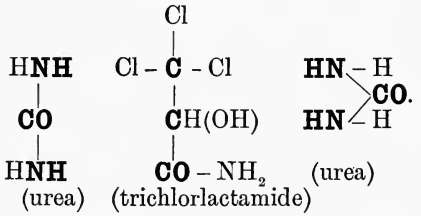
(parabanic acid) (oxaluric acid)

By prolonged boiling with water this is split up into oxalic acid and urea:



(oxaluric acid) (urea) (oxalic acid)

By analysis, therefore, we learn that uric acid contains two urea molecules (which are split off at different stages of oxidation) united together by a chain of carbon atoms (represented by the oxalic acid). It is therefore a diureide. *By Synthesis.*—Diureides are usually prepared by the condensation of hydroxy acids with urea, and in the case of uric acid, lactic acid is the hydroxy acid used, or still better, a substitution product of it, viz., tri-chlor-lactamide. By heating urea with this body the following reaction ensues:



The groups printed in thick type unite to form uric acid (after Hopkins).

Estimation of Uric Acid. Hopkins' Method.—*Principle.*—This has already been described in the elementary course (p. 259).

Reagents and Solutions necessary.—1. Pure ammonium chloride. (Must be entirely soluble in water and must not contain any iron salt.)

2. Pure ammonium sulphate. When the uric acid is to be estimated by titrating it against potassium permanganate, it is absolutely necessary that all traces of chlorine be removed from the ammonium urate precipitate. This is done by washing it with an ammonium sulphate solution (9 parts saturated solution + 1 part water).

3. A twentieth normal solution of potassium permanganate made by dissolving 1·581 gr. potassium permanganate in 1 litre of distilled water. 1 c.c. of this solution = 3·75 mg. uric acid.

Determination.—Weigh out 30 grammes powdered ammon. chlor. and add it to 100 c.c. of urine, stirring briskly until all the salt is dissolved. Now add a few drops of strong ammonia and allow the mixture to stand until the precipitate of ammonium urate has settled at the bottom of the beaker, and the supernatant fluid is quite clear. Then filter the supernatant fluid through a small filter, and wash the beaker with a saturated ammon. chloride solution so as to remove all traces of the precipitate, the washings being also passed through the filter paper. Finally, wash the precipitate itself with saturated ammon. chloride solution and, when the last washings have drained through, puncture the apex of the filter paper with a pointed glass rod, and wash the precipitate into the beaker in which the precipitation was performed by means of a fine jet of water which has

previously been brought to the boil in a wash bottle. In order to remove the last traces of precipitate from the filter paper it is necessary to open out the latter so that no precipitate remains in the folds.

The estimation of the uric acid contained in the solution of ammonium urate thus obtained, may now be determined by one of two methods:

1. *Weighing the uric acid directly.*—For this method it is necessary that the water used in the removal of the precipitate should not amount to more than 30 c.c.; if more than this amount has been employed, the liquid should be concentrated on the water-bath till this bulk has been attained. The urate is now decomposed by adding 1 c.c. of HCl (con.) and heating just to boiling point. The fluid is then set aside for the uric acid to crystallise out, and this is collected on a weighed filter (see p. 492), washed with cold distilled water, dried and weighed.

2. *Titrating the decomposed urate with standard potassium permanganate.*—Since chlorides have a certain power of decolourising permanganates, it is necessary that all traces of ammon. chlor. be removed from the precipitate. This is accomplished by washing it with a saturated solution of ammonium sulphate instead of ammonium chloride. The contents of the filter are then transferred to a small Erlenmeyer flask by means of a jet of hot water, and the volume brought up to 100 c.c., the water employed for this purpose being also used to thoroughly wash out the filter. Now add to the solution in the flask 15 c.c. concentrated sulphuric acid, and immediately titrate with the permanganate. The principle of the reaction is that the permanganate oxidises the uric acid, and, in doing so, loses its colour; whenever all the uric acid has been oxidised the permanganate will retain its red colour. The standard solution is, therefore, run into the acidified solution, when it will be noticed that, on the first additions, the red colour immediately disappears, but that, as more and more is added, the red takes longer to disappear, until at last a drop of the permanganate retains its colour and gives to the fluid on shaking a diffused pink flush. This is the end reaction. The number of c.c. used is now read off, and the amount of uric acid is thus calculated. For clinical purposes the titration method is certainly the one to be employed, the only part about which there is any difficulty (on account of its slowness in filtering) being the washing of the precipitate with ammonium sulphate solution.

The Estimation of the Total Alloxuric Bodies. Modified Camerer's Method.—*Principle.*—Ammoniacal silver nitrate, in the presence of neutral salts, or better, of magnesium mixture, combines with all the alloxuric bodies to form an insoluble salt of definite composition (see p. 206). The nitrogen in this can be estimated by Kjeldahl's

method, and the result expressed as total alloxuric nitrogen. This result is exceedingly useful in studying the metabolism of alloxuric bodies. If it be desired to determine the uric acid and the bases separately, a slight modification of the process is necessary.

Solutions necessary.—1. Magnesia mixture. This consists of 1 part crystallised magnesium chloride, 2 parts chloride of ammonium, dissolved in 8 parts of water and made strongly alkaline with 4 parts of ammonia. If the mixture be not quite clear (from the presence of magnesium hydrate) more ammon. chlor. should be added.

2. Ammoniacal silver nitrate. Dissolve 26 gr. silver nitrate in about 300 c.c. water, add ammonia to this until the precipitate of silver oxide, which first forms, redissolves. Dilute the solution to one litre.

3. Kjeldahl's apparatus and solutions.

Determination.—240 c.c. proteid free urine are mixed with 30 c.c. magnesia mixture, and the solution is made up to 300 c.c. by the addition of a 20 per cent. ammonia solution. This process is best done in a measuring cylinder. After the precipitate has settled, which it does in a few minutes, it is filtered through a dry folded filter and two portions of the filtrate are taken amounting to 125 c.c. each. Each of these corresponds to 100 c.c. of the original urine. They are both treated in exactly the same way, and should yield similar results. Each is mixed with 10 c.c. ammoniacal silver nitrate, and the mixture, after the precipitate has settled somewhat, filtered through an ash-free filter paper (of 10 c.m. diameter). The last traces of the precipitate are removed from the beaker by means of weak ammonia water. The next stage consists in washing the precipitate with distilled water until it is free from ammonia, as the presence of this would vitiate the determination of the nitrogen. In order to do this, the precipitate should be allowed to stand exposed to the air over night so that it may become partially dried, in which state the washing with water is much easier than when the precipitate is moist, for then it forms a gummy mass. The washing must be continued until the washings no longer react alkaline to litmus. In order to remove the last traces of ammonia, the filter paper, with the precipitate on it, is carefully removed to a Kjeldahl's combustion flask; about 50 c.c. of water are added, and then a little magnesium oxide. The mixture is then boiled whereby the magnesia expels the ammonia. The boiling is continued until only about 10 c.c. of fluid remain, and then sulphuric acid, etc., are added, and the nitrogen determined.

To Determine the Bases and Acid separately, a large quantity of urine (500 c.c.) is mixed with one-tenth its bulk of magnesium mix-

ture, the precipitated phosphates filtered off, and an aliquot part of the filtrate mixed with ammoniacal silver nitrate. The resulting precipitate of all the alloxuric bodies is collected on a filter paper, washed, transferred to a beaker, and decomposed by boiling it with a solution of sulphide of potassium prepared of such a strength that 10 c.c. are necessary for every 100 c.c. urine employed.[1] The sulphide of silver which is hereby formed is removed by filtration, and the filtrate acidified with hydrochloric acid, and slowly evaporated to small bulk (10 c.c.). The fluid is allowed to stand a few hours, when all the *uric acid* will have crystallised out. This is collected on a weighed filter, washed with cold water, and determined gravimetrically; or, it may be dissolved in an alkali and determined by the permanganate method. The *filtrate* from which the uric acid has been separated, combined with the water used to wash the latter, is made strongly alkaline with ammonia, and the *bases* are precipitated by adding silver nitrate. The precipitate is collected on an ash-free filter paper, washed free of ammonia, boiled in a Kjeldahl's flask with magnesia and water and the basic nitrogen determined. 1 gramme of nitrogen corresponds to 2·62 grammes xanthin.

As has been explained in the elementary course, it is of primary importance not only to know how much total alloxuric nitrogen is being excreted in the urine, but also, how much is being taken in the food. It is then possible to determine how much alloxuric nitrogen is derived from the tissues, or, in other words, we determine the *endogenous moiety*. There can be no doubt that it is owing to the neglect of this precaution, that there exist so many discordant observations concerning the metabolism of uric acid in health and in disease. It is of little use to know that the uric acid is greater in one person than in another for the difference may simply be due to the diet; on the other hand, a difference in the endogenous moiety would indicate a corresponding difference in the metabolism of the tissue nucleins.

CHAPTER XII.

URINE—Continued.

Creatinin: Quantitative Determination (Salkowski's Method).—*Principle.*—An alcoholic extract of evaporated phosphate-free urine is mixed with an alcoholic solution of zinc chloride, which combines with

[1] *Potassium sulphide solution.* Dissolve 15 gr. KOH in a 1000 c.c. distilled water; divide the solution into two equal parts and saturate one of these with H_2S gas; now mix the two halves.

creatinin to form a salt of known composition. By collecting this salt on a weighed filter, or better, by estimating its nitrogen, the amount of creatinin which it contains can be determined.

Solutions necessary.—1. Alcoholic zinc chloride. A concentrated watery solution of zinc chloride is poured into 90 per cent. alcohol until the specific gravity of the latter rises to 1·20.

2. Milk of lime—prepared by shaking quicklime with water.

3. 5 per cent calcium chloride solution.

Determination.—480 c.c. urine are mixed with milk of lime until faintly alkaline in reaction, and then with calcium chloride solution until no further precipitation of phosphates results. The volume is

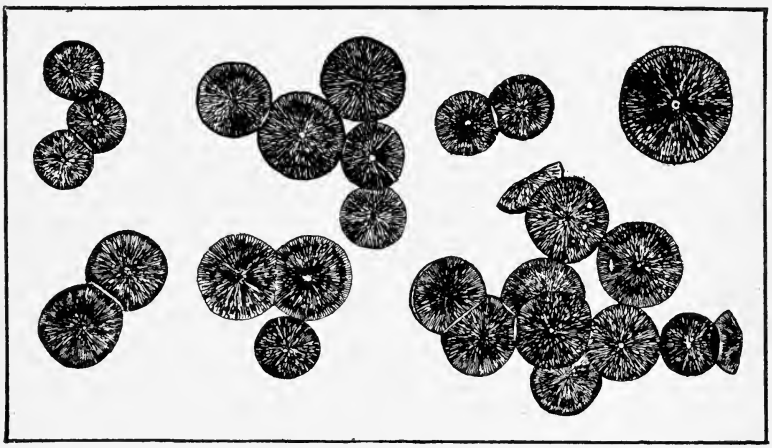


Fig. 273.—Creatinin zinc chloride. × 300.

made up to 600 c.c. by adding water, and the solution is filtered. 500 c.c. of the filtrate are rendered slightly acid by the careful addition of 10 per cent. acetic acid, then placed in a large evaporating dish, and evaporated, at first over a flame, but afterwards on a water-bath, until the volume is about 40 c.c. The resulting syrup is then transferred to a stoppered measuring cylinder of at least 200 c.c. capacity, the last trace of the fluid being washed into the cylinder by means of absolute alcohol. The solution is made up to 200 c.c. with absolute alcohol, and is then allowed to stand 24 hours so that all the sodium chloride may become precipitated. The solution is filtered through a dry filter paper, and of the filtrate two samples of 80 c.c. each are measured into small beakers, and mixed with 1 c.c. of alcoholic zinc chloride. The beakers are covered with a ground glass plate and placed in a cool place for at least three days, the contents being briskly stirred at least once a day. The creatinin zinc chloride separates out as a dirty

white precipitate adhering to the sides of the beaker. On microscopic examination the crystals consist of fine needles, which are grouped into rosettes or sheaths (see Fig. 273). The precipitate is collected, either on a weighed filter (see p. 492), or, if it be desired to determine the nitrogen present, on an ash-free filter.

