Experimental Physiology

SCHAFER'S

ESSENTIALS OF HISTOLOGY

Descriptive and Practical for the Use of Students

FOURTEENTH EDITION

Edited by

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Experimental Physiology

SIXTH EDITION

BY

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WITH NINETY-SEVEN ILLUSTRATIONS

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PREFACE

THIS book contains precise directions for performing most of the fundamental experiments in physiology, except those of a chemical nature. Most of the experiments can be carried out by the individual elementary student; some are suitable only for advanced students, while others are intended to take the form of demonstrations. It is left to the teacher of the subject to make such selection from the exercises as will meet the requirements of his own students.

The text has again been thoroughly revised and some obsolete matter removed. Teaching experience indicates that the description of electrical apparatus in the early part of the book cannot yet be regarded as obsolete and so, somewhat reluctantly, has been retained. An introductory chapter on the recording and reporting of experiments has been added, and considerable additions have been made to the sections on involuntary muscle, the heart, the circulation, and the special senses. Some of these additions, together with further observations on the actions of drugs of physiological interest, bring the work into line with recent advances in the physiology of the autonomic nervous system.

Long known as "Schafer's Experimental Physiology" the title has been altered to accord with this usage. But the character of the book remains essentially unchanged in accordance with what the Editor believes would have been Schafer's wish.

WEIGHTS AND MEASURES

ENGLISH MEASURES EXPRESSED IN THE METRIC SYSTEM.

WEIGHT (Avoirdupois)

1 grain (gr.)=0·0648 gram (or gramme).
1 oz.=28·35 grams (437·5 grains).
1 oz. (Troy)=31·1 grams (480 grains).
1 lb.=453·59 grams (7,000 grains).
1 cwt.=51 kilograms.

LENGTH

1 inch=25·40 millimeters.
1 foot=30·48 centimeters.
1 yard=0·914 meter (or metre).
1 mile (1,760 yards)=1·6 kilometers.

VOLUME

1 minim=0·0592 milliliter.
1 fluid ounce=28·4 milliliters.
1 pint=568·2 milliliters.
1 gallon=4·546 liters.
1 cubic foot=28·317 liters.

UNITS OF THE METRIC SYSTEM EXPRESSED AS ENGLISH MEASURES.

WEIGHT

1 microgram (0·001 mg.) (γ)=0·000015 grain.
1 milligram (mg.)=0·015 gr. (about $\frac{1}{65}$ gr.).
1 gram (g. or G.)=15·432 grains.
1 kilogram (1,000 grams)=2·2 lbs.

LENGTH

1 millimeter=0·03937 inch (about $\frac{1}{25}$ inch).
1 centimeter=0·3937 inch (about $\frac{2}{5}$ inch).
1 meter=39·37 inches.

VOLUME

1 microl (0·001 ml.) (λ)=0·0169 minim.
1 milliliter or mil (ml.) (1·000028 c.cm.)=0·352 fluid ounce.
=16·894 minims.
1 liter (or litre) (1000·028 c.cm.)=1·76 pints.

RELATION OF CENTIGRADE AND FAHRENHEIT THERMOMETERS

Freezing Point	0° C.	32° F.
Boiling Point	100° C.	212° F.

1° Centigrade=1·8° Fahrenheit.

CONTENTS

EXPERIMENTAL PHYSIOLOGY

CHAPTER I

INTRODUCTORY: THE CONDUCT AND REPORTING OF EXPERIMENTS

It is axiomatic that for any real appreciation of a science the student must himself perform some of the fundamental experiments and repeat some of the fundamental observations on which that science is based. No amount of mere reading can ever replace this first-hand practical acquaintance with the subject-matter of a science and the chief types of experimental procedure by which it is investigated.

Before embarking on a course of Experimental Physiology the student will already have made a practical study of chemistry and physics and should be acquainted with the principles of the experimental method, with the use of inductive logic, and with the meaning of the terms "hypothesis," "theory," and "fact." If, as is most probable, he is not acquainted with these matters he should repair that omission now.[1]

The method of physiology is to study, in the first instance, the manner in which the simplest units of any system or organ work—that is, how they react to changes in their environment—and is thus analytical. The aim of physiology is to give a description, as complete as possible, of the way in which the body as a whole works, and is thus synthetic or integrative. As analysis must precede synthesis in the building-up of physiological theory, so, in the conduct of a course of experimental physiology, analytical experiments on simple preparations isolated from the body, and on isolated organs and tissues within the body, precede experiments illustrating the integrative or synthetic aspects of the activity of the different organs and systems as studied in the intact animal or man. This book is concerned chiefly with the analytical aspects of experimental physiology. It is assumed that the student will subsequently give detailed attention to the more synthetic aspects by working through some of the exercises in Lamb's "Human

[1] By reading at least the first part of Claude Bernard's "Introduction à l'Étude de la Médecine Expérimentale." All students are strongly advised to read this great work. An English translation is available.

Experimental Physiology,"[1] to which work this present book may be considered to serve as an introduction.

Apparatus.—For ordinary analytical class experiments it is assumed that each working place has a recording drum driven either from bench shafting or by a motor incorporated in the apparatus, a myograph with lever and weights for ordinary muscle-nerve experiments, another myograph and lever for use with the frog-heart, a sliding induction coil, a make-and-break key, a short-circuiting key, a commutator, one or more Leclanché cells or accumulators, a pair of electrodes, and the wires necessary for making connexions.

In most of the experiments to be described, records are made on highly glazed paper smoked by a gas flame charged with benzene vapour. The paper is carried on the recording drum. Records made by such means are rendered permanent by varnishing.

Before making any experiment involving the use of recording apparatus, the student should become thoroughly acquainted with the working of the recording drum. In particular he should examine the gears and driving pulleys and realise the range of speeds provided by the different combinations of driving shaft and drum pulleys, when the drum is in low and in high gear respectively. The electrical contacts and their adjustments should also be noted.

Electrical apparatus is extensively used in experimental physiology. Separate chapters of this book are devoted to a description of such apparatus, and the student is advised to study these chapters thoroughly if he wishes to save himself trouble throughout the course.

Instruments.—Dissecting instruments, etc., must be supplied by the student. Instruments used for anatomical work must on no account be used for physiological dissections. Following are the minimum requirements: 1 pair of strong and 1 pair of fine dissecting scissors (straight blades); 1 pair of strong dissecting forceps; 1 pair of fine dissecting forceps, preferably with curved points; 1 pair of dividers. Each instrument should be carefully examined at the time of purchase: scissors, to ensure that they cut to the points; forceps, that they bite to the points. In the case of the fine forceps, ensure that the teeth engage properly and that the points do not splay out when the limbs are firmly closed.

Conduct of experiments.—Many details relating to the conduct and recording of experiments are noted in the directions throughout the book, but the following general rules should be observed in all experiments:—

1. Always fit up and test the necessary apparatus before beginning the dissection.

[1] "An Introduction to Human Experimental Physiology," by F. W. Lamb, Longmans, Green & Co. Ltd.

2. Take the greatest care to avoid handling of and damage to tissues which are to be the subject of experiment. Ensure that the preparation you are working with is kept moist with Ringer's solution throughout the whole course of the experiment.
3. Write full details relating to experimental procedures in a notebook *at the time the observations are made.* Where experiments are recorded graphically essential data should also be marked on the smoked paper as the observations are made: such data marked on the tracing must, of course, be supplemented by written notes.
4. Before the tracing is varnished the name of the student, the date, and a short description of the experiment should be marked on the surface. Tracings should be removed from the drying rack as soon as possible after varnishing.

Reporting of experiments.—An experiment is in no sense completed by the varnishing of the record, or by the insertion of this, however admirable it may be, into a notebook. The student should realise at the outset that *the accurate analysis of his results and the drawing of inferences from them is one of the main parts of each exercise.*

For the reception of records and reports a notebook with strong covers should be provided. It is desirable that all records, diagrams, tables, graphs and calculations be inserted on the left-hand pages, leaving the right-hand pages free for the written accounts. Accounts of experiments not recorded graphically, as well as those shown as demonstrations, must also be entered in the notebook, which will thus contain a complete record of the work done in the class.

The following rules relating to the reports of experiments are of the greatest importance:—

1. Each experiment should be written up as soon as possible after it has been made, when the details are still fresh in the mind and the notes taken at the time are still intelligible.
2. In the written accounts attention should be paid to the following points:—
   (*a*) The objects of the experiment and the methods used should be briefly described.
   (*b*) The results should be analysed *in detail.*
   (*c*) *The student must draw his own inferences and conclusions from his results.* It is not sufficient merely to copy textbook statements. The conclusions must be the student's own, from his own results. His results, and, therefore, his conclusions, may differ from those given in the textbooks. Such differences should be noted and, if possible, accounted for.

In conclusion, students are advised to lose no opportunity of comparing their results with those of their colleagues. Individual variation is one of the most striking and important phenomena encountered in biological study; this fact and its significance should be appreciated by the student at as early a stage of his physiological studies as possible. The complexity of physiological reactions—even when reduced to their simplest components and manifestations—and the consequent importance and difficulty of making adequate controls in their experimental investigation, is another outstanding fact which should be kept constantly in mind. Appreciation of these fundamental facts, and strict attention to the rules detailed above, will serve to develop not only habits of accuracy in the observation and analysis of physiological and clinical phenomena, but habits of critical judgment in their interpretation, and in the interpretation of the results of others. The acquisition of such habits will prove of the greatest service to the student in his future career, and the earlier they are acquired the better.

CHAPTER II

AMŒBOID AND CILIARY MOVEMENT[1]

Amœboid movement.—The fresh-water amœba. Find an amœba in a drop of pond water under the microscope, and study its movements. Make an outline sketch of the amœba at intervals of half a minute.

Amœboid movements and other changes in leucocytes.—1. Mount on a slide a drop of frog's blood diluted with an equal amount of frog-Ringer (see Appendix). Bring a leucocyte under observation with the high power of the microscope, and sketch at intervals the changes in shape which it undergoes.