In either case the precipitate is washed with absolute alcohol two or three times, and is then either dried and weighed, or is transferred along with the filter paper to a Kjeldahl's flask, and the nitrogen determined.

100 parts creatinin zinc chloride = 62·44 parts creatinin.
100 parts nitrogen correspond to = 269 parts creatinin.

The 80 c.c. alcoholic extract employed above correspond to 192 c.c. of the urine employed. By adopting the method described above (*i.e.* by taking aliquot parts of the various filtrates) the necessity of washing the various precipitates is obviated and much time is thereby saved.[1]

Creatinin also forms a double salt *with mercuric chloride* having, according to Johnson, the formula $4(C_4H_7N_3O \,.\, HCl \,.\, HgO)3HgCl_2$. This salt may be obtained by adding to urine a twentieth of its bulk of a cold saturated solution of sodium acetate, and a fourth of its bulk of a cold saturated mercuric chloride solution. An immediate precipitate of inorganic bodies and urates falls down, and if this be filtered off, the filtrate, on standing, gradually develops a further precipitate, said by Jonston and Colls to consist of pure mercuric creatinin. It has been suggested to collect this precipitate, dry and weigh it, and calculate therefrom the amount of creatinin present. On testing the method as above described I found that the results, when compared with those obtained by Salkowski's method, were very unreliable, and that if the first filtrate were warmed as suggested by Halliburton, in order to accelerate the precipitation of the creatinin compound, a black mass of reduced mercuric oxide was almost invariably obtained. Moreover, if the precipitate were collected and dried, and an estimation of its mercury contents made, it was found that the precipitate was far from pure. I found, however, that the impurities were, to a large extent at least, insoluble in 10 per cent. HCl, so that by washing the Johnson's precipitate with acid of this strength, an almost pure solution of the mercury salt in hydrochloric acid was obtained. This solution was then neutralised and its mercury contents estimated by a titrimetric method described by Hannay in Sutton's *Volumetric Analysis*, p. 241, and from this the amount of creatinin was calculated. The results thus obtained, when

[1] This method of estimating creatinin although the most accurate is almost prohibitive on account of the expense of absolute alcohol.

compared with those obtained by Salkowski's method showed the method to be a fairly reliable one. Before adopting it as a standard method, however, it will be necessary to study more fully the exact formula of the mercury compound, as there can be no doubt that this is variable.

The excretion of endogenous creatinin is increased after severe muscular work. It is markedly diminished in amount in certain blood diseases associated with enlargement of the spleen. Its excretion in dogs after removal of the spleen is, however, not affected. It is said also to be decreased in patients suffering from muscular atrophy, but I have been unable to verify this.

Ammonia.—*Estimation* (Schlosing's Method).—25 c.c. of urine are placed in a flat glass dish with vertical sides, and mixed with 20 c.c. milk of lime. The vessel is placed on a ground glass slab, and is covered by a glass triangle, resting on which is another vessel containing 20 c.c. of one-fifth normal or deci-normal sulphuric acid. The whole is covered with a bell jar, and left standing several days. The lime expels the free ammonia, which is at once taken up by the sulphuric acid. The amount of acid which has not been neutralised, is then determined by titration, as described on p. 244.

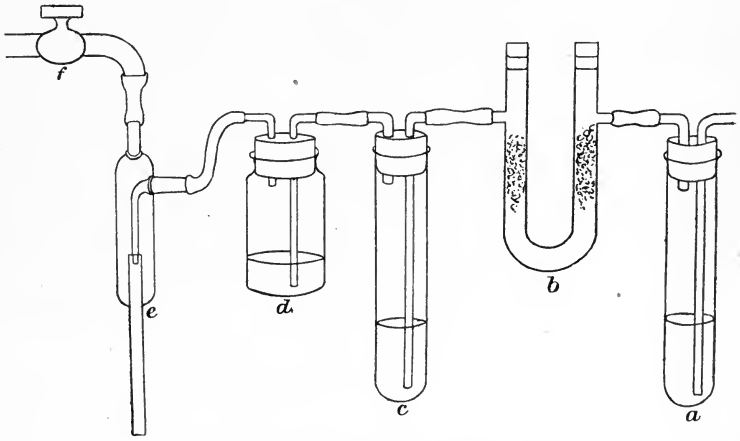


FIG. 274.—Folin's apparatus for estimating ammonia.

A much quicker and more accurate method for estimating ammonia is **Folin's.**

It depends on the fact that free ammonia is liberated from its salts when a solution of the latter is made faintly alkaline, and, by bubbling a fast current of air through such a mixture, the ammonia is carried away and may be collected and measured by passing this air through standard acid.

The technique of the method is as follows: 25 c.c. urine are placed in a large test-tube *a* ($2\frac{1}{2}$-3 cm. diam. and 20-30 cm. long) and are mixed with 8-10 gr. sodium chloride and 5-10 c.cm. petroleum (to prevent excessive frothing), and *lastly* with 1 g. sodium carbonate. The test-tube is closed by an indiarubber stopper through which pass two tubes, the one for the air inlet passing to the bottom of the test-tube, the other connecting the top of the test-tube with a wide tube (**U** tube) *b* containing a loosely packed cotton-wool plug (to catch any particles of fixed alkali which might be sucked over with the air current). This safety tube is connected with a second test-tube *c* (of the same size as the first) containing 5 c.c. *n*/10 H_2SO_4 + 5 c.c. H_2O, the tubing being so arranged that the air bubbles through the acid. A third tube or bottle *d* arranged as the second and also containing 10 c.c. *n*/20 acid follows this, otherwise all the ammonia would not be caught by the acid. The tubing connected with this tube goes to a Bunsen's air pump *e* attached to a tap *f*. A quick stream of air (600-700 litres per hour) is made to pass through the apparatus for $1\frac{1}{2}$ hours. The acid in the two last test-tubes is then washed into an Erlenmeyer's flask and titrated with *n*/10 or *n*/20 alkali. For titrating Folin recommends 2 drops of a 1% solution of Alizarin red (for 200-300 c.c. fluid), the titration being carried just till a pink (not a violet) colour appears. This indicator is not so easily affected by CO_2, ammonia salts, etc., as others are.

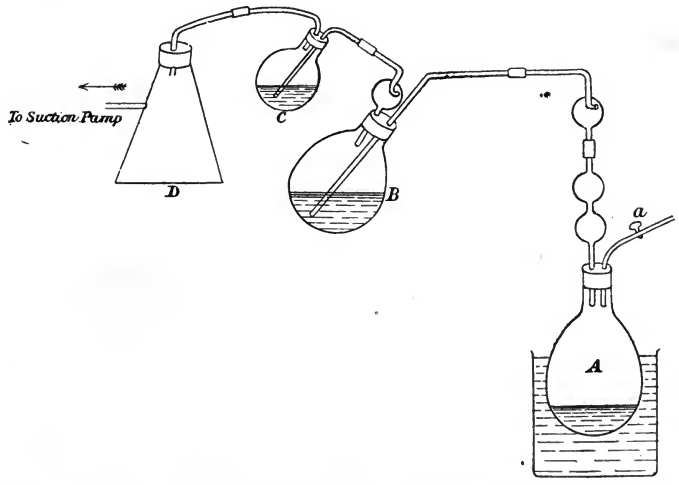


FIG. 275.—Shaffer's method of estimating ammonia in urine. Beddard's modification.

Shaffer's vacuum distillation method of estimating ammonia in urine is as accurate as **Folin's** and much more rapid.

Place 50 c.c. urine in a round bottom $\frac{1}{2}$ litre flask *A*, add 20 grams sodium chloride to prevent decomposition and 50 c.c. methyl alcohol to reduce the boiling point of the mixture. In flask *B* place 50 c.c. or less *n*/10 acid and in *C* 10 c.c *n*/10 acid, diluted in both cases with a little water. The flasks may be tilted obliquely and should be large enough to prevent loss of acid by jumping over during the violent commotion which is set up by the rapid passage of steam. If such loss should occur, the acid may be recovered by rinsing out the flask *D*. When the apparatus is ready, 1 gram of dry sodium carbonate is added to the liquid in flask *A*, the stopper is rapidly inserted and the suction started. The pump will quickly reduce the pressure to about 30 mm. and the liquid in *A*, which is warmed up to about 40° C. in a water-bath, will begin to boil. The temperature of the bath must be maintained and should not be allowed to rise above 50° C. for fear of decomposing urea. When the boiling has continued for fifteen minutes, all the ammonia will have been given off and the operation is stopped by slowly letting in air by the stopcock *a*. The acid in *B* and *C* is titrated, after a few drops of a 1 per cent. solution of Alizarin red have been added as the indicator.

Variations in Amount Excreted.—While discussing the metabolism of urea, it will be remembered that one of its chief precursors is ammonium salts. This fact would lead us to expect that the administration of those salts by the mouth would be followed by an increase in the amount of urea excreted. This is found, however, not to be the case with all the salts of ammonia, but only with those in which the acid radicle present is easily dislodged; whereas in those salts, such as chlorides, in which the acid radicle is very firmly attached, there is no increased excretion of urea, but instead of this an increased excretion of ammonia. It must be pointed out, however, that this statement regarding ammonium chloride is true only in the case of carnivorous and omnivorous animals. In the case of herbivorous animals, there are so many strong bases taken with the food that the ammonium chloride at once undergoes double decomposition in the tissues, the chlorine combining with sodium to form sodium chloride, and the ammonium combining with carbonic acid to form ammonium carbonate, which becomes at once transformed into urea. The administration of ammonium chloride to rabbits along with their ordinary food has, therefore, no effect on the excretion of ammonia, but causes that of urea to rise; but if the rabbit be starved, *i.e.* be caused to live on its own tissues, then an immediate increase in the ammonia excretion follows the administration of this salt, and the urea excretion remains unchanged.

A *second* condition which leads to an increased excretion of ammonia salts, is the presence of free acids in the tissues. This occurs in severe forms of Diabetes mellitus, where oxybutyric acid exists; or after the administration of mineral acids by the mouth. Now the presence of free acids in the tissues is incompatible with life,[1] and in order to neutralise the free acid some of the ammonia, which otherwise would have been transformed into urea, is utilised. An examination of the urine in these cases will show an increase in the ammonia excretion, with a corresponding decrease in urea. Of course, where other bases are present in sufficient amount to neutralise the acids present in the blood, the ammonia is left alone.

A *third* condition which influences the excretion of ammonia, and more especially its relationship to urea, is disease of the liver. Thus in acute yellow atrophy, where the liver cells become inactive, there is an enormous increase in the amount of ammonia excreted and a corresponding decrease in the urea. In phosphorus poisoning the same condition exists, the protoplasm of the cells being replaced by fat, and thereby rendered incapable of transforming the ammonia into urea. In less severe disease of the liver it seems probable that a tendency to the condition exists, viz. an increase of ammonia at the expense of urea. An estimation of these two bodies, therefore, should always be made where obscure disease of the liver exists, and although a disturbance in the relationship between them does not of necessity imply hepatic disease, it nevertheless furnishes a valuable aid to the diagnosis in doubtful cases.