2. *Effect of temperature increase on leucocytes.*—Place the slide on a " warm stage " and heat gently to about 25° C., as indicated by the melting of a piece of " 25° " paraffin wax placed alongside the preparation on the slide. Observe the effect of warmth in accelerating the movements of the leucocytes.

3. *Ingestion of particles by leucocytes.*—Take a very small quantity of yeast and shake it up in frog-Ringer. Mix a minute drop of the yeast and salt solution with a drop of frog's blood and examine under the microscope. Observe the ingestion of yeast torulæ by leucocytes. Sketch one or two corpuscles which have ingested torulæ. Particles of carbon (Indian ink) may be used instead of yeast for this experiment.

4. *Mammalian leucocytes.*—These may be investigated in the same way as frog leucocytes, but the preparation must be maintained at a temperature of about 37° C. on the warm stage.

Ciliary movement.—Gently scrape some epithelium from the roof of a frog's mouth, and shake the scrapings in a drop of frog-Ringer. Observe with the microscope the ciliated cells and the movements of their cilia.

Experiments on ciliary movement. 1. *Transport of material by cilia.*—(*a*) In a frog which has just been killed, cut through the attachments of the lower jaw and carry an incision down the œsophagus to the stomach. Cut this organ across, seize the cardiac end with forceps, and dissect out the œsophagus together with the pharynx and a part of the mucous membrane of the mouth. Pin out the œsophagus and pharynx and adjacent parts of the buccal membrane on a flat cork

[1] Most of the observations and experiments recorded in this chapter are conveniently made in connexion with Histology. Details of the methods are given in Schafer's " Essentials of Histology."

with the inner surface uppermost. Rinse with frog-Ringer. Sprinkle a few grains of charcoal over the buccal end of the preparation, and notice that the charcoal is carried down as far as the stomach by the action of the cilia. In the same way, pieces of cork or wax, or even small flat pieces of heavy materials such as lead, may be passed over the surface.

(*b*) Open a marine mussel (*Mytilus edulis*) by cutting through the adductor muscle. Remove all the organs except the gills and mantle. Wash one of the shells with its attached gills, place it in a dish of sea water, and add some particles of Indian ink or carmine to the water with a pipette. Note how the particles are moved along by the action of the cilia.

2. *Effect of temperature on ciliary movement.*—(*a*) Fasten with pins two pieces of thread one centimeter apart across the above preparation of œsophagus, and slightly raised above it. Rinse the membrane with frog-Ringer at room temperature. Drop a granule of charcoal on the buccal end, and with a watch record the number of seconds which the charcoal takes to pass over the interval between the threads. Now rinse the membrane with *ice-cold* Ringer and repeat the experiment. Repeat again after rinsing with *warm* Ringer (25° C.). Note the differences in time taken to traverse the space marked off by the threads. Lastly, rinse with Ringer heated to 50° C., and repeat the experiment. The ciliated cells are killed at this temperature, and the charcoal is no longer carried along.

(*b*) The gill cilia of *Mytilus* may also be used for demonstrating the effect of temperature upon ciliary activity. Mount in sea water a gill filament of *Mytilus* or a fragment of gill comprising several filaments. Study the action of the largest cilia. Now place the preparation upon the warm stage and observe the effect of gradually raising the temperature. Use a low power of the microscope.

By using an eyepiece with a micrometer scale attached, the effect of temperature changes upon the rate of transport of particles by the cilia can be investigated.

3. *Effects of ions on ciliary movement.*—The effects of changes in ion ratios, and the effects of drugs on ciliary activity can be studied on the cilia of the frog, but even more satisfactorily on the gill cilia of *Mytilus*. For this purpose a supply of gill filaments may be placed in a dish of sea water at about pH 8·0, and a series of small watch-glasses is arranged to receive the solutions it is desired to investigate. In one of these place some normal sea water and a gill filament. Observe the movements of the frontal cilia with a low power. Another watch-glass contains the solution to be tested. Place it on the microscope stage, transfer the filament to it, and record any change in the ciliary movements. Determine if the effect is reversible by replacing the

filament in normal sea water. Investigate particularly (*a*) the effect of rendering sea water more alkaline (pH 8·5 to 9·5); (*b*) the effect of rendering it slightly acid (pH 5 to 6·8); (*c*) the effect of Ca deprivation.

4. *The effects of gases and vapours on ciliary activity.*—Cement with sealing-wax a piece of small glass tubing to a slide so that one end of the tube comes nearly to the centre and the other end projects beyond the edge of the slide. To do this the slide must be heated, and sealing-wax melted on to it. The glass tube is then made hot and applied to the slide, embedding itself as it does so in the sealing-wax. Apply a ring (half an inch in diameter) of modelling wax or plasticine so as to include the end of the tube and rising well above it. Make a deep notch, or a hole, in the ring for the exit of gas. Place a drop of water in the ring. Put a gill filament on a cover-glass in the least possible quantity of sea water: invert the cover-glass over the ring and (with another slide) press it down evenly and gently. The preparation now hangs in a *moist chamber* within which it can be studied through the cover-glass, and into which gases or vapours can be passed and their effects observed.

Pass CO_2 through the chamber, and after observing the effect replace it by air. Repeat with ether vapour and with chloroform vapour. The slide must be securely clamped to the stage of the microscope.

CHAPTER III

THE ELECTRICAL APPARATUS IN COMMON USE IN PHYSIOLOGICAL WORK

Batteries.—A voltaic element or cell consists, in its simplest form, of two metals, such as zinc and copper, or of zinc and carbon, immersed in a suitable fluid such as dilute sulphuric acid; the movements of ions which occur under these circumstances in the fluid produce a

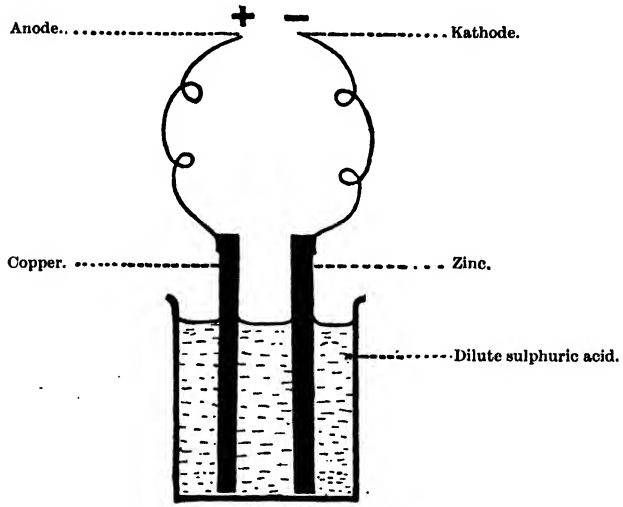

FIG. 1.—Voltaic couple.

disturbance of electrical equilibrium which manifests itself as a difference of electrical potential or pressure at the metals. If wires are attached to the parts of these outside the fluid it is found that the end of the wire connected with the copper is charged with positive electricity, and that connected with the zinc is charged with negative electricity; these ends are called the positive pole, or *anode*, and the negative pole, or *kathode*, respectively. The anode is said to be in a condition of higher potential and the kathode in one of lower potential, and when they are joined electrical equilibrium tends to re-establish itself in the circuit thus closed. It is common to speak of a current as

flowing from the anode to the kathode outside the cell and from the zinc to the copper inside.[1] The amount of this current depends upon the difference of potential produced within the cell. This is diminished by any increase of resistance to the flow of electricity, whether occurring within the cell or in the outside circuit. Electromotive force (E.M.F.) is measured in volts; thus the E.M.F. of a Daniell cell is 1·079

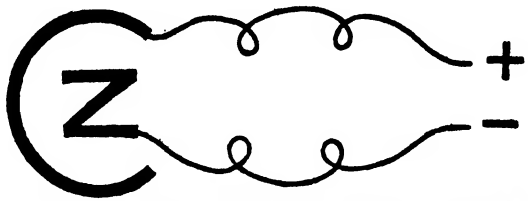

FIG. 2.—Diagram of a voltaic couple. **Z**, Zinc; **C**, Copper.

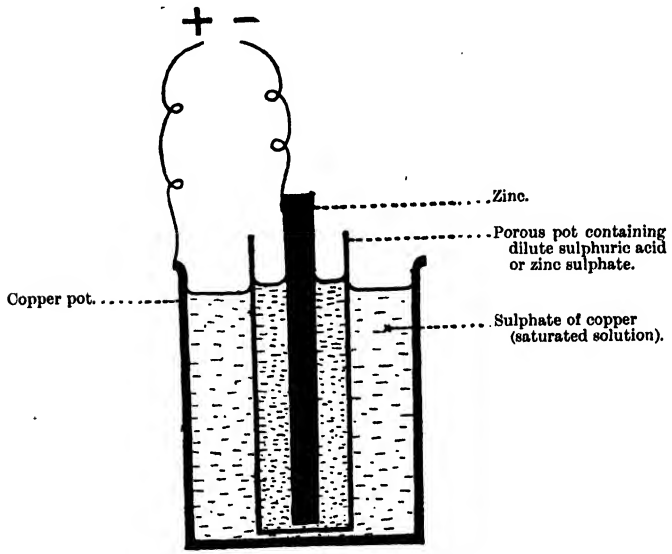

FIG. 3.—Daniell cell.

volts. It may be increased by coupling two or more cells together in series, the zinc of one connected with the copper of the next, and so on When two or more cells are connected, whether in series or in parallel, they form what is known as a *voltaic or galvanic battery.*

The structure of the Daniell cell is shown in Fig. 3. Another

[1] *Within the cell* the electrical potential is highest at the zinc, which is therefore here the anode, and lowest at the copper, which is here the kathode.

constant cell is Leclanché's (Fig. 4), in which the acid is replaced by ammonium chloride and the place of the positive plate is taken by carbon, which is surrounded by manganese dioxide. The so-called "dry" cells are modified Leclanché's. In both the Daniell and Leclanché cells the negative plate is zinc.

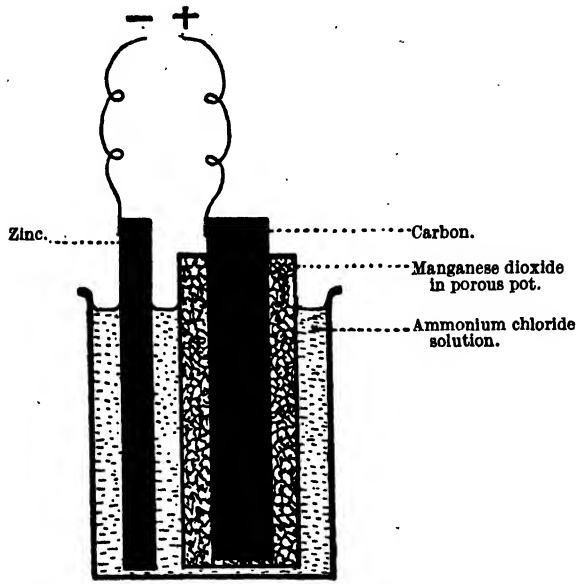

FIG. 4.—Leclanché cell.

Accumulators.—For physiological, as for many other purposes, the voltaic cell has been largely superseded by the accumulator or storage cell. The type generally employed has plates of lead and of lead covered by lead peroxide immersed in dilute sulphuric acid: the positive plate is that covered by the lead peroxide. The E.M.F. of such a cell when fully charged is somewhat more than 2 volts, but falls rapidly to exactly 2 volts, and is maintained constant at this value throughout the greater part of the discharge. The charging of an accumulator is effected by passing a current of suitable amperage from the mains through the cell in the opposite direction to the accumulator current.

Electrodes.—The wires used in physiological experiments are always insulated with gutta-percha or rubber, or with silk or cotton; in the latter case the insulation is rendered more effectual by dipping the covered wire into molten paraffin. For experimental purposes it is usual to place the ends of the wires (which must be clean and free

from the insulating material) in some sort of holder, so that they can be more readily applied to the tissue which is to be investigated; these ends are usually termed the *electrodes*.[1] They are often made of platinum set in a vulcanite holder; but a pair of pins, with fine wires soldered to their heads, constitutes a readily improvised and efficient pair of electrodes for most class purposes. Such pin-electrodes can be passed through a small paraffined cork, or fixed in parallel fashion with Chatterton's cement, with their cleaned points projecting for a few millimeters. Such an arrangement enables them to be conveniently handled.

FIG. 5.—Pin-electrode.

To determine which of the two electrodes in any case is the anode and which the kathode, they are placed in contact with a piece of blotting-paper moistened with starch solution containing iodide of potassium (*pole-testing paper*). Iodine is set free at the anode and turns the starch blue. Or, filter paper which has been impregnated with phenol-phthalein may be used; a red coloration appears under the kathode, sodium being set free there.

FIG. 6.—Simple cork electrode-holder.

Non-polarisable electrodes.—Like the plates of the cell itself, metallic electrodes are capable of becoming polarised when they are in contact with the moist tissues and a current is passed continuously between them in one direction. For some experiments it is necessary to obviate this polarisation of electrodes by employing electrodes which are not polarisable.

For this purpose we make use of the fact that where there is no contact of metals with fluids which can produce dissimilar ions at their surface of contact, polarisation is absent or negligible.

Non-polarisable electrodes can be made by taking two small pieces of glass tubing open at both ends, either straight—du Bois-Reymond's type—(Fig. 7) or bent—Burdon-Sanderson's type—(Fig. 8), and, one end of each having been plugged with china clay made into a paste with normal salt solution, the tube is filled with saturated solution of zinc sulphate: an amalgamated zinc rod (to which one of the wires of the circuit is soldered or otherwise attached) is plunged into the zinc sulphate.[2]

[1] The term *electrode* means literally the "path" of the electric current, and in this sense the wires throughout are electrodes. But it has come to mean technically the point at which the current enters or leaves a *metallic conductor*.

[2] The rod is amalgamated by dipping it for a few seconds into a solution of mercury in nitric acid, washing under a tap, and polishing with cotton-wool.

Connexion between the clay plug and the tissue may be direct, or may be effected by means of a piece of cotton wick wetted with normal salt solution.

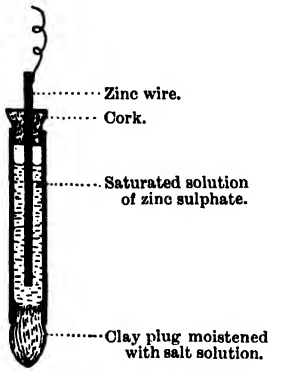

FIG. 7.—Non-polarisable electrode.

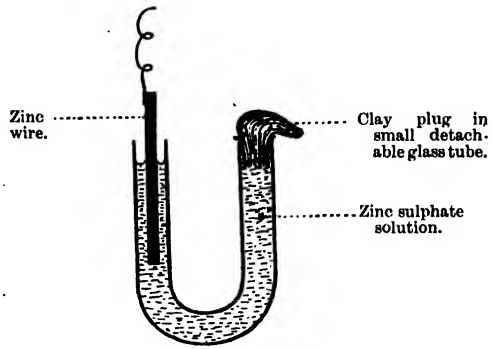

FIG. 8.—Sanderson's pattern of non-polarisable electrode.

A convenient form of non-polarisable electrode for class use is that of Porter, who uses a boot-shaped tube of unglazed porcelain which is soaked with normal saline and filled with saturated solution of zinc sulphate; the amalgamated zinc rod is passed into the leg of the boot.

Another type of non-polarisable electrode is afforded by silver wires which have been coated electrolytically with silver chloride. They are applied to tissues in the same way as are ordinary pin electrodes. Frequent rechloriding is advisable.

Keys.—Any apparatus which is used for interrupting or diverting the course of a current is called a *key* or *switch*. The keys used in physiological experiments are arranged to close and open a circuit (make and break the current); two wires forming part of the circuit being connected together either through a pool of mercury (*mercury key*—Figs. 9 and 10); or by contact between a platinum plate and platinum point (*contact key*—Fig. 11), as in the Morse key; or by friction contact between two brass surfaces (*friction key*), as in that known as du Bois-Reymond's (Fig. 12), and in the ordinary electric-light switches. Keys are used in two ways, viz.: either simply to close or open the circuit (*direct method*—Figs. 10 and 11); or by bridging across a part of the circuit to offer a passage with very little resistance through the key, so that most of the current is diverted from the main circuit and from the electrodes (*short-circuit method*—Fig. 12). For this purpose du Bois-Reymond's key is especially well suited.

Commutators.—A key which is constructed so as to cause a current

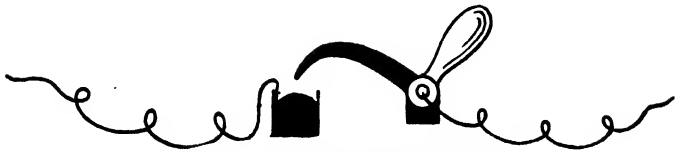

FIG. 9.—Diagram of mercury key.

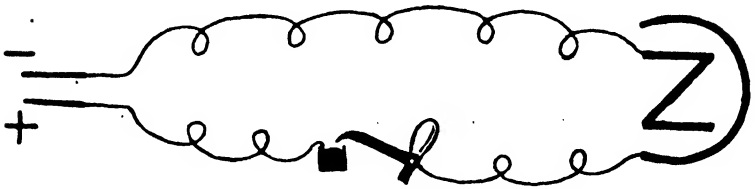

FIG. 10.—Mercury key in a battery circuit.

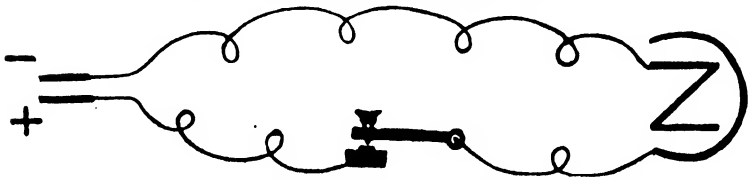

FIG. 11.—Contact key in a battery circuit.

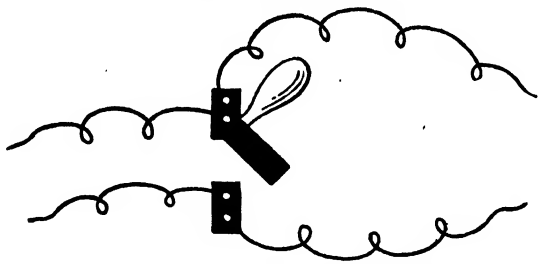

FIG. 12.—Diagram of short-circuiting key of du Bois-Reymond.

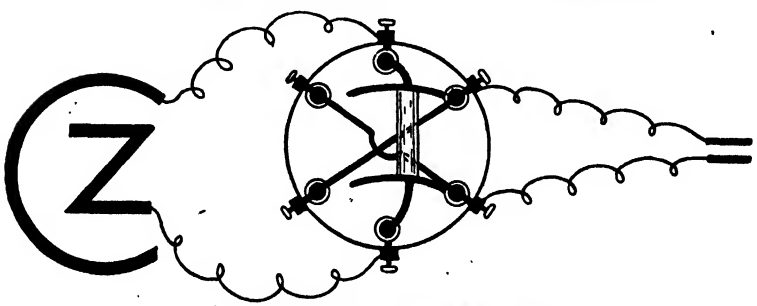

FIG. 13.—Diagram of Pohl's commutator.

to flow either in one direction or in the reverse direction in part of a circuit is called a *reverser* or *commutator*. One of the most frequently

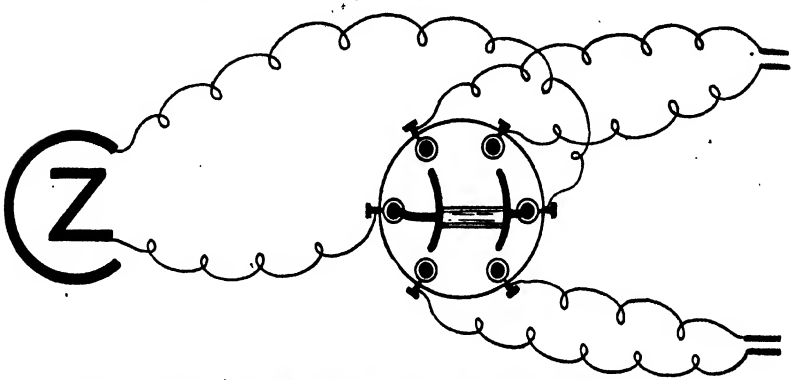

FIG. 14.—Pohl's commutator used as a two-way switch (cross wires removed).