CHAPTER XIII.

URINE—Continued.

Estimation of the Inorganic Constituents of Urine.—Each of these may be determined by the usual gravimetric chemical methods, but, in order to carry out this satisfactorily, a considerable amount of chemical technique, as well as the use of an accurate chemical balance, is necessary. It is usual, therefore, to employ instead a titrimetric method which yields, in most cases, sufficiently accurate results for physiological purposes, and which involves much less time than does the gravimetric method. The principle of the method

[1] Thus, the presence of free acids in diabetes probably accounts for the occurrence of diabetic coma, of which complication the majority of diabetics die. Similar symptoms of coma are produced by injecting acids into the blood vessels.

consists of the addition of a standardised solution of some well-known precipitant of the body which is to be determined, to a measured quantity of urine. A standardised solution is one of such a strength, that each cubic centimetre of it corresponds to a definite amount of the body which it is desired to determine. When sufficient of this solution has been added to precipitate all the substance, and a slight excess of it is accordingly present in the fluid—this being determined by some indicator—the number of c.c. of the precipitant employed is read off, and this result, multiplied by the standard of the solution, gives the amount of substance in the number of c.c. of the urine employed.

Estimation of Chlorides.—*The Standard Solution.*—Dissolve 29·075 grammes of fused argentic nitrate in a litre of distilled water: 1 c.c. = 0·01 gr. NaCL.

The Indicator.—A saturated solution of neutral potassium chromate. This gives a red precipitate with $AgNO_3$.

Titration.—Place 10 c.c. of urine, diluted to 100 c.c. water, in a porcelain basin, and add a few drops of the potassium chromate solution, till a distinct yellow colour is produced. The standard solution is now run in from the burette. As this solution comes in contact with the urine a red colour is produced, which disappears on stirring, until a slight excess is present, when an orange tint persists. From the number of c.c. of the standard solution employed 1 c.c. is subtracted, since the urine contains besides chlorides certain substances which combine with silver nitrate before the chromate does. A more accurate estimation of chlorides may be made by mixing 5-10 c.c. urine with 1 gr. Na_2CO_3 (Cl. free) and 2 gr. KNO_3 (also Cl. free); evaporating to dryness in a platinum crucible; incinerating the residue till a white ash is formed; cooling and dissolving in water. This watery solution is then titrated as above, but no deduction made from the reading of the burette. By this method the organic constituents of the urine are removed.

Estimation of Phosphates.—*The Standard Solution.*—Dissolve 35·5 gr. of uranium nitrate in a litre of water: 1 c.c. = 0·005 gr. phosphoric acid (P_2O_5). On account of the nitric acid which is liberated when this solution reacts with the phosphates, it is necessary to add to the urine a solution of sodium acetate, which will absorb the free acid and prevent it dissolving the uranium phosphate, for all phosphates are soluble in free nitric acid.

This solution is made by dissolving 100 gr. sodium acetate in 900 c.c. water, and adding to this solution 100 c.c. glacial acetic acid.[1]

[1] The acetic acid is added to make certain that all the phosphates are present as acid salts, since alkaline calcium phosphate decomposes in boiling solution into

The Indicator.—Tincture of Cochineal (Tinct. Cocci. B.P.). This gives a green colour with uranium, but not so long as any unprecipitated phosphates exist in the solution.

The Titration.—Place 50 c.c. urine in a porcelain basin, add 5 c.c. sodium acetate solution and cochineal until the solution is distinctly red. Now bring the solution to the boil and run in the standard solution from a burette, until a faint green colour persists even after boiling.

This result gives the *total phosphates* (*i.e.* alkaline and earthy).

The Earthy Phosphates may be determined by the following method :—200 c.c. urine are mixed with ammonia till strongly alkaline and left standing over night. The earthy phosphates are thus precipitated. They are filtered off, washed with dilute ammonia, removed from the filter paper to a basin by means of a wash bottle, and the precipitate dissolved by boiling it with a few drops of acetic acid. The resulting solution is made up to 50 c.c. and the estimation carried out as above.

By subtracting the earthy from the total phosphates, we obtain the amount of the *alkaline phosphates.*

Sulphates.—The volumetric method for the determination of sulphates is very unsatisfactory, on account of there being no coloured indicator for the end of the reaction. It will therefore be necessary to describe the gravimetric as well as the volumetric method.

I. The Volumetric Method.—The Standard Solution.—30·5 gr. crystallised barium chloride are dissolved in a litre of distilled water; 1 c.c. = 0·01 gr. of sulphuric acid SO_3.

The Indicator.—A 20 per cent. solution of sulphate of potassium or sodium.

The Titration.—100 c.c. urine are boiled in a flask with 5 c.c. of pure hydrochloric acid so as to decompose the ethereal sulphates, which otherwise do not give a precipitate with barium chloride. Now run in about 5 c.c. of the standard solution from a burette, boil and allow to stand till the precipitate has settled, then add a few drops more and see if the further precipitate is produced. If there be any doubt of this, remove some of the clear fluid to a watch glass which is placed on a piece of black glazed paper, and add a drop of the barium chloride solution. If a precipitate results replace the contents of the watch glass in the flask, and add more barium chloride. The titration must be carried on in this method until no more precipitate is obtained with barium chloride. A sample of the supernatant fluid is then mixed in

the normal phosphate which is insoluble in water and would not, therefore, react with the uranium.

the watch glass with a drop of the sulphate solution, when only a very slight haze should be obtained. It is necessary to repeat the determination several times before an accurate result can be obtained.

II. Gravimetric Method. The Total Sulphates.—Boil 100 c.c. of urine with 5 c.c. HCl as before, and add to the resulting solution a solution of barium chloride till no more precipitate is produced. Filter off this precipitate of barium sulphate through an ash-free filter paper, and wash it with boiling water till the washings no longer give a precipitate with sulphuric acid. Expose the filter paper and precipitate in an air-bath at 100° C., and when dry remove them to a crucible which is heated, at first gradually, but afterwards with a strong flame. When the mass has become white allow it to cool in a desiccator, then add a few drops of pure sulphuric acid and again heat very gradually to redness, taking great care that none of the contents of the crucible are lost by spirting.

The sulphuric acid is added to convert into sulphates any sulphides which may have formed.

Now cool in a desiccator, weigh and deduct the weight of the crucible, when the remainder will correspond to the amount of barium sulphate formed, 100 parts of which correspond to 34·33 parts of sulphuric acid (SO_3).

The Ethereal Sulphates. (Salkowski's method).—The inorganic sulphates are precipitated by means of an alkaline solution of barium chloride, the resulting barium sulphate is filtered off, and the filtrate is acidified with hydrochloric acid, and heated just to boiling point over an asbestos plate. The heat decomposes the ethereal sulphates, which at once combine with the excess of barium salts present.

In making a quantitative determination 100 c.c. urine should be mixed with 100 c.c. barium chloride solution; and of the filtrate only 100 c.c., corresponding to 50 c.c. original urine, should be taken for the estimation, as, by so doing, the necessity of washing the precipitate is obviated.

CHAPTER XIV.

THE METHODS FOR THE ESTIMATION OF GENERAL METABOLISM.

METABOLISM is the subject which treats of the changes undergone by the food stuffs after they are absorbed from the intestine. There

are two subdivisions of the subject; the one called, *general metabolism*, has to do with the building up or breaking down of the tissues. It derives its information from a comparison of the amount of the various food stuffs absorbed, with the amount of their excretory products. The other, called *special metabolism*, has to do with the exact chemical changes which absorbed food stuffs undergo, and the localisation of the actual organ or organs in which the various changes are effected.

Space will only permit us to indicate some of the methods employed in studying general metabolism, and to describe briefly how the results obtained may be interpreted. The actual methods of analysis have already been fully described in the previous chapters, and in the following description reference will be made to the pages on which the most suitable method for each determination can be found.

General Metabolism.—In order to study this a balance sheet must be drawn up, on one side of which is placed the *intake* (the amount of food and oxygen absorbed), and on the other the *output* (the amount of the various bodies excreted in the urine, faeces, breath, and sweat).

I. **The Intake.**—The value of a diet can be expressed either as its chemical value, or as its physical value. The *chemical value* means the amount of proteid, fat, carbohydrate, and salt which it contains. This is determined by referring to analytical tables of the various food stuffs (especially serviceable for this purpose are the tables of König. The amount of the various food stuffs administered can then be easily determined by multiplying the percentage given on the tables by the amount of food given. When it is desired to be specially accurate an actual analysis of the food is necessary, and when the metabolism of proteid is being specially studied, it is customary to determine the amount of nitrogen which the food stuff contains (Kjeldahl's method, p. 243), and this multiplied by 6·3 gives the amount of proteid.

The *physiological heat value* of a diet means the number of calories which it can yield during its metabolism in the body. To find the total heat value of the diet, all that is necessary is to multiply the physiological heat values of the administered food stuffs by the amount of each which the diet contains.

The Form in which the Food Stuffs are best given for Metabolism Experiments. Proteid.—This is usually given as meat, from which all the visible fat and tendon are, as far as possible, removed. When calculating the amount of proteid from the nitrogen present, the gelatin and extractives which the meat contains may be neglected, for gelatin, in the presence of an excess of proteid, has the same metabolic value as native proteids, and the extractives exert no influence on the

metabolism since they pass through the tissues unchanged. Proteid may also be administered as white of egg or milk.

Carbohydrate.—That is best given as bread a day old, and always obtained from the same source, so that its composition is constant.

Fat.—About 1 per cent. fat has to be reckoned as contained in the meat prepared as above. The rest is best given as butter.

When the investigation is being carried out on an animal, the whole diet should be weighed out in the morning, after collecting the previous day's excreta. It is seldom necessary to cook the food, but where there is difficulty in persuading the animal to take some unpalatable food substance, this latter may be mixed with the soup prepared from the meat. When the experiments are being carried out on man, it is of course necessary to cook the meat, and frequently also some of the other food stuffs. The various constituents must be weighed out before cooking, as it is impossible to know, after the food has been prepared, the proportion of the substances used in cooking. For observations extending over any length of time the diet should be carefully chosen, and exactly the same amounts given each day.

II. **The Output.**—By referring to the following schema it will be seen, that the only food stuff which contains nitrogen and sulphur is proteid. We have, therefore, two excretory products from the amount of which we can determine proteid metabolism. In the case of carbohydrates and fats, on the other hand, there is no exclusive end product, so that, in order to estimate the metabolism of these two bodies, it is necessary to make a calculation.

$$\text{Proteids}\left\{\begin{array}{l} \text{S}\text{———}\text{H}_2\text{SO}_4 \\ \text{Fats} \left\{\begin{array}{l}\text{N}\\ \text{C}\\ \text{O}\end{array}\right\}\text{CO}_2 \\ \text{Carbo.} \left\{\begin{array}{l}\text{H}\\ \text{O}\end{array}\right\}\text{H}_2\text{O} \end{array}\right\}\text{urea, etc.}$$

1. **Proteid.**—The output of this is determined:

(*a*) **From the Amount of Nitrogen Excreted.**—Nearly the whole of this occurs in the urine in which it is determined by Kjeldahl's method (p. 243). A certain amount, however, appears in the faeces. With an ordinary diet most of this latter comes from the unabsorbed proteid, and must accordingly be deducted from the amount administered in order to ascertain the actual amount absorbed. A certain amount of it, however, comes from nitrogenous bodies, which are excreted into the intestine from the blood. The actual amount of this excretory nitrogen has been determined by feeding an animal with a proteid free diet, and for man it amounts on an average to 1 gr. per diem. During starvation it only amounts to 0·2 gr. so that it is obvious that it comes

from the digestive juices poured into the intestine for the digestion of the food.[1]

The amount of nitrogen excreted in the sweat is so small as to be negligible. The urinary nitrogen, plus one gramme per diem, as nitrogen excreted into the intestine, gives us, therefore, the total amount of nitrogen excreted. Since proteid contains 16 per cent. of nitrogen, each gramme of nitrogen corresponds to 6·25 gr. of proteid, and since meat contains on an average 3·4 per cent. of nitrogen, each gramme of the latter will correspond to 30 gr. of muscle.