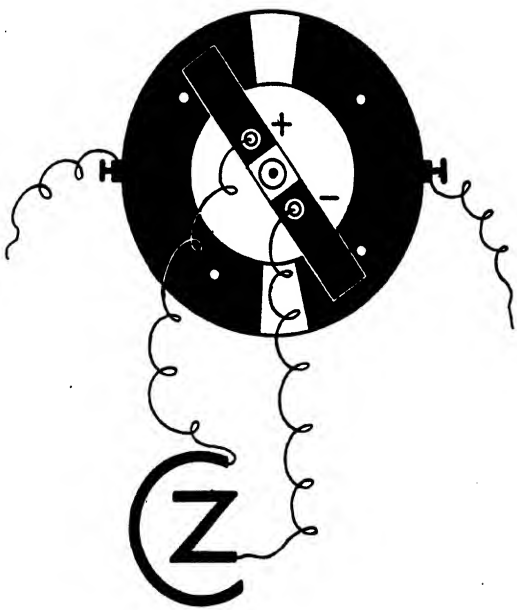

FIG. 15.—Rotatory commutator.

used is Pohl's (Fig. 13), which consists of a plate of vulcanite or other non-conducting material in which are six cups filled with mercury connected with terminals. Four of the cups are joined diagonally,

two and two, by crossed wires. A rocking double bridge of copper serves, on being moved to one side or the other, to effect the reversal.

If the crossed wires are removed the Pohl can be used as a switch for diverting a current into one or other of two circuits (Fig. 14).

Other commutators have friction-contacts in place of mercury: of these the simplest is Waller's (Fig. 15), which has a rotating action; and Malcolm's (Fig. 16), which has a sliding action.

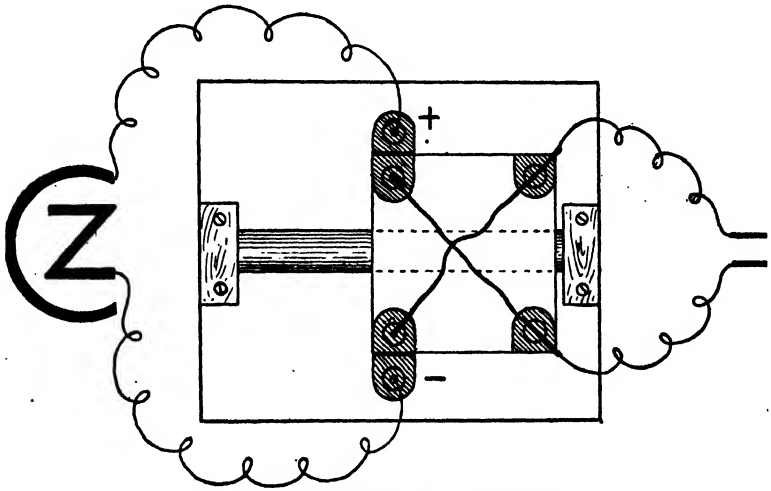

FIG. 16.—Diagram of sliding commutator.

Rheochords.—A *rheochord* is an apparatus for dividing a constant current by offering a circuit of relatively small resistance which is capable of being varied so that a certain proportion only of the current shall pass through the experimental circuit. It usually consists of a german-silver or platinum-iridium wire of a known resistance (*e.g.*, 20 ohms), to the ends (Fig. 17, *a* and *b*) of which the wires from a cell are connected; a certain difference of potential is thereby produced at the ends of the wires. With one of these ends (*a*) another wire is connected; this forms part of the experimental circuit through which a portion of the cell current is to be conducted; this circuit is completed through a rider (*r*) which slides along the rheochord wire.

When *r* is in contact with *b* the whole difference of potential between *a* and *b*—which depends upon the E.M.F. of the cell or battery and the resistance of the rheochord wire relative to that of the experimental circuit—is operative in producing a current through the preparation. When *r* is at the middle of the rheochord wire only one-half of this difference of potential comes into play, and so in proportion to the distance between *a* and *r* as compared with the whole length of the

wire. Thus if the wire is 100 centimeters long and r is placed at one centimeter from a, only $\frac{1}{100}$ of the total difference of potential will be operative and a proportional current will be diverted into the experimental circuit. If r is in contact with a no current is led through the preparation.

When this kind of rheochord is used, the resistance of the experimental circuit must always be relatively very great: as is invariably the case in physiological experiments where an animal tissue forms part of the circuit.

The wire may be stretched straight as in Fig. 17, or, to economise space, it may be zigzagged upon a board (Fig. 18), or arranged spirally round a vulcanite cylinder, or circularly round a disc.

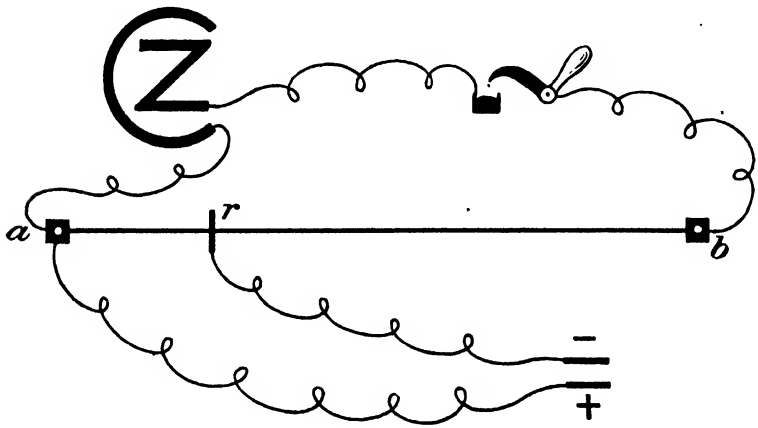

FIG. 17.—Diagram of rheochord.

Measurement of E.M.F., etc.—To determine the E.M.F. between any two points in an experimental circuit a voltmeter is employed, placed in parallel between the points. The strength of current flowing in a circuit is measured in amperes. In the circuits used in physiology the current being of the order of thousandths of an ampere is measured with a milliammeter, which is placed in series in the circuit. To determine the resistance of a circuit it is sufficient to know the E.M.F. of the cell and the current flowing, the resistance being calculated from these data by Ohm's Law.

Induction coil.—If the wires of two separate circuits are near and parallel with one another and if, in the first or *primary* circuit, the current of a cell is either made or broken by the closing or opening of a key, an induced E.M.F. is set up in the other or *secondary* circuit at the instant of such closing or opening, but not during the passage of the

primary current. Any sudden alteration in potential in the circuit of the primary coil is also effective in producing an induced E.M.F. in the secondary coil, the strength of the induced E.M.F. being a factor of the rate of change of potential in the primary.

In order to multiply the induction effect the two circuits usually take the form of closely coiled wires (Fig. 19) (that of the secondary

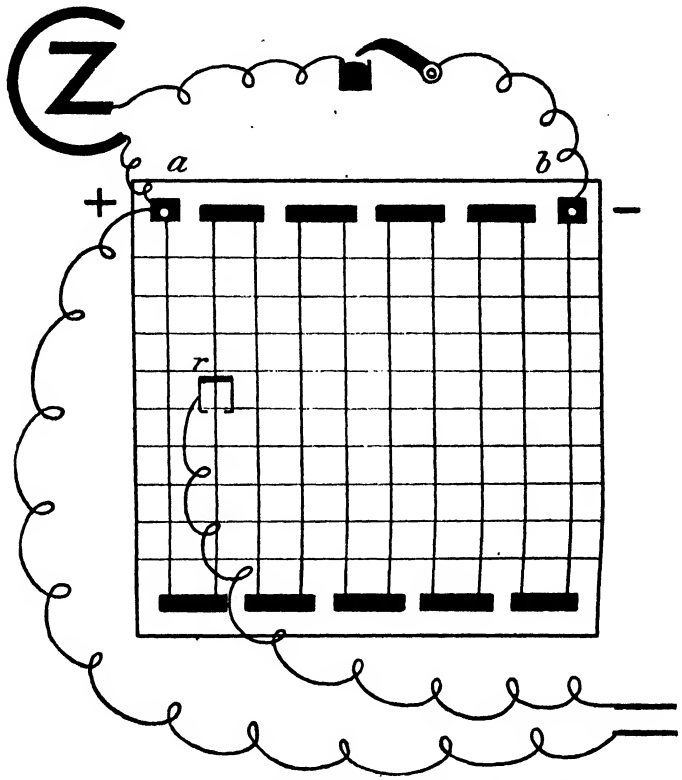

FIG. 18.—Diagram of the Oxford form of rheochord. *a*, *b*, rheochord wire zigzagged upon a mahogany board, marked by cross lines into hundredths; *r*, rider.

circuit being very fine and having very numerous coils), and still further to increase the effect the primary coil is generally wrapped round a core formed of a bundle of soft iron wires which are magnetised and demagnetised on the closing and opening of the primary circuit, thereby enhancing the induction effects. *The induced or secondary current thus produced is of very short duration and of very small amperage, but has a high electromotive force (voltage).*

For physiological purposes the induction coil was arranged by *du Bois-Reymond* so that the secondary circuit can be made to slide nearer to or farther from the primary circuit; since with a battery of a given voltage the nearer or farther the coils are from one another the greater or less is the strength of the induced E.M.F. The variation is not, however, proportional to the distance, but approximately inversely as the square of the distance.

For producing single make and break induced shocks the primary circuit is closed and opened with a simple key (Fig. 19). For multiple induced shocks most coils are fitted with an apparatus for automatically breaking and making the primary circuit (Neef's hammer). This

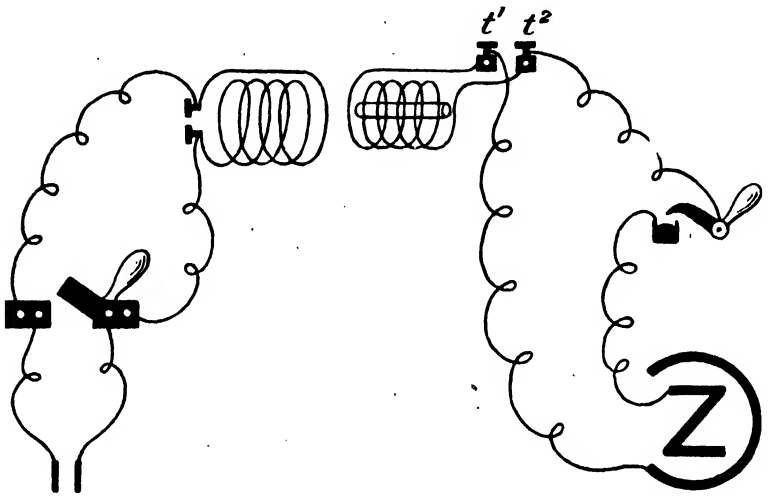

FIG. 19.—Induction coil arranged for single shocks.

will be understood from the diagram shown in Fig. 20. The current is conveyed from the terminal t^3 to a steel spring *sp*, having a bar of soft iron at its free end, and the current passes from the spring, which has a plate of platinum upon it, to the platinum point of a screw s^1, and thence through the primary coil. Before passing back to the cell it is conducted through a small electro-magnet *m*; the electro-magnet being thus set in action, draws down the iron bar and with it the spring, which leaves the screw and breaks contact so that a *break* induced E.M.F. is set up in the secondary coil. But, the circuit being broken, the electro-magnet *m* is no longer active, the bar springs up again, and contact is re-established between the spring and screw; this produces a *make* induced E.M.F. in the secondary coil. Thus the spring vibrates to and fro, and break and make induced E.M.F.'s are

set up in the secondary coil many times a second, according to the rate of vibration of the spring.

These make and break shocks are unequal owing to self-induction effects in the primary circuit—the so-called "extra currents"—due to the primary coil itself. At make of the primary circuit the self-induced E.M.F., being in the opposite direction to the battery-current, retards

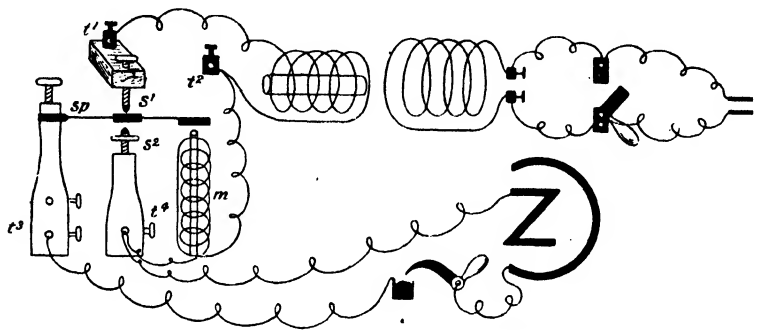

FIG. 20.—Diagram of du Bois-Reymond coil arranged for faradisation with Neef's hammer. (For description see text.)

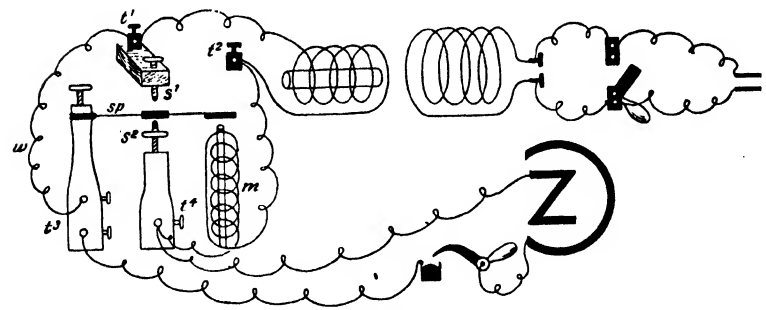

FIG. 21.—Diagram of the arrangements employed in the Helmholtz modification of du Bois-Reymond's induction coil. (For description see text.)

the rate of rise of potential in the primary circuit and thus diminishes the make induced E.M.F. in the secondary circuit. At break a self-induced E.M.F. tends to flow in the same direction as the battery-current, and would oppose its fall, but the battery-current no longer exists. The break of the battery-current, therefore, produces a larger effect, so that the break induction shock is stronger than the make. The inequality is reduced by a modification introduced by Helmholtz. In this arrangement (Fig. 21) a wire w connects the terminals t^1, t^3; the screw s^1 is

raised altogether away from the spring, and does not come into use; the screw s^2 is brought nearly up to the spring. The current passes by the wire *w* from the terminal t^3 directly to t^1, thence through the primary coil and through the electro-magnet *m*, which draws down the iron bar and brings the spring in contact with the screw s^2. A large part of the current now goes directly back to the cell through this contact, and is diverted from the primary coil and electro-magnet. This greatly weakens the current through the primary coil, and the equivalent of a *break* induced shock is obtained in the secondary circuit. But the electro-magnet is also weakened, so that the bar and spring fly up. This breaks the short-circuiting contact which was established between the spring and s^2, and the whole current again passes through the primary coil, producing the equivalent of a *make* induced shock in the secondary circuit, and so on automatically. It will be observed that the primary circuit is never actually broken, but only weakened by shunting. This permits both the break and the make self-induced E.M.F. to be effective, and tends to equalise the make and break stimuli.

It is obvious that tetanic stimuli applied to a tissue by means of the arrangement shown in Fig. 20 are bound to produce a certain amount of electrolysis of the tissue, since the potential between the electrodes at break is always very much greater than at make, with the result that the make current, while flowing in the opposite direction to the break, is not sufficient to reverse the polarisation occasioned by the break current. With the Helmholtz arrangement, on the other hand, the make and break shocks being almost equal tend to neutralise the polarising effects of each other, and thus damage to the stimulated tissue is reduced to a minimum.

The Helmholtz arrangement should always be employed for tetanic stimulation.

CHAPTER IV

SIMPLE EXPERIMENTS ILLUSTRATING THE USE OF ELECTRICAL APPARATUS IN PHYSIOLOGY

1. CONNECT up a cell with a pair of wires, introducing a simple key into the circuit (Fig. 10). Place the free ends of the wires on the tongue, and close and open the key. Note the sensation of taste during the flow of the current.

2. Connect up a battery of at least five cells in series, introducing a simple key into the circuit and employing copper wires as electrodes. Hold the free ends of the wires on the tongue and close and open the key. Note the effects (*a*) at the moment the circuit is made, (*b*) during flow of the current, and (*c*) at the moment of breaking the current. Determine at which electrode—anode or kathode—the effect is obtained (1) at make and (2) at break of the circuit.

3. Connect a cell with the upper terminals t^1, t^2 of the primary coil of the inductorium, introducing a simple key into the circuit. Connect a pair of electrodes through a short-circuiting key with the terminals of the secondary coil, and slide this coil to some distance from the primary (Fig. 19). Place the electrodes on the tongue. Alternately close and open the key in the primary circuit. Notice that induction shocks are obtained on making and breaking the primary circuit, but not during the passage of the current. Determine the distance of the secondary coil from the primary at which a make-shock and a break-shock can just be felt on the tongue. Notice that the break-shocks are much sharper than the make.

This is partly due to the self-induced potential in the primary circuit (see p. 19), and partly to the fact that with the keys generally used the opening of the primary circuit is more sudden than its closure.

4. To show the existence of the self-induced E.M.F.S, remove the secondary coil altogether and connect up the primary coil with a battery and keys in the way shown in Fig. 22. Place the electrodes on the tongue. Make and break the circuit by closing and opening the key k^1. If this is done when the primary coil is included in the circuit (*i.e.*, with k^2 open as in the diagram) the shock is sharp owing to the " extra " self-induced potentials, but if the coil (inductance) is shunted out by closing k^2 the shock is hardly perceptible to the tongue. (In many induction coils the inductance of the primary circuit is so low that this experiment fails to give satisfactory results.)

5. Instead of placing the simple key in the primary circuit (as in Fig. 19) place it in a side circuit (Fig. 23). On closing and opening the key, potentials are still induced in the secondary circuit, although the current through the primary coil is not made and broken, but only altered in strength. The make and break shocks in the secondary coil are now more uniform, but are both weaker. This is the same effect as

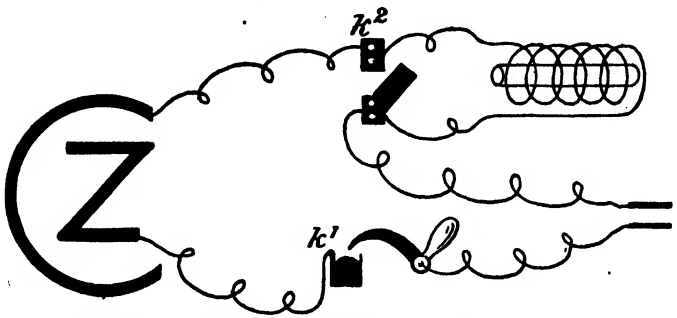

FIG. 22.—Experiment for showing effect of inductance in primary circuit.

is obtained for rapidly interrupted shocks by the use of the Helmholtz Wire (see p. 19). (Since this experiment involves short-circuiting of the battery, it is inadvisable to perform it where the source of current is a lead accumulator.)