(*b*) **From the amount of Sulphur Excreted.**—Proteids contain 1 per cent. of sulphur. This is excreted in the urine as sulphuric acid, and the amount of this excreted bears a constant relationship to that of nitrogen, viz. 1 of sulphuric acid for every 5·2 gr. of nitrogen. Being less in amount, its determination is not nearly so accurate as that of urea, but it affords us a valuable *control* in estimating proteid metabolism, and is the only way by which we can estimate this when nitrogenous bodies other than proteid are contained in the diet.

2. **Fat and Carbohydrate.**—The end products of the metabolism of both these bodies are water and carbon dioxide gas, and, of these two bodies, the only one which it is possible to estimate with anything like accuracy is the latter. Proteid, however, also contributes to the excretion of carbon dioxide, so that, before we can know how much carbohydrate and fat are being oxidised in the body, we must find out what proportion of the total carbon excreted is derived from the metabolism of the proteid.

To estimate the total amount of carbon excreted, the expired air must be collected, and a determination of the amount of carbon dioxide which it contains made by one of the methods described. The obtained result multiplied by 0·273 gives the amount of *carbon excreted in the breath.* A certain amount of *carbon is also excreted in the urine.* This latter amount could be directly determined by making an elementary analysis of the dried urine, but such a method would, of course, be too laborious for metabolism work. In order to determine this amount of carbon all that is necessary is to multiply the nitrogen excreted by 0·67, for it has been determined that, for every gramme of nitrogen excreted, there is this amount of carbon, and that this ratio is a constant one.

[1] In accurate metabolism determinations it is necessary to collect the faeces for each day, to dry them slowly on a sand bath, and then to make the following determinations:

(*a*) The total amount of nitrogen.
(*b*) The total amount of fat (*i.e.* extract with Soxhlet's apparatus)
(*c*) The total amount of solids.

Having estimated what the total excretion of carbon is, we must now ascertain how much of it comes from proteid. To do this multiply the total amount of nitrogen excreted by 3·3 (since proteids contain approximately 52·8 of carbon, and 16 of nitrogen). If this amount of carbon be deducted from the total amount excreted, the remainder corresponds to the carbon derived from the combustion of fat and carbohydrate. As to which of these two bodies it is from which the carbon really comes, we have no means of telling definitely, but since there is very much more fat than carbohydrate in the tissues we usually reckon it as fat. Each gramme of carbon corresponds to 1·3 grammes of fat (because fat contains 76·5 grammes carbon).

3. **The Amount of Energy given out by the Body.**—The energy is liberated in the body partly as heat, and partly as muscular work. The amount actually lost as heat may be determined by placing the animal in a respiration calorimeter, but it is impossible to estimate directly, with anything like accuracy, the amount lost as mechanical work.

There are, however, certain *indirect methods* by which the total amount of energy liberated may be determined, and these are as follows: (*a*) By comparing the amount of food stuffs taken in with the amount which reappears in the excreta, we can find out how much of each food stuff has actually undergone metabolism in the tissues. It is now quite easy to find how much energy this corresponds to, by *multiplying the amount of each food stuff metabolised by its caloric value.* (Where the diet contains both fat and carbohydrate, and where an accurate balance of intake and output of carbon does not exist, we must reckon the excess or deficit as fat, since there is much evidence to show that the amount of carbohydrate in the body remains pretty constant.)

(*b*) The extent of oxidation in the tissues is determined, not by the amount of oxygen inspired,[1] but by the activity of the tissues. We can, therefore, employ the *amount of oxygen absorbed by the tissues as an index of the amount of energy liberated in them.* In order to do this, however, it is necessary to remember that the amount of energy liberated, when different food stuffs are burnt, is not the same; thus 100 gr. of oxygen are necessary for the combustion of 35 gr. of fat, the amount of energy hereby liberated amounting to 325 calories

[1] This statement may be true for slight variations in the amount of oxygen supplied, but where the partial pressure of oxygen is increased to one atmosphere a very marked depression in the output of CO_2 results. These experiments were carried out on mice, the determinations being made by the gravimetric method (Hill and Macleod).

The same amount of oxygen will burn up 84·4 gr. of carbohydrate, and yield thereby 346 calories, or 74·4 gr. proteid yielding 362 calories. It is therefore necessary, before employing the oxygen absorbed as an index of the amount of energy liberated, to ascertain that, when the determination is being made, the food stuffs undergoing oxidation are always the same. This can be ascertained by estimating the respiratory quotient, the value of this being influenced mainly by the nature of the food stuff undergoing combustion at the time (see p. 145). So long as the *R.Q.* remains constant, any increase or diminution in the amount of oxygen absorbed represents more or less energy liberated. In order that we may be able to compare the oxygen assimilation of different individuals under the same conditions, Zuntz has suggested that the determination should be made the first thing in the morning, immediately on awakening, and twelve hours after the last diet (which should not contain much carbohydrate) has been taken. The estimation should be made by Zuntz respiratory apparatus. The amount of oxygen absorbed, and of carbon dioxide exhaled, is then reckoned for each kilo. body weight, and for each minute. The normal amounts for man are 3 to 4·5 c.c. *O*, and 2·5 to 3·5 c.c. CO_2.

Example of a Metabolism Investigation.—It is desired to know whether a diet containing 125 grammes proteid, 50 grammes fat, and 500 grammes carbohydrate is sufficient for a man doing a moderate amount of work.

INTAKE.

	Carbon.	Nitrogen.	Calories.
Proteid,	62 gr.	20 gr.	512·5
Carbo.,	200	—	2050·0
Fat,	38	—	465·0
Total,	300 gr.	20 gr.	3027·5

OUTPUT.

	Carbon.		Nitrogen.
In urine,	11 gr.	(16·5 × 0·67)	16·5 gr.
In faeces,	5		1·0
In the breath,	254		—
Total,	270 gr.		17·5 gr.

Retained in Body.—30 grammes carbon and 2·5 grammes nitrogen. This amount of nitrogen represents 2·5 × 6·25 = 15·6 grammes *proteid*, or 75 grammes *muscle*. Now, this amount of proteid will account for

8·25 grammes carbon; so that $30 - 8{\cdot}25 = 21{\cdot}75$ grammes carbon represents $21{\cdot}75 \times 1{\cdot}3 = 28{\cdot}3$ grammes *fat*. On this diet, therefore, the subject retains in his tissues 15·6 gr. proteid and 28·3 gr. fat per diem.

To express this result in terms of energy liberated, we know that 3027·5 C. were supplied and that all these have been used except $15{\cdot}6 \times 4{\cdot}1 = 64$ retained as proteid, and $28{\cdot}3 \times 9{\cdot}3 = 263{\cdot}2$ retained as fat; or *in toto* 327·2 C. We find, therefore, that $3027{\cdot}5 - 327{\cdot}2 = 2{,}700$ C. have been required.

CHAPTER XV.

FIBRINOGEN. FIBRIN FERMENT. AMMONIA AND SUGAR IN BLOOD.

Preparation of Fibrinogen.—Blood plasma obtained by any of the methods detailed on page 190 except the natural salt method, is thoroughly mixed with an exactly equal amount of a cold saturated solution of sodium chloride. By this means, half saturation with sodium chloride is obtained in the mixture and in this the fibrinogen is precipitated, whereas the other globulins remain in solution. The precipitate is collected on a folded filter (see p. 491), washed quickly with a half-saturated solution of sodium chloride and redissolved by adding water to it. The salt adhering to the precipitate forms with the water added to the latter, a weak saline solution and in this the fibrinogen dissolves. This process of purification may be repeated several times, but the operations must be very quickly carried out as, otherwise, the precipitate of fibrinogen becomes insoluble in a weak saline solution (Hammarsten's method).

Preparation of Fibrin Ferment.—Blood serum or some defibrinated blood is mixed with twenty times its bulk of alcohol. A copious white precipitate of all the proteids is thereby obtained. This precipitate is allowed to stand under the alcohol for two months. After this time all the precipitated proteids except fibrin ferment become coagulated so that they are no longer soluble in their original solvents. The fluid is then pipetted off and the sediment collected on a filter, and, after the spirit has drained off, ground up in a mortar with water. The resulting extract is filtered and contains the fibrin ferment.

The Estimation of Ammonia in Blood.—*Folin's Method*—The method described on p. 468 for urine can be employed for blood with the following modifications:

1. The test-tube containing the blood must be kept on ice during the estimation.

2. Half its volume of methyl alcohol must be added to the blood to prevent frothing which is apt to be excessive on account of the proteids it contains. As the estimation proceeds the frothing frequently becomes more marked but can be diminished by adding more alcohol.

3. Only 5 c.c. $n/10$ acid and water should be placed in the absorption tubes.

4. The air current must be kept up for 5 hours.

5. Before titrating the absorption tubes must be warmed to 30-35° C. to drive off the CO_2 which the air current drives out of the blood and which would influence the titration.

The method depends on the fact that such ions as Ca, Mg and Na (metal ions) do not produce hydrolysis of proteids and so do not dislodge any ammonia, whereas they decompose the ammonia salts. Hydroxyl ions, on the other hand, produce hydrolysis of proteids and would give too high a result. Lime-water therefore could not be used as the alkali.

The Estimation of Sugar in Blood.—To estimate sugar in blood it is necessary that the proteids and haemoglobin be removed. This is most easily done by *Waymouth Reid's method.* Into a beaker of about 600 c.c. capacity are placed 250 c.c. of a 7 per cent. solution of phosphotungstic acid containing 2 per cent. HCl and the whole is weighed. The blood is then added, the contents well stirred, and the beaker again weighed. The difference in weight gives the amount of blood added. The beaker is then heated on a sand bath (or better still an oil bath), its contents being meanwhile briskly stirred. The proteids including the haemoglobin are, by this treatment, precipitated and form at first a brown gummy mass floating in a clear liquid. After a little the coagulum becomes brittle and sinks to the bottom of the beaker. Great care must now be taken that the beaker does not crack. When all the coagulum has settled to the bottom, the beaker is cooled and the supernatant fluid filtered through paper into an evaporating dish, the paper well washed into the same dish, the contents of the latter nearly neutralised with NaOH, but left faintly acid, and the evaporating dish then placed on a boiling water bath.

While the above fluid is evaporating the brittle proteid precipitate is removed from the beaker to a mortar, ground up with some water till a chocolate-like paste is obtained and then washed on to a large

suction filter plate and sucked dry. It is washed with water three times. The washings are then transferred to a 2 litre flask, nearly neutralised and boiled down to a small volume (50 c.c.) with the flask on the slant. The evaporated washings are then mixed with the contents of the evaporating dish (the evaporated supernatant fluid) and the whole brought to a volume of 50 c.c., after which it is cooled, almost neutralised, filtered through a small filter paper, the filter washed and the volume of the filtrate and washings brought up to 100 c.c. The sugar is then estimated in this by one of the methods described on page 274.

CHAPTER XVI.

THE PHOTOGRAPHIC SPECTRA OF HAEMOGLOBIN AND ITS DERIVATIVES. THE SPECTROPHOTOMETER.

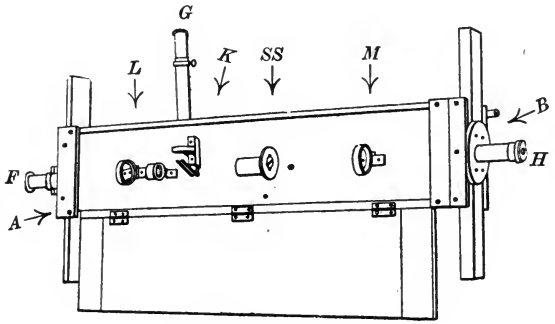
Photographic Spectra of Haemoglobin.—Certain absorption bands in the violet end of the spectrum are characteristic of haemoglobin and its derivatives. These may be demonstrated by photographing the violet end when solutions of haemoglobin or certain of its derivatives are placed between the slit and the source of light. They may also be shown by projecting the rays issuing from the telescope upon a fluorescent screen according to the following method.

Very considerable illumination is required. A beam of sunlight projected by a heliostat is probably the best illumination as certain of the Fraunhöfer lines (H & K) may be seen, though indistinctly, and thus fix the position of the absorption bands appearing. In default of this an electric arc lamp may be used, and if actuated by the continuous current the positive pole should be arranged to send a beam through the slit of the collimator of an ordinary compound spectroscope. The slit must be opened very widely. The eyepiece of the telescope is removed, and the issuing rays can be focussed on a screen having a coating of barium platino-cyanide fixed a few inches from the eyepiece. The spectrum, with the beam from the positive pole of an arc lamp, is continuous, but if the arc lamp, using an alternating current, be adopted, certain absorption bands will be seen in the green fluorescence which replaces the violet end. They do not greatly interfere, however, with the development of the absorption bands characteristic of oxyhaemoglobin and the haemoglobin derivatives, and these bands appear-

ing in the region between the Fraunhöfer lines G & H vary somewhat in position according to the particular substance examined.

The Spectrophotometer.—The spectrophotometer is an instrument by means of which the amount of light absorbed in any part of the spectrum as the result of passing through some absorbing solution may be measured. The amount of light thus absorbed depends upon the concentration of the solution and the thickness of the absorbing stratum.

The instrument may be used then for two purposes. (1) To measure the amount of light absorbed by some medium in any given portion of the spectrum, (2) to compare the concentration of solutions of the same



276.—The Spectrophotometer.

substance at different strengths, and therefore by comparing the concentration of an unknown solution with that of a standard to estimate the strength of such solution.

Glazebrook's form of the instrument consist of a flat rectangular box. At one end *A* is a slit. Light passing through this is focussed by the lens *L* as a parallel pencil on the direct vision prisms *SS*. At *G* is a second slit, and light passing through this is directed by the mirror *K* to form a parallel pencil below that from *A* upon the prisms. By adjusting *K* the spectra may be made to coincide. The emergent beams are focussed by *M* and the line of separation may be made distinct by movement of the eyepiece *H*. At *G* and *F* are polarising Nicol's prisms, *F* being its principal axis vertical, *G* horizontal. An analysing Nicol is at *H*, and when the pointer of the rotating eyepiece is at zero the principal axis of this is vertical. The light passing through *A* is wholly transmitted through the analyser at positions 0° and 180°, that from *G* is totally extinguished. At 90° and 270° the whole of that from *G* passes, but none from *A*. By means of a sliding slit at *B* any desired portion of the spectrum may be viewed. Between 0° and 90°

some portion will exist, when the amount of light passing from the two sources is equal. Let the angle when this happens be called θ. Now, suppose a cell of 1 cm. internal width, containing an absorbing substance, be placed between L and M, some new position of the pointer which may be called θ_1 will now permit equal passage of light. A certain amount of light (k) will be lost by absorption of the substance, and this may be ascertained from the equation

$$k = 1 - \frac{\tan^2\theta_1}{\tan^2\theta}.$$

The light absorbed in different portions of the spectrum by a solution of haemoglobin may thus be estimated.

(1) To estimate the concentration of an absorbing substance the method suggested by Lea may be adopted.

A standard solution of the substance is examined in a cell of known thickness, and the position of the pointer determined when the intensity of the two images is identical. The unknown strength is then taken and placed in a cell, the thickness of which can be varied. This is substituted for the standard. The thickness of the cell is adjusted till the position of equal intensities is the same as when the standard was under examination.

If c be the quantity of absorbing material in a unit of volume, and M the thickness of the cell in the case of the standard, and c^1 and m^1 in the case of the unknown strength, then

$$c^1 = \frac{cm}{m^1}.$$

Thus the construction of the unknown can be estimated.

(2) If a cell of variable thickness be not available a definite quantity of the unknown solution may be placed in the cell of known thickness. This is diluted (if stronger than the standard) with a known quantity of the diluent till the position of the pointer is the same as when the standard was examined. This affords a more delicate means of ascertaining exact quality of strength than the naked eye. From the amount of diluent added the strength of the original absorbent can be calculated.

(3) One other method of estimating concentration is available. Place a vessel of 1 cm. thickness containing the standard between one Nicol and the source of light and ascertain θ_1, the angle where equality of brightness obtains. Remove the standard and substitute the unknown strength of absorbing substance in a similar cell and ascertain the angle (θ_2) where equal brightness is observed. Let θ be the angle of equality when no absorbing medium is being examined. If C_2 be the

concentration of the unknown strength, and C_1 the concentration of the standard, then C_2 may be ascertained from the equation

$$C_2 = C_1 \frac{\log \tan \theta - \log \tan \theta_2}{\log \tan \theta - \log \tan \theta_1}.$$

In using the spectrophotometer the mean of at least three observations as to the position of the pointer in any particular case should be taken. If the substances under examination give marked absorption bands it is desirable to work in a part of spectrum where such bands are absent. With oxyhaemoglobin the green about λ510 is best.

CHAPTER XVII.

THE PIGMENTS OF URINE.

When fresh normal urine is examined by means of the spectroscope it usually presents no absorption bands, a diffuse absorption of the violet end being alone conspicuous.

The yellow colour of the urine is to be regarded as due almost entirely to the presence of a preformed pigment, **urochrome**. If this pigment be removed from the urine, the colour of the urine is largely lost. It may be separated from urine by saturating urine with ammonium sulphate and filtering. The filtrate which contains the pigment is shaken with alcohol, and by such repeated extractions from the saline solution practically all the pigment may be removed. The urochrome may now be precipitated by adding an excess of ether. The substance is readily soluble in water and when examined by the spectroscope shows no absorption bands.

Urobilin is present in very small quantities in normal urine and the amount normally present is generally in the condition of a chromogen. In abnormal conditions the urine may tend to have a brownish tint added to the ordinary rich orange colour and such urine frequently contains urobilin. A solution of urobilin or urine rich in urobilin will present the spectrum shown in Fig. 277, 1. If a concentrated solution of urobilin in sodium hydrate be taken and hydrochloric be added till the mixture is slightly acid, a turbid condition of the liquid results owing to imperfect re-solution of

the pigment in the acid. Examined spectroscopically a band is seen in the position of the E-line, in addition to the normal band at the junction of the green and blue (Fig. 277, 2). If the liquid be filtered the E-band will be no longer seen.

As regards the connection of urobilin and urochrome, it is important to remember that when urochrome is acted upon by aldehyde a urobilin-like substance is produced, and if urobilin be oxidised with potassium permanganate a substance similar to urochrome is formed.

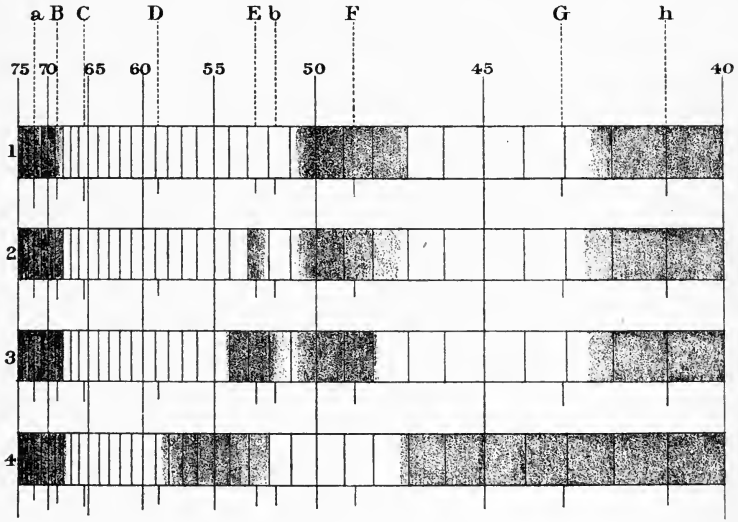


FIG. 277.

1. Acid urobilin in strong solution.
2. Urobilin precipitated by acid from its alkaline solution and partially redissolved. The so-called **E**-band spectrum.
3. Uroerythrin.
4. Uroerythrin in pink urate sediments.

The pink colour possessed by a deposit of urates is due to another pigment **uroerythrin**. This pigment is never excreted in large amount, but it possesses in high degree a colouring power. If a pink urate deposit be dissolved in warm water the urates may be precipitated by saturation with ammonium chloride carrying down the pigment. This may now be extracted with alcohol, and on shaking the alcoholic solution with chloroform to which one drop of acetic acid has been added the pigment passes into the chloroform. It now gives the spectrum seen in Figure 277, 3. If the pink urate deposit be simply

dissolved in warm water the spectroscopic appearance is different and represented by Figure 277, 4.

Haematoporphyrin is normally present in very small amount in urine. After certain drugs it may be present in comparatively large amounts. Even in acid urine it is present in the condition in which it shows the so-called alkaline spectrum (p. 199, Fig. 145, 11).

APPENDIX.

ANALYTICAL TABLES.

(Outline of Method for Detection of Various Physiological Chemical Substances in a Mixture.)

The following Physical Properties should be noted :

I. **Appearance.**

A. **Powder.**—Dust some on to a slide and examine under the microscope for starch grains and crystals. Dissolve some in a suitable solvent.

B. **Solution.**

1. **Opaque**—may be due to :
   (*a*) suspended fat globules—clear up with ether ;
   (*b*) certain inorganic salts—clear up with mineral acid ;
   (*c*) certain proteids.
2. **Opalescent**—may be due to :
   (*a*) glycogen or starch—iodine reaction ;
   (*b*) certain proteids.
3. **Deeply coloured**—suspect blood.

II. **Reaction.**

A. **Acid**—may be due to:

(*a*) free acid
(*b*) acid salt } apply Congo red test (p. 220).

If due to free acid, ascertain whether this be

1. a mineral acid or
2. an organic acid } apply Gunsberg's and the tropaeolin test (p. 221).

If due to an organic acid, apply Uffelmann's test for lactic acid.

B. **Alkaline** test for carbonic acid (effervescence with mineral acid), ammonia (smell, etc.), caustic alkali.

The following Chemical Tests should now be applied to Suitable Quantities of the Solution.

I. **For Carbohydrates.**

1. Apply **Trommer's test.**

A. **Positive**—indicates **monosaccharides, lactose,** or **maltose.**

B. **Negative,** but complete solution of cupric hydrate obtained on adding caustic alkalie, indicates **cane sugar.** Confirm for this by boiling some of the solution with a mineral acid for a minute or so, and applying Trommer's test to the product—reduction indicates cane sugar. The original solution will also taste sweet.