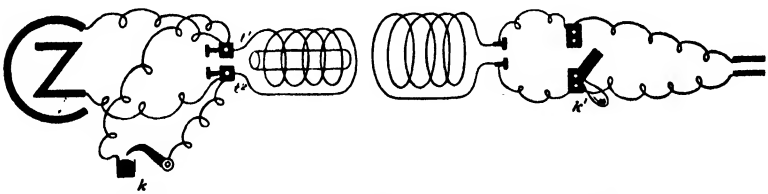

FIG. 23.—Diagram of experiment to show the effect of closing and opening a side circuit to primary coil. k, key in side circuit; k^1, key in secondary circuit.

6. Take the secondary coil out and place it across the direction of the primary coil instead of in its usual position. The making and breaking of the primary circuit now produce no effect on the secondary circuit, but induced currents begin to show themselves if the secondary coil is placed obliquely to the primary, and are strongest when the two coils are again co-axial.

7. Connect up the cell with the terminals t^3, t^4 of the induction coil (as in Fig. 20), introducing a simple key into the circuit. Set the Neef's hammer in vibration. The electrodes from the secondary coil are to

be applied to the tongue, and the distance of the secondary from the primary coil found at which the induced shocks can just be felt. Determine that these are the break shocks by raising and lowering the hammer by the hand, thereby making and breaking the primary circuit (the mercury key being closed).

8. Detach one of the wires of the electrodes from the secondary coil so that only one electrode is connected with that coil. Slide the coil home. Pass a strong current through the primary coil and set Neef's hammer going as in the last experiment. It will be found that shocks are faintly felt by the tongue, although only the one electrode is in connexion with the secondary coil and the secondary circuit is broken (*unipolar induction*). The explanation of this is that the body acts as a condenser which becomes charged and discharged through the electrode applied to the tongue. It is on account of this possibility of stimulating through only one pole that a simple key is never used in the secondary circuit, but always a short-circuiting key, which is introduced in the manner shown in Fig. 19. No shocks can pass to the electrodes when the key is closed, since the coil is then short-circuited; only when the key is open are the shocks conducted to the electrodes. On the other hand, in the primary or cell circuit a simple key is always used; were a short-circuiting key placed here the cell would rapidly run down.

9. Connect up a cell with the induction coil, using Helmholtz's modification (Fig. 21). As in experiment 7, find the distance of the secondary from the primary coil at which the induced shocks can just be felt on the tongue, and determine that the make and break shocks are now nearly equal by raising and lowering the spring by the hand. Both are markedly diminished as compared with the ordinary arrangement.

CHAPTER V

MUSCLE-NERVE PREPARATIONS: SIMPLE EXPERIMENTS ON EXCITATION OF TISSUES

Gastrocnemius-sciatic preparation.—The gastrocnemius muscle with its attached sciatic nerve, the hyoglossus muscle (with or without its nerve), and the sartorius muscle are those generally used for experiments. To obtain the gastrocnemius preparation proceed as follows:

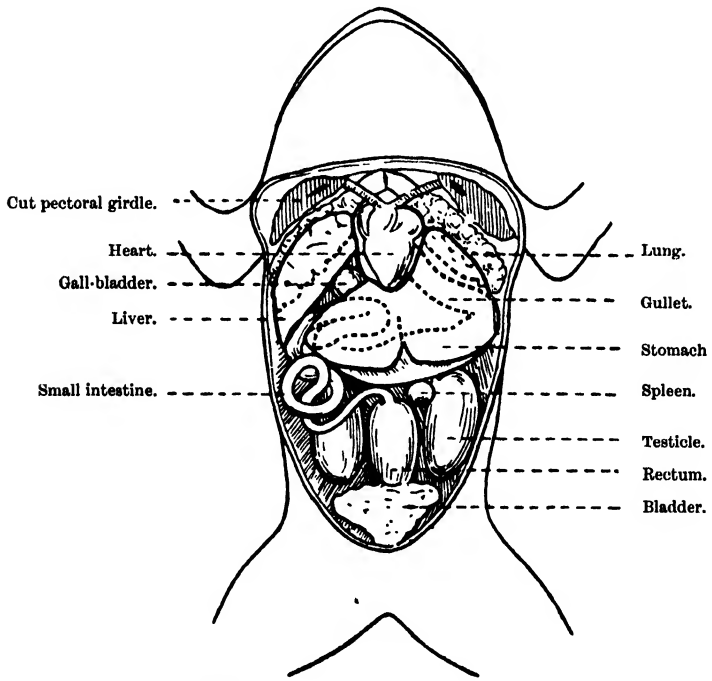

FIG. 24.—Viscera of frog. The liver is shown in outline, and the parts concealed by it are indicated by dotted lines.

Destroy the central nervous system of a frog by cutting through the spinal cord at the occipito-atlantoid ligament and passing a blanket pin into the skull and down the cord. Notice that the muscles of the

trunk and limbs are thrown into contraction while the cord is being destroyed (mechanical stimulation). Lay the frog on its back on a flat cork or glass plate. Make a free V-shaped incision through the skin and body wall in the abdomen, thus exposing the abdominal viscera. Note the liver, stomach, intestines, ovaries and oviducts (or testes), bladder (Fig. 24). Cut through the lower end of the intestine and through its attached mesentery. On raising it, two elongated red bodies—the kidneys—are seen at the back of the abdomen, partly covering the nerves which are passing down to the hind limbs. Remove the kidneys

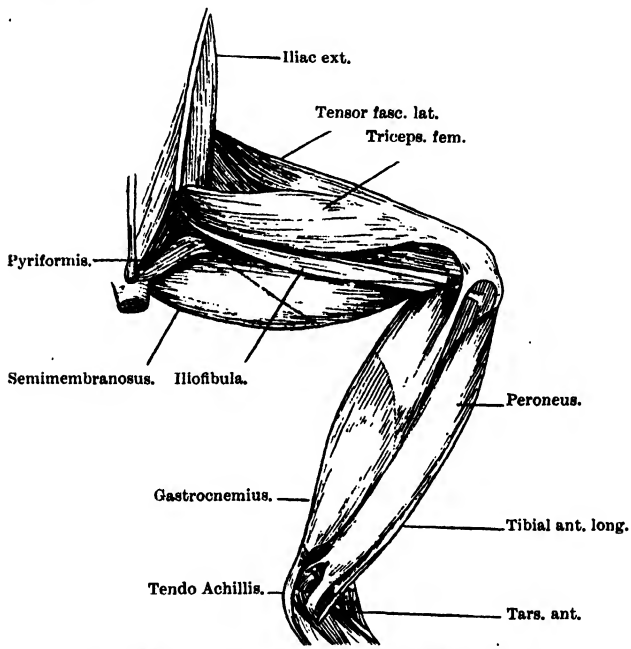

FIG. 25.—Muscles of frog-leg; dorsal aspect (Gaupp).

without touching the nerves. Now hold the frog up by its legs so that the viscera hang towards the head, and cut the whole trunk across with strong scissors at a level near the posterior end of the vertebral column and well above the urostyle: the cut separates the fore part of the trunk and the viscera from the hind part (pelvis and hind limbs). Holding the separated hind part by the vertebral column with a pair of strong forceps, grip the cut edge of the skin with a cloth, and strip the skin off the legs. Lay the preparation on a clean glass plate or on a piece of paraffined paper on the frog-cork. Note the several muscles which are seen on the front and back of the lower limbs (Figs. 25, 26).

Divide the vertebral column of the preparation in the sagittal plane with strong scissors and continue cutting in this plane as far as the pelvis. Complete the separation of the preparation into two parts by severing the femur from the ilium on one side. Place this leg aside for use later. With the other leg proceed as follows: Separate the muscles at the back of the thigh by the aid of two pairs of forceps, keeping to the medial of the two chief intermuscular septa; the sciatic nerve will now be seen, accompanied by the femoral vessels. On no

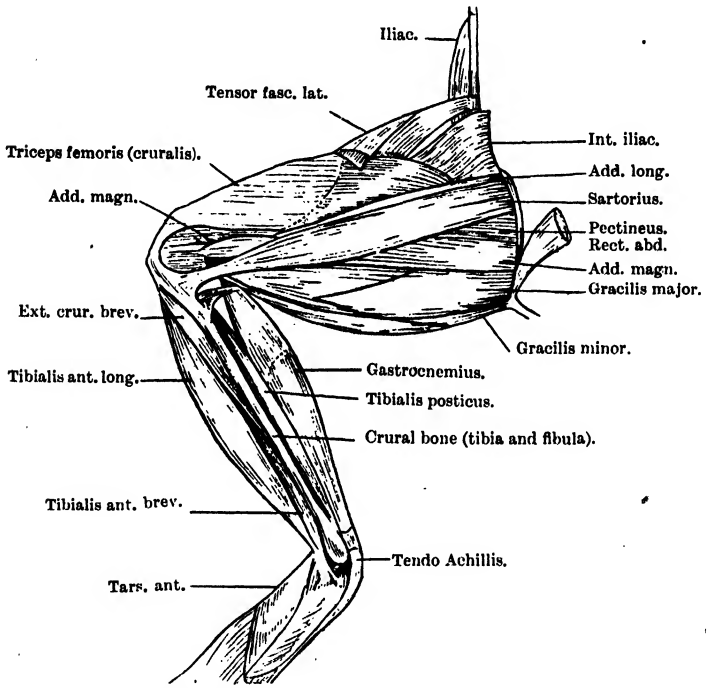

FIG. 26.—Muscles of frog-leg; ventral aspect (Gaupp).

account touch the nerve, but separate the muscles from it so as to expose it freely. Cut away the urostyle from the lower end of the vertebral column, taking care not to injure the nerves issuing from the spinal cord, which now come to view, passing down to form the sciatic. Grasping the spinal column with forceps, lift it up obliquely, but not at too sharp an angle, along with these nerves. Gradually dissect them out from above down, snipping the lateral branches with scissors (without touching the main nerve) until the back of the knee is reached. Notice that as each muscular branch is snipped the muscle which it supplies contracts (mechanical stimulation). Cut through the femur

about a centimeter above the knee and clear the attachments of the thigh muscles from this end. Tie a thread round the tendo Achillis and cut this away from the calcaneum. Holding by the thread, tear the muscle upwards away from the tibia, and then sever this bone just below the knee.

You now have a preparation consisting of the knee-joint with portions of the femur and tibia, the gastrocnemius muscle, the sciatic nerve, and part of the vertebral column.

Place a piece of paraffined paper, or blotting paper, on the frog-cork, and lay the nerve out clear of the muscle, fixing a pair of electrodes so that the nerve lies across them. Keep both muscle and nerve—but especially the latter—wet with frog-Ringer, but do not let this drip on to the table or apparatus.

The sartorius preparation.—For certain experiments the sartorius muscle is preferable to the gastrocnemius. The sartorius is a thin, flat muscle which crosses obliquely over the front of the thigh. It is readily isolated by cutting its tibial attachment away with a piece of the bone, raising this, and snipping through the fascia on either side of the muscle, thus separating it right up to its iliac attachment. Notice the twitch which occurs when the nerve, which enters the under surface about its middle, is cut through. The upper end of the muscle may be left attached to the ilium, or its bony attachment may be cut away with it and the muscle thus completely isolated. Its uppermost part contains no nerve-fibres, and can be used to show that, independently of nerve, muscle responds to all forms of stimulation (electrical, mechanical, thermal, and osmotic).

> The "double sartorius" preparation is often useful. This consists of both sartorii attached to the ilium. Each muscle is isolated as before up to its iliac attachment. The other muscles of the thigh attached to the ilium are severed on each side, the head of the femur being disarticulated at the same time. The ilium serves as a means of clamping the preparation or of pinning it to a frog-board.

The hyoglossus preparation.—The hyoglossus is a very thin muscle lying in a lymph-space of the tongue. It may be used instead of the sartorius in experiments upon the action of drugs on skeletal muscle. In a large frog, killed by pithing, cut away the whole of the lower jaw, along with the tongue and hyoid bone. Tie a thread round the tongue near its tip and another near its fixed extremity, and cut this away from the hyoid. The tongue thus separated includes the hyoglossus muscles, which run through it from the hyoid bone, and the preparation can be used in the same way as the gastrocnemius muscle, the hyoid end being fixed by a pin to the myograph cork and the tip

connected by its thread with the myograph lever. A smaller weight must be used than in the case of the gastrocnemius, since the hyoglossus muscles are far weaker. Probably the weight of the lever alone will be sufficient. Insert pin-electrodes near the fixed end so that induction shocks will stimulate all the fibres of both hyoglossus muscles.

If it is desired to stimulate the muscles through their nerves (hypoglossal), the skin over the lower jaw should be first removed and the hypoglossal nerves identified and isolated for a short distance before cutting away the jaw. Then proceed as above.

Mammalian muscle-nerve preparation.—The simplest mammalian muscle-nerve preparation consists of a strip of rabbit diaphragm with the phrenic nerve which supplies it. The preparation is set up in a

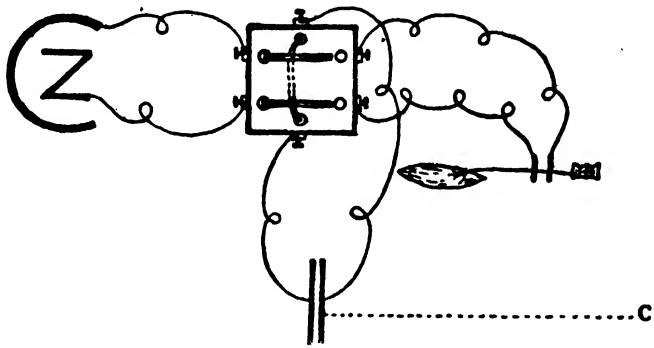

FIG. 27.—Stimulation of nerve by condenser discharge.

bath similar to that of Fig. 55, one end of the muscle-strip being attached to a rigid support submerged in the beaker and the other to a light isometric lever. The bath is filled with Locke solution and maintained at 37° C.

Another mammalian preparation, which contains both skeletal and plain muscle innervated by the same nerve, consists of the *lower* 4 centimeters or so of the rabbit œsophagus together with the cervical vagus. This is set up in the same way as the diaphragm strip. The reactions of both skeletal and plain muscle to nerve stimulation and to drugs can be investigated simultaneously with this œsophagus-vagus preparation.

The *upper* part of the cat's œsophagus contains only plain muscle innervated by the vagus.

Simple experiments on the excitation of tissues.—With the gastrocnemius-sciatic preparation the following experiments, which are, for the most part, similar to those already performed upon the tongue, are to be made. Note down all your results.

Excitation by galvanic current.—(*a*) Determine that making or breaking the circuit of a cell or battery is a stimulus to the nerve, whereas the passage of the current usually [1] produces no obvious effect. Note that the effect at make of the current is greater than the effect at break.

It is proper to use non-polarisable electrodes whenever a galvanic current is led through a preparation (see p. 11).

(*b*) Kill or damage a centimeter or so. of the nerve near the vertebral column either by dipping the end of the nerve in hot water or by squeezing it with forceps. The damaged part is now unable to react to a stimulus but will still conduct current. Place the damaged end over one electrode, the second electrode being on a normal portion of nerve. Make and break the current (1) with the damaged portion on the anode, and (2) with the damaged part on the kathode. In (1) there will be excitation at make only, and in (2) at break only; *i.e.*, at make of a galvanic current excitation takes place at the kathode, while at break it takes place at the anode. (See also Chapter XII.)

Excitation by induced current. Determination of excitability of a nerve.—An induction shock is a stimulus; the break induction shock is a far stronger stimulus than the make. Find the minimal effect of each by sliding the secondary coil to the necessary distance from the primary, and make a note as to the respective positions of the secondary coil. This gives a rough measure of the excitability of the nerve.

Determine its excitability to tetanisation (using the Neef's hammer with and without the Helmholtz wire) in the same way.

Unipolar induction.—It is possible to stimulate the nerve when it is connected by only one wire with the secondary coil; hence the necessity for using a short-circuit key to prevent *unipolar induction* (see p. 23). It is best for this experiment to place the secondary coil close to the primary and to make use of the automatic interrupter.

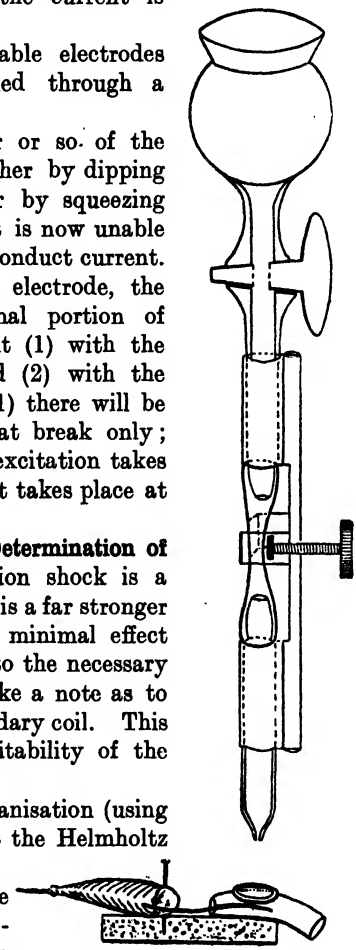

FIG. 28.—Diagram of mercury dropper to produce mechanical stimulation of a nerve. Natural size.

[1] For apparent exceptions see Chapter XII.

Excitation by condenser discharge.—The discharge of a condenser through a nerve acts as a stimulus. Arrange the apparatus as shown in Fig. 27, in which C is a condenser made by covering a sheet of glass with tinfoil on both sides. The sheets of tinfoil are connected momentarily with a battery of two accumulators, and then, by turning the switch, are connected with the nerve, the battery being cut off by the same movement.

Mechanical excitation.—A nerve can be stimulated by mechanical means—*e.g.*, by tapping it gently or by allowing mercury to drop upon it (Fig. 28). The effect of a mechanical stimulus is also seen whenever a nerve is cut or pinched, but a severe injury abolishes its conducting functions.

Thermal excitation.—A nerve is stimulated if touched with a hot wire; or with a wire cooled to below 0° C.

Osmotic excitation.—A nerve can be stimulated by withdrawal of water, caused by placing brine or glycerine upon it. The salt and the glycerine both act by abstracting water.

Excitation by drying.—The loss of water by evaporation also acts as an excitant to nerve. When the nerve begins to dry, its muscle twitches. *This twitching is a frequent source of puzzle to the beginner; it is a sign that he has not been careful to keep the nerve moist.*

Addition of water may also act as a stimulus, especially with muscle. If distilled water is injected into the blood-vessels of an animal, all the muscles are thrown into contraction: this is followed by paralysis. The effects are in part due to the abstraction of calcium salts from the tissue.

CHAPTER VI

THE RECORDING OF MUSCULAR CONTRACTIONS
THE SIMPLE MUSCLE CURVE

MUSCULAR contractions are recorded upon a metal drum covered with highly glazed paper, and caused to revolve by clockwork, or some other form of motor, at a regular rate. With a drum of 6 inches diameter one revolution in a second is a conveniently fast speed. The

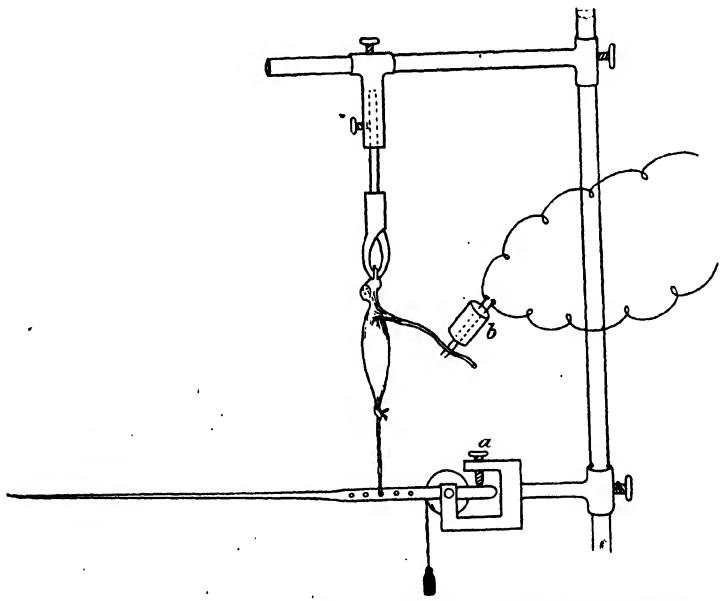

FIG. 29.—Muscle-nerve preparation suspended and attached to a straight myograph lever. *a*, after-loading screw; *b*, electrodes.

glazed paper is blackened by holding a gas flame containing benzene vapour against it while the drum is revolving. The paper must fit evenly and tightly, or it will become burnt.

The contractions of the muscle is amplified by a lever (myograph lever), which may be straight (Fig. 29) or may take the crank form

(Fig. 30). In this case the fulcrum of the lever is near one end of a cork plate, to which the muscle is fastened by a pin passed through

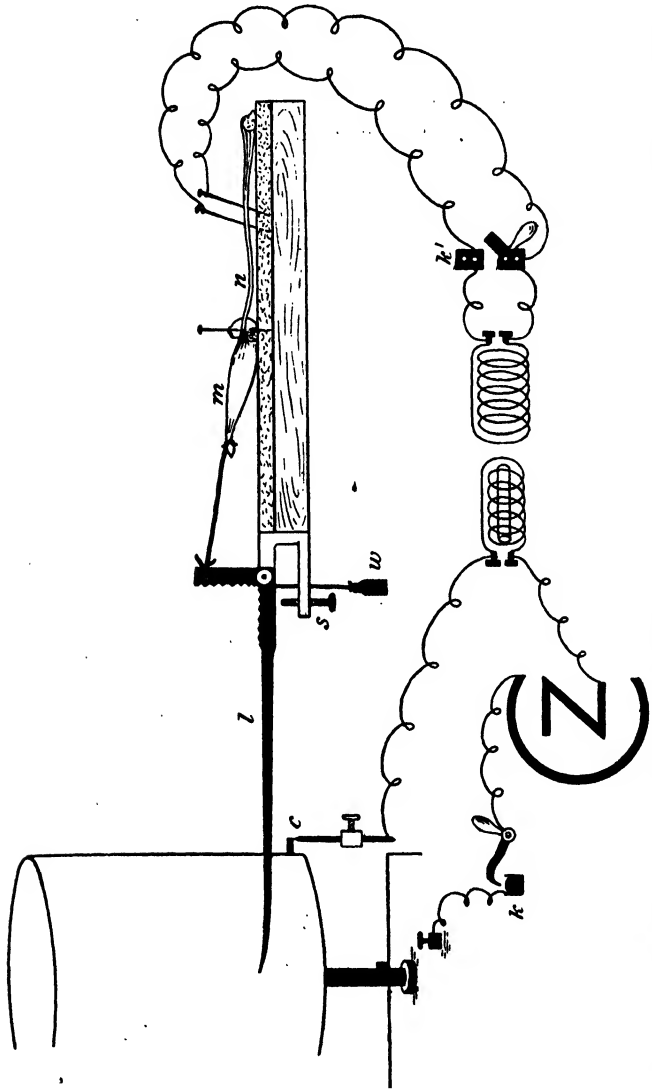

FIG. 30.—Arrangement of apparatus for obtaining an isotonic muscle-curve. *c*, drum contact in primary circuit; *k*, key in primary circuit; *k'*, du Bois-Reymond key in secondary circuit; from this key wires pass to the pin-electrodes on which the nerve, *n* is placed; *m*, muscle; *l*, crank-lever; *w*, weight; *s*, after-loading screw (not in use).

the knee-joint; the tendon is attached to the short vertical arm of the lever by means of a thread. The cork plate should be covered with paraffined paper or with blotting paper moistened with Ringer's

solution. The lever should be weighted with a 10 or 20 gram weight attached to it close to the fulcrum, and should be so adjusted as to be nearly horizontal, but with the free end a little lower than the fulcrum. The muscle is kept stretched by the weight, so that the connecting thread is taut. Under these circumstances the muscle is said to be *free-loaded* or *free-weighted.*

There is a screw near the fulcrum to support the lever in certain experiments. The screw can be adjusted so that the muscle and thread are not freely stretched, and only become so after the muscle has begun to contract; the muscle is then described as *after-loaded.*

The following points must be attended to in every graphic record in which a lever is employed:—(1) On no account must the lever point be directed obliquely upwards: the result of doing this is to distort the curves which are recorded; (2) the lever must be arranged tangentially to the curve of the drum with the writing point slightly curved in towards the surface of the drum; (3) the myograph stand, which carries the cork plate and lever upon a vertical rod capable of being turned on its axis, must always be furnished with a *stop,* so that the point of the lever can always be brought against the drum with exactly the same amount of pressure as that with which it is originally adjusted; (4) the myograph stand must always be on the right-hand side of the drum, so that the lever extends from right to left, and the drum itself must always be arranged to move in the direction of the hands of a watch, never in the reverse direction.

Record of contraction on a stationary surface. *Effect of strength of stimulus upon extent of contraction.*—Arrange the myograph and preparation in the manner shown in Fig. 30, but without including the drum in the primary circuit, which should be adjusted for giving single make or break shocks by closing or opening the primary key. The electrodes from the secondary coil may be applied either to the nerve, as in the figure, or directly to the muscle near its attachment to the femur. The muscle contractions are recorded as slightly curved vertical lines (ordinates) upon the stationary surface, which may be moved a millimeter or two by hand after each record. Stimulate (*a*) by closing, (*b*) by opening the primary key, beginning with the secondary coil at such a distance from the primary that no effect is obtained, and gradually diminishing the distance until the effects are maximal. The ordinates obtained represent the extent of contraction of the muscle (as magnified by the lever) with different strengths of stimulus. Beyond a certain point no further contraction is obtained. The contractions are then said to be maximal. The submaximal contractions are due to the fact that with the weaker induction shocks only some of the

fibres are stimulated, so that the whole of the muscle is not thrown into contraction.

The tracings show the relation of the extent of contraction to the strength of the stimulus (see also p. 45).

The simple muscle-curve. *Effect of a single maximal nerve volley upon a muscle.*—Arrange the muscle and myograph lever as in the last experiment, but with the drum in the primary circuit of the induction coil in the manner shown in Fig. 30, so that, as the drum revolves, a contact (*c*) which projects from it, by just touching another contact fixed outside, instantaneously makes and breaks the circuit at each revolution. With a very rapidly revolving drum the two induction shocks thereby produced act as a single stimulus, since the second falls within the refractory period (see p. 43) which immediately follows the first. With a slower rate of drum the make-shock must be made subminimal. This is effected by first bringing the two drum contacts together, and then making and breaking the primary circuit by means of the primary key: the secondary coil is moved away from the primary until the make-shock produces no effect on the preparation.[1]

Before the lever is allowed actually to touch the cylinder, determine that all the apparatus is in working order, and at what distance of the secondary from the primary the induction shock is effective in causing a maximal contraction when the drum is made to revolve. Adjust the position of the recording cylinder on its spindle so that (1) the curve when recorded will avoid the join in the smoked paper, and (2) the base line, or abscissa, of the record will lie some 2 or 3 centimeters from the bottom of the paper. (*In all experiments the position of the records on the drum should be arranged so as to make the most economical use of the paper and give maximum space for repetition of observations.*)

Now bring the lever point so as lightly to touch the blackened paper, *using the stop of the myograph stand to prevent the point pressing too hard against the paper.* When the stop is used in this way the lever point can be removed at any time from the paper and brought back again so as to press with exactly the same force as before; *it is absolutely essential to make use of the stop in all recording experiments in which comparisons of different curves upon the same surface have to be made;* failure to do so will invalidate the result. Remove the lever point from the paper.

The primary and short-circuiting keys both being closed, start the drum revolving and bring the lever point against the drum so that it describes a horizontal line (abscissa). During the *next* revolution open the short-circuit key *but close it again the instant the muscle has contracted*;

[1] It is possible to employ a single induction shock as the stimulus by introducing a break key into the primary circuit and making the pin open this key as the drum revolves.

immediately afterwards remove the lever point from the drum before this has had time to perform another revolution. A simple muscle-curve will thus be described.

To mark the point of stimulation, move the drum slowly round by hand until the two contacts touch and are on the point of parting (as in the diagram, Fig. 30); bring the lever point against the smoked surface as far as the stop will allow, and raise the lever about a centimeter by the finger. The distance between this mark, which indicates the moment when the stimulus was put into the nerve, and the rise of the curve, which indicates the commencement of the contraction of the muscle, gives the *latent period.* To measure this period, as well as the duration of the contraction and relaxation of the muscle, set the drum revolving *at the same rate as before*, and allow a tuning-fork of known rate, *e.g.*, one hundred vibrations per second, to record a time tracing, either directly or by an electro-magnet, putting the writing point attached to the tuning-fork or electro-magnet against the drum during a single revolution only. Cut through the paper without scoring the surface of the drum. Lay it on the table, and write upon it name, date, and description. Then pass it through the varnishing trough, and hang it up to dry. When dry make and note down all the requisite measurements. The part of the tracing which is to be kept may now be cut out and pasted