C. **Negative,** and no solution of cupric hydrate. Absence of monosaccharides and disaccharides.

2. **Add Iodine Solution.**

(*a*) a blue colour which disappears on heating, and returns on cooling indicates *starch.*

(*b*) a port-wine colour which disappears on heating, and returns on cooling indicates *dextrin* or *glycogen.* Confirm for polysaccharides by heating some of the original fluid for about fifteen minutes with a mineral acid, and testing for *sugar* in the hydrolysed fluid.

To distinguish between Starch, Glycogen and Dextrin.—Shake up some of the original powder with *cold* water and filter. By this treatment glycogen and dextrin will dissolve, starch will not. Wash the filter paper thoroughly with water, then add a drop of Iodine solution—a blue stain indicates starch. Add Iodine solution to the filtrate—a red colour indicates dextrin or glycogen; if the former body be present the filtrate is clear, opalescent if the latter.

To distinguish between Dextrose, Maltose, and Lactose.

(1) Prepare **osazone crystals** (p. 417) and examine under the microscope—dextrosazone gives long thin needles; maltosazone, short thick needles; lactosazone, needles of varying length and thickness (Fig. 266).

(2) **Barfoed's reaction** may also be tried. Dextrose reduces this with ease; lactose and maltose not so readily.

II. **For Proteids.**

1. Apply the **Biuret reaction**—(*a*) A violet colour indicates native proteids or albuminoids; (*b*) a rose pink colour, proteose or peptone.
2. Apply **Millon's** and the **Xantho-proteic** tests.

(*a*) A well-marked reaction indicates proteids of Kossel's 3rd and 4th groups. (*b*) A faint reaction (combined with a distinct biuret, and the absence of coagulation on boiling) points to **gelatine** (2nd group). (Confirm by seeing if the solution gelatinises on cooling).

If the Biuret Test gives a Violet Colouration,

A. **Add a drop or so of dilute acetic acid and boil.** A coagulum points to **native proteids.** To ascertain which of these is present (*i.e.* albumin or globulin), half saturate some of the solution with $(NH_4)_2SO_4$. A precipitate indicates **globulin**; filter; if the filtrate still gives a coagulum on boiling, **albumin** is present (for details, see p. 176).

B. **Carefully neutralise some of the solution.** A precipitate may be:

1. **Alkali albumin**—original fluid alkaline
2. **Acid albumin**—original fluid acid

} the precipitate re-dissolves on adding excess of acid or alkali.

3. **Nucleo albumin**—original fluid alkaline
4. **Mucin**—original fluid alkaline

} precipitate does not disappear on adding a moderate excess of acid.

To distinguish between Nucleo Albumin and Mucin.—This is possible only when a large amount of these bodies is present. The acetic acid precipitate is collected on a filter paper, washed with acidulated water, and divided into two portions *a* and *b*.

(*a*) Boil with 20 per cent. HCl for 10 minutes; cool; neutralise; apply Trommer's test. A positive reaction points to *mucin*.

(*b*) Melt in a crucible with fusion mixture; after the ash cools, dissolve it in nitric acid and add molybdate of ammonia solution. A yellow precipitate on warming indicates **Nuclein.**

If the Biuret Test gives a Rose Pink Colouration, add a few drops of concentrated pure nitric acid.

A. A **white precipitate,** which clears up on warming and returns on cooling, points to **Proteose.** Confirm by the salicyl sulphonic acid test (p. 270).

If proteose be present, saturate some of the original fluid, from which native proteids have been separated by boiling, with sodium chloride. A precipitate indicates **primary proteoses.** Filter and add a drop of acetic acid; a precipitate points to **secondary proteoses.**

B. **No precipitate** with nitric acid, but a distinct pink biuret reaction points to **Peptone.** Confirm by saturating the original fluid with ammonium sulphate, filtering and applying the biuret test to the filtrate (see p. 172).

When two or more Proteids are present, the following method will be found very useful.

Add a few drops of salicyl sulphonic acid to several c.c. of the original fluid. A white precipitate may indicate native proteid or proteoses. Boil. The proteoses dissolve, whereas the native proteid becomes coagulated. Filter hot. If a precipitate forms in the filtrate on cooling, it indicates **Proteoses.** Filter off this precipitate and apply the biuret test to the filtrate. A rose pink colouration indicates **Peptone.**

III. **For Fats.**—In watery solution fat may be dissolved as a *soap.* The presence of this can be detected by pouring some of the original fluid into about 20 c.c. of 20 per cent. H_2SO_4 contained in a small beaker, and heated to near boiling point. If soap be present a film of fatty acid will form on the surface of the fluid.

IV. **The following substances should also be tested for.** I. **Bile salts**—Pettenkofer's reaction (p. 233); II. **Bile Pigments**—Gmelin's test (p. 234).

V. **Urea** (1).—Add some fuming nitric to some of the original fluid. Effervescence points to urea.

(2) Repeat with hypobromite solution.

(3) If 1 and 2 be positive, confirm by obtaining urea nitrate crystals. To do this evaporate about 30 c.c. of the original fluid to small bulk,

extract residue with six times its bulk of methylated spirit, evaporate this extract to dryness, dissolve residue in 3-4 c.c. distilled water, and add to the resulting fluid a few c.c. of *pure* nitric acid, meanwhile keeping the test-tube cool by holding it under the tap. Crystals of urea nitrate separate out if urea is present. Examine under microscope (Fig. 154).

VI. **Uric Acid.**—Apply Murexide test (p. 258).

VII. **Blood Pigment.**—(1) Examine by means of the spectroscope. *A*, the original fluid; *B*, the same after reduction; *C*, the same after the addition of caustic alkali and heating. By this latter method alkali haematin is formed. This itself does not give a very distinct absorption band, but if a reducing agent (NH_4HS) be added to it haemochromogen is formed, which has two very distinctly marked bands in about the same position as those of oxyhaemoglobin.

(2) Apply the guaiac and ozonic ether test (p. 277).

When it is desired to ascertain whether Ferments be present it is necessary to add a piece of coagulated egg-white, or of washed fibrin to the original fluid, and to place the mixture on a water bath heated to body temperature. If, after an hour, the digest gives a distinct proteose reaction, and this was not obtained in the original fluid, the presence of a proteolytic ferment may be assumed; **pepsin**, if the original fluid react acid, and **trypsin**, if it react alkaline. If proteoses are present in the fluid itself, Mett's method (p. 450) must be employed to identify the ferment.

For the detection of **Amylolytic** and **Steatolytic** ferments, the methods described on page 216 must be employed.

For the detection of the various substances which may occur in the urine, the tests and reactions described in chapters xviii. and xix., Part II., must be applied.

SOME FORMS OF APPARATUS USED IN CHEMICAL PHYSIOLOGY AND NOT ALREADY DESCRIBED.

1. **Folded Filter.**—Where rapid filtration of solutions containing proteids is necessary, a folded filter should be used. This is made by folding the filter paper into a quarter and then folding each quarter inwards on itself, so that each is now divided into two. The filter is then completed by again folding each portion in a direction opposite to the primary folds, by which means the paper folds together like a fan. The folds are pressed, and then the fan is opened out, and is ready for placing in the funnel (Fig. 278).

2. **Suction Filter.**—This is also used for rapid filtration. A strong triangular flask with a side tube is fitted with an indiarubber cork, through which fits the stem of a funnel. The funnel is provided either with an ordinarily folded filter paper made of specially hardened paper, or it carries a small perforated porcelain

disc, bevelled so as to fit the funnel accurately. This disc is covered with a circularly cut piece of filter paper of slightly larger diameter than the disc, so that its margin lies on the funnel. A suction pump (such as that of Bunsen) is attached, by pressure tubing, to the side tube of the flask, and the fluid to be filtered is poured on to the filter. The suction causes the edge of paper to adhere to the glass of the funnel, and the fluid filters perfectly clear.

3. **Weighing Filters.**—These are employed where it is desired to weigh a precipitate. The simplest form consists of a small hardened filter paper which has been folded and placed on a watch glass in a desiccator for several hours, so as to dry it. To weigh it, a second watch glass is placed over the one holding the filter paper, and the whole is transferred to the scale pan. After weighing, the filter paper is fitted to a small funnel, and the precipitate collected on it removed to a watch glass, dried in a desiccator, and weighed as before. The difference in weight gives the amount of precipitate.

Another method is to make an asbestos filter, dry it and, after weighing, to filter the fluid through it under suction. When filtration is complete, the precipitate is washed, dried and weighed. An asbestos filter is made in the following way. A piece of glass tubing of 1½-2 cm. diameter is drawn out to a tube of about 3 mm. width. The wide portion should be from 6-8 cm. long, and the stem about 4 cm. The neck, where the stem and wide portion meet, is loosely plugged with cotton wool and, above this, is placed loosely packed asbestos threads for about 1-1½ cm. The asbestos should have been previously purified by boiling it with strong alkali and acid and then washing with water. The plug of cotton wool prevents any pieces of asbestos being sucked out of the filter. The suction pressure employed with these filters should be gradually applied as, otherwise, some of the precipitate may be sucked through them.

4. **Separating Funnel.**—This is used for separating two fluids which do not mix. In physiological chemistry it is mainly employed for separating ethereal and watery solutions from one another. Its shape will be seen in Fig. 279. While shaking ether, it is necessary frequently to open the *tap* so as to prevent the stopper being blown out. Before doing this, the funnel is, of course, inverted. To separate water and ether, the funnel is placed in an upright position so as to allow the water to sink. When a sharp line forms between the two fluids the stopper is removed and the tap turned so as to drain off the water. When nearly all this has been removed, the tap is almost closed so that the outflow may be the more easily controlled. After all the water has been removed, the ethereal fluid should be shaken, whereby, it will often be found, more water separates out.

Desiccators.—There are various forms of these. The two types represented in Figs. 280, 281 are perhaps the most useful, the former being employed for desiccation alone, the latter for desiccation in vacuo. In the conical under-part of the desiccator depicted in Fig. 280 is placed some highly deliquescent substance such as concentrated sulphuric acid or fused calcium chloride. A piece of wire gauze is placed on the floor of the upper chamber, and on this rests the vessel containing the substance to be dried. A glass lid with ground-glass edges is fitted on to the ground-glass edge of the upper chamber, the junction being made air-tight by smearing the applied surfaces with vaseline or resin ointment. The other desiccator (Fig. 281) is provided with an opening whereby it can be connected, by means of tubing, etc., with a suction pump. The deliquescent substance is contained in a vessel placed in the lower chamber. This vessel

supports the wire gauze, and care must be taken, when moving the apparatus, that no acid comes in contact with the gauze. The indiarubber tubing connecting the chamber with the air pump should be provided with a clasp, so that the chamber can be disconnected from the pump.

Hot-Air Bath and Water Bath.—The forms depicted in Figs. 282, 283 will be found most useful for physiological chemical purposes.

PERCENTAGE AVERAGE COMPOSITION OF SOME OF THE MORE IMPORTANT FOOD STUFFS.

(Adapted from various sources.)

	Water.	Proteids.	Fats.	Carbo-hydrates.	Salts.
Beef (best quality)	72	21	6	——	1
Biscuits	8	15	1·3	73·4	1·7
Bread (wheaten)	40	8	1·5	49·2	1·3
Butter (fresh)	12	2	85	——	1
Cheese	41	28	23	1	7
Eggs	73·5	13·5	11·6	——	1·4
Fish (salmon)	76	15	7	——	2
Fish (sole)	86	12	0·5	——	1·5
Flour (fine wheaten)	16·5	13	1·5	68·3	0·7
Lentils	12·5	24·8	1·8	58·4	2·5
Milk (cow's)	86·9	4·7	3·5	4·2	0·7
Mutton	76	18	5	——	1
Oatmeal	15	13	6	63	3
Peas	15·6	22	2	58	2·4
Potatoes	74	2	0·2	21·8	1
Rice	10	5	0·1	84·4	0·5

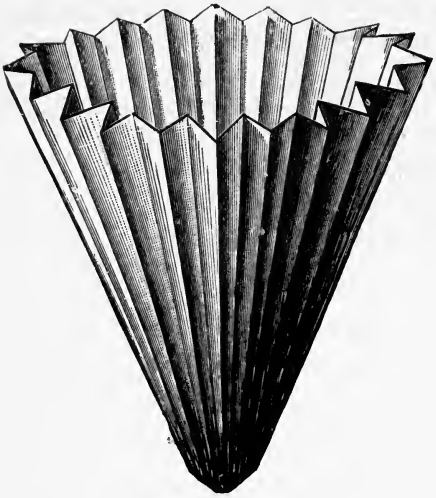


FIG. 278.—Folded filter

FIG. 279.—Separating funnel.

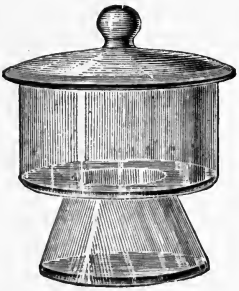


FIG. 280.—Desiccator.

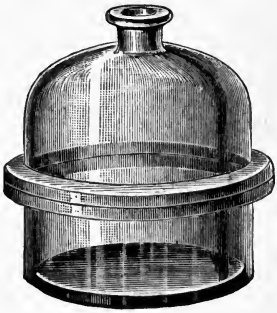


FIG. 281.—Desiccator for drying *in vacuo*.

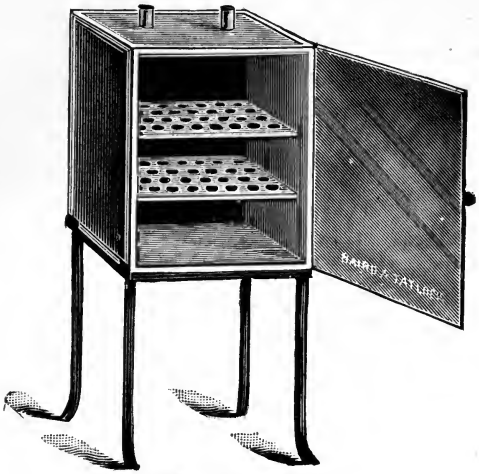


FIG. 282.—Hot-air bath.

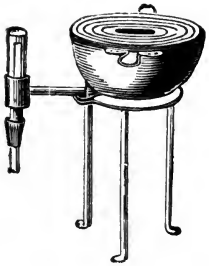


FIG. 283.—Water bath.

FIG. 284.—Measuring cylinder.

FIG. 285.—Burettes.

SCHEDULE OF EXPERIMENTS.

The following schedule of experiments on mammals is for the convenience of students of the Western Reserve University. All animals experimented on must be kept under deep anæsthesia. The class is divided into groups of four for this work. Each lesson occupies four hours, one of which is devoted to a conference on the results of the experiments.

1. Proofs of the circulation of the blood (Chap. XXX.), omitting the excision of the heart.

Introduce cannula in carotid and record mean arterial pressure (Chap. XXXV.). Kill animal and make a careful dissection of vagus nerve and sympathetic chain.

2. Demonstrate that the mean arterial pressure depends on the pumping action of the heart (Fig. 110 and Fig. 231) and on the peripheral resistance (cut cervical spinal cord).

3. Record arterial and venous pressures simultaneously (Chap. XXXIV.), and study effect of the conditions enumerated on p. 128.

3′. Record aortic and intracardiac pressures by Hürthle's and Fick's manometers (Chap. XXXIX. advanced course).

(*N.B.*—3 and 3′ are done alternately by the groups.)

4. Demonstrate the effect of respiration on the blood pressure (Fig. 109). Produce asphyxia (α) by clamping trachea, (β) by injecting curare. Do this with vagi intact and vagi cut (Chap. XXXV.). (Have respiratory bellows ready.)

5. Demonstrate vaso-motor fibres in cervical sympathetic of rabbit (Chap. XXXIII.). Study action of depressor nerve in the same animal (Chap. XXXV.).

6. Demonstrate presence of vaso-motor fibres in sciatic (lectures and Chap. XLI. advanced).

6′. Demonstrate vaso-motor fibres in renal nerves; study conditions influencing ureter outflow (Chap. XLI. advanced).

7. Demonstrate the station of synapsis for the vaso-motor fibres to upper limb (Chap. XLI. advanced, last paragraph; also Chap. XLIII., action of nicotin).

8. Demonstrate pressor fibres in—(α) sciatic, (β) vagus.

Demonstrate the effect of gravity on the blood pressure of the dog (Chap. XXXV.).

9. Demonstrate the effect of suprarenal extract and of a nucleo-albumin solution on the blood pressure (Fig. 235). Experimentally explain the action of each.

10. Estimate the velocity of circulation by methylene blue experiment on rabbit, and by Ludwig's stromühr in dog (Chap. XXXI.).

INDEX.

PARTS I. AND III.

EXPERIMENTAL PHYSIOLOGY.

The numerals in thick type indicate that the subject is illustrated by diagrams or tracings.

INDEX.

PARTS II. AND IV.

PHYSIOLOGICAL CHEMISTRY.

The numerals in thick type indicate that the subject is illustrated by figures.

GLASGOW: PRINTED AT THE UNIVERSITY PRESS BY ROBERT MACLEHOSE AND CO. LTD.

Recently Published Medical Works.

LECTURES ON DISEASES OF CHILDREN. By ROBERT HUTCHISON, M.D. Edin., F.R.C.P., Assistant Physician to the London Hospital and to the Hospital for Sick Children, Great Ormond Street. With numerous Illustrations. Crown 8vo. 8s. 6d. net.

FOOD AND THE PRINCIPLES OF DIETETICS. By ROBERT HUTCHISON, M.D. Edin., F.R.C.P. Fifth Impression. Illustrated. Demy 8vo. 16s. net.

HUMAN EMBRYOLOGY AND MORPHOLOGY. By ARTHUR KEITH, M.D. Aberd., F.R.C.S. Eng., Lecturer on Anatomy, London Hospital Medical College; formerly Hunterian Professor, Royal College of Surgeons, England, and Examiner in Anatomy, University of Aberdeen. xii + 324 pages, with 252 Illustrations and an Index. Demy 8vo. New and Enlarged Edition. 12s. 6d. net.

THE PHYSIOLOGICAL ACTION OF DRUGS. An Introduction to Practical Pharmacology. By M. S. PEMBREY, M.A., M.D., Lecturer on Physiology in Guy's Hospital Medical School, and C. D. F. PHILLIPS, M.D., LL.D. With 68 Diagrams and Tracings. Demy 8vo. 4s. 6d. net.

PHOTOTHERAPY. By PROFESSOR NIELS R. FINSEN, Copenhagen. Translated from the German Edition, and with an Appendix on the Light Treatment of Lupus by JAMES H. SEQUEIRA, M.D. Lond., M.R.C.P. With Full-page Plates. Demy 8vo. 4s. 6d. net.

MANUAL OF HUMAN PHYSIOLOGY. By LEONARD HILL, M.B., Lecturer on Physiology at the London Hospital Medical School, Hunterian Professor Royal College of Surgeons. xii + 484 pages, with 173 Illustrations. Crown 8vo. 6s.

LECTURES ON THEORETICAL AND PHYSICAL CHEMISTRY. By Dr. J. H. VAN 'T HOFF, Professor of Chemistry at the University of Berlin. Translated by Dr. R. A. LEHFELDT. Demy 8vo.

Part I. CHEMICAL DYNAMICS. 12s. net. Part II. CHEMICAL STATICS. 8s. 6d. net. Part III. RELATIONS BETWEEN PROPERTIES AND CONSTITUTION. 7s. 6d. net.

A TEXT-BOOK OF PHYSICS. With Sections on the Application of Physics to Physiology and Medicine. By R. A. LEHFELDT, D.Sc. viii + 304 pages. Crown 8vo. 6s.

LONDON: EDWARD ARNOLD.

MR. EDWARD ARNOLD'S

LIST OF

Scientific and Technical Books.

FOOD AND THE PRINCIPLES OF DIETETICS. By ROBERT HUTCHISON, M.D. Edin., F.R.C.P., Assistant Physician to the London Hospital. Seventh and Revised Edition. Illustrated. Demy 8vo., 16s. net.

LECTURES ON DISEASES OF CHILDREN. By ROBERT HUTCHISON, M.D. (Edin.), F.R.C.P., Assistant Physician to the London Hospital and to the Hospital for Sick Children, Great Ormond Street, London. With numerous Illustrations. Second Impression. Crown 8vo., 8s. 6d. net.

PRACTICAL PHYSIOLOGY. By M. S. PEMBREY, M.A., M.D., Lecturer on Physiology at Guy's Hospital; A. P. BEDDARD, M.A., M.D., Demonstrator of Physiology, Guy's Hospital; J. S. EDKINS, M.A., M.B., Lecturer in Physiology and Demonstrator of Physiology, St. Bartholomew's Hospital; LEONARD HILL, M.B., F.R.S., Lecturer on Physiology, London Hospital Medical School; and J. J. R. MACLEOD, M.B. Copiously Illustrated. New and Revised Edition. Demy 8vo., 12s. 6d. net.

RECENT ADVANCES IN PHYSIOLOGY AND BIO-CHEMISTRY. By LEONARD HILL, M.B., F.R.S.; A. P. BEDDARD, M.A., M.B.; J. J. R. MACLEOD, M.B.; BENJAMIN MOORE, M.A., D.Sc.; and M. S. PEMBREY, M.A., M.D. Demy 8vo., 18s. net.

HUMAN EMBRYOLOGY AND MORPHOLOGY. By A. KEITH, M.D., F.R.C.S. Eng., Lecturer on Anatomy at the London Hospital Medical College. With 316 Illustrations. New, Revised, and Enlarged Edition. Demy 8vo., 12s. 6d. net.

SURGICAL NURSING AND THE PRINCIPLES OF SURGERY FOR NURSES. By RUSSELL HOWARD, M.B., M.S., F.R.C.S., Lecturer on Surgical Nursing at the London Hospital. Crown 8vo., with Illustrations, 6s.

THE PHYSIOLOGICAL ACTION OF DRUGS. An Introduction to Practical Pharmacology. By M. S. PEMBREY, M.A., M.D., Lecturer on Physiology in Guy's Hospital Medical School; and C. D. F. PHILLIPS, M.D., LL.D. Fully Illustrated. Demy 8vo., 4s. 6d. net.

PHOTOTHERAPY. By N. R. FINSEN. Translated by J. H. SEQUEIRA, M.D. With Illustrations. Demy 8vo., 4s. 6d. net.

A MANUAL OF HUMAN PHYSIOLOGY. By LEONARD HILL, M.B., F.R.S. With 173 Illustrations. xii+484 pages. Crown 8vo., cloth, 6s.

A PRIMER OF PHYSIOLOGY. By LEONARD HILL, M.B. 1s.

A MANUAL OF PHARMACOLOGY FOR STUDENTS. By W. E. DIXON, M.A., M.D., B.S., B.Sc. Lond., etc., Assistant to the Downing Professor of Medicine in the University of Cambridge. With Numerous Diagrams. Demy 8vo., 15s. net.

THE CHEMICAL SYNTHESIS OF VITAL PRODUCTS AND THE INTER-RELATIONS BETWEEN ORGANIC COMPOUNDS. By Professor RAPHAEL MELDOLA, F.R.S., of the City and Guilds of London Technical College, Finsbury. Two vols. Vol. I. now ready. Super Royal 8vo., 21s. net.

LONDON: EDWARD ARNOLD, 41 & 43 MADDOX STREET, W.

LECTURES ON THEORETICAL AND PHYSICAL CHEMISTRY. By Dr. J. H. VAN 'T HOFF, Professor of Chemistry at the University of Berlin. Translated by Dr. R. A. LEHFELDT. In three volumes. Illustrated. Demy 8vo., 28s. net; or obtainable separately, as follows:

Vol. I.—**Chemical Dynamics.** 12s. net. Vol. II.—**Chemical Statics.** 8s. 6d. net.
Vol. III.—**Relations between Properties and Composition.** 7s. 6d. net.

THE ELEMENTS OF INORGANIC CHEMISTRY. For use in Schools and Colleges. By W. A. SHENSTONE, Lecturer in Chemistry at Clifton College. 554 pages. New Edition, Revised and Enlarged. 4s. 6d.

A COURSE OF PRACTICAL CHEMISTRY. Being a Revised Edition of 'A Laboratory Companion for Use with Shenstone's Inorganic Chemistry.' By W. A. SHENSTONE. 144 pages. Crown 8vo., 1s. 6d.

A TEXT-BOOK OF PHYSICAL CHEMISTRY. By Dr. R. A. LEHFELDT, Professor of Physics at the East London Technical College. With 40 Illustrations. Crown 8vo., cloth, 7s. 6d.

A TEXT-BOOK OF PHYSICS. With Sections on the Applications of Physics to Physiology and Medicine. By Dr. R. A. LEHFELDT. Fully Illustrated. Crown 8vo., cloth, 6s.

PHYSICAL CHEMISTRY FOR BEGINNERS. By Dr. Ch. M. VAN DEVENTER. Translated by Dr. R. A. LEHFELDT. 2s. 6d.

A FIRST YEAR'S COURSE OF EXPERIMENTAL WORK IN CHEMISTRY. By ERNEST H. COOK, D.Sc., F.I.C., Principal of the Clifton Laboratory, Bristol. With 26 Illustrations. Crown 8vo., cloth, 1s. 6d.

AN EXPERIMENTAL COURSE OF CHEMISTRY FOR AGRICULTURAL STUDENTS. By T. S. DYMOND, F.I.C., Lecturer on Agricultural Chemistry in the County Technical Laboratories, Chelmsford. With 50 Illustrations. 192 pages. Crown 8vo., cloth, 2s. 6d.

THE STANDARD COURSE OF ELEMENTARY CHEMISTRY. By E. J. COX, F.C.S. With 90 Illustrations. 350 pages. Crown 8vo., cloth, 3s. Also obtainable in five parts, limp cloth. Parts I.-IV., 7d. each; Part V., 1s.

A PRELIMINARY COURSE OF PRACTICAL PHYSICS. By C. E. ASHFORD, M.A., Headmaster of the Royal Naval College, Dartmouth. Fcap. 4to., 1s. 6d.

ADVANCED EXAMPLES IN PHYSICS. By A. O. ALLEN, B.A., B.Sc., A.R.C.Sc., Lond., Assistant Lecturer in Physics at Leeds University. 1s. 6d.

PHYSICAL DETERMINATIONS. A Manual of Laboratory Instructions for the Determination of Physical Quantities. By W. R. KELSEY, B.Sc., A.I.E.E., Principal of the Taunton Technical College. 4s. 6d.

ELECTROLYTIC PREPARATIONS. Exercises for use in the laboratory by chemists and electro-chemists. By Dr. KARL ELBS, Professor of Chemistry at the University of Giessen. Translated by R. S. HUTTON, M.Sc. Demy 8vo., 4s. 6d. net.

THE ELECTRIC FURNACE. By HENRI MOISSAN, Professor of Chemistry at the Sorbonne. Authorized English Edition. Translated by A. T. DE MOUILPIED, M.Sc., Ph.D. With Illustrations. Demy 8vo., 10s. 6d. net.

ELECTRICITY AND MAGNETISM. By C. E. ASHFORD, M.A., Headmaster of the Royal Naval College, Dartmouth, late Senior Science Master at Harrow School. With over 200 Diagrams. Crown 8vo., 3s. 6d.

MECHANICS. By W. D. EGGAR, Science Master at Eton College. Crown 8vo., 3s. 6d.

MAGNETISM AND ELECTRICITY. An Elementary Treatise for Junior Students, Descriptive and Experimental. By J. PALEY YORKE, of the Northern Polytechnic Institute, London. With nearly 150 Illustrations. 3s. 6d.

VALVES AND VALVE-GEAR MECHANISMS. By W. E. DALBY, M.Inst.C.E., M.I.M.E., Professor of Engineering, City and Guilds of London Central Technical College. Royal 8vo. Fully Illustrated. 21s. net.

THE BALANCING OF ENGINES. By W. E. DALBY, M.A., B.Sc., M.Inst.C.E., M.I.M.E. With 173 Illustrations. Demy 8vo., 10s. 6d. net.

THE STRENGTH AND ELASTICITY OF STRUCTURAL MEMBERS. By R. J. WOODS, Master of Engineering, Royal University of Ireland, Fellow of the Royal Indian Engineering College, and Assistant Professor of Engineering Cooper's Hill College. Demy 8vo. 10s. 6d. net.

TRAVERSE TABLES. With an Introductory Chapter on Co-ordinate Surveying. By HENRY LOUIS, M.A., A.R.S.M., F.I.C., F.G.S., etc., Professor of Mining and Lecturer on Surveying, Armstrong College, Newcastle-on-Tyne; and G. W. CAUNT, M.A. Demy 8vo., 4s. 6d. net.

THE CALCULUS FOR ENGINEERS. By JOHN PERRY, M.E., D.Sc., F.R.S., Professor of Mechanics and Mathematics in the Royal College of Science, etc. Crown 8vo., cloth, 7s. 6d.

ELECTRIC AND MAGNETIC CIRCUITS. By ELLIS H. CRAPPER, M.I.E.E., Head of the Electrical Engineering Department in the University College, Sheffield. viii + 380 pages. Demy 8vo., 10s. 6d. net.

AN INTRODUCTION TO THE THEORY OF OPTICS. By Professor ARTHUR SCHUSTER, Ph.D., F.R.S., Professor of Physics at the University of Manchester. With numerous Diagrams. Demy 8vo., 15s. net.

ASTRONOMICAL DISCOVERY. By H. H. TURNER, Savilian Professor of Astronomy in the University of Oxford. Demy 8vo., 10s. 6d. net.

VECTORS AND ROTORS. With Applications. Being Lectures delivered at the Central Technical College. By Professor O. HENRICI, F.R.S. Edited by G. C. TURNER, Goldsmith Institute. Crown 8vo., cloth. 4s. 6d.

THE PRINCIPLES OF MECHANISM. By H. A. GARRATT, A.M.I.C.E., Head of the Engineering Department of the Northern Polytechnic Institute, Holloway. Crown 8vo., cloth, 3s. 6d.

ELEMENTARY PLANE AND SOLID MENSURATION. By R. W. K. EDWARDS, M.A., Lecturer on Mathematics at King's College, London. For use in Schools, Colleges, and Technical Classes. 304 pages. Crown 8vo., 3s. 6d.

FIRST STEPS IN QUANTITATIVE ANALYSIS. By J. C. GREGORY, B.Sc., A.I.C. Crown 8vo., cloth, 2s. 6d.

GEOMETRICAL CONICS. By G. W. CAUNT, M.A., Lecturer in Mathematics, Armstrong College, Newcastle-on-Tyne, and C. M. JESSOP, M.A., Professor of Mathematics, Armstrong College. Crown 8vo., 2s. 6d.

FIVE-FIGURE TABLES OF MATHEMATICAL FUNCTIONS. By J. B. DALE, M.A. Camb., B.A. Lond., Assistant Professor of Mathematics, King's College, University of London. Demy 8vo., 3s. 6d. net.

LOGARITHMIC AND TRIGONOMETRIC TABLES. (To Five Places of Decimals.) By J. B. DALE, M.A. Demy 8vo., 2s. net.

AN ELEMENTARY TREATISE ON PRACTICAL MATHEMATICS. By JOHN GRAHAM, B.A., Demonstrator of Mechanical Engineering and Applied Mathematics in the Technical College, Finsbury. Crown 8vo., cloth, 3s. 6d.

PRELIMINARY PRACTICAL MATHEMATICS. By S. G. STARLING, A.R.C.Sc., B.Sc., Head of the Mathematics and Physics Department of the West Ham Municipal Technical Institute; and F. C. CLARKE, A.R.C.Sc., B.Sc. 1s. 6d.

A NOTE-BOOK OF EXPERIMENTAL MATHEMATICS. By C. GODFREY, M.A., Headmaster of Royal Naval College, Osborne, and G. M. BELL, B.A., Senior Mathematical Master, Winchester College. Fcap. 4to., paper boards, 2s.

HOUSE, GARDEN, AND FIELD. A Collection of Short Nature Studies. By L. C. MIALL, F.R.S., Professor of Biology, University of Leeds. With numerous Diagrams. Crown 8vo., 6s.

THE EVOLUTION THEORY. By AUGUST WEISMANN, Professor of Zoology in the University of Freiburg-im-Breisgau. Translated by Professor J. ARTHUR THOMSON and MARGARET THOMSON. With numerous Illustrations and Coloured Plates. Two Vols. Royal 8vo., 32s. net.

ANIMAL BEHAVIOUR. By C. LLOYD MORGAN, LL.D., F.R.S., Principal of University College Bristol, author of 'Animal Life and Intelligence,' etc. With numerous Illustrations. Large crown 8vo., 10s. 6d.

HABIT AND INSTINCT. By C. LLOYD MORGAN, LL.D., F.R.S. With Photogravure Frontispiece. viii + 352 pages. Demy 8vo., cloth, 16s.

A TEXT-BOOK OF ZOOLOGY. By G. P. MUDGE, A.R.C.Sc. Lond., Lecturer on Biology at the London Hospital Medical College. With about 200 original Illustrations. Crown 8vo., cloth, 7s. 6d.

ELEMENTARY NATURAL PHILOSOPHY. By ALFRED EARL, M.A., Assistant Master at Tonbridge School. Crown 8vo., cloth, 4s. 6d.

A CLASS-BOOK OF BOTANY. By G. P. MUDGE, A.R.C.Sc. Lond., F.Z.S., and A. J. MASLEN, F.L.S., Lecturer on Botany at the Woolwich Polytechnic. With over 200 Illustrations. Crown 8vo., 7s. 6d.

THE BECQUEREL RAYS AND THE PROPERTIES OF RADIUM. By the Hon. R. J. STRUTT, Fellow of Trinity College, Cambridge. With Diagrams. Demy 8vo. 8s. 6d. net.

A MANUAL OF ALCOHOLIC FERMENTATION AND THE ALLIED INDUSTRIES. By CHARLES G. MATTHEWS, F.I.C., F.C.S., etc. Fully Illustrated. Crown 8vo., cloth, 7s. 6d. net.

WOOD. A Manual of the Natural History and Industrial Applications of the Timbers of Commerce. By G. S. BOULGER, F.L.S., F.G.S. Fully Illustrated. Crown 8vo., 7s. 6d. net.

PSYCHOLOGY FOR TEACHERS. By C. LLOYD MORGAN, LL.D., F.R.S. xii + 251 pages. Crown 8vo., 3s. 6d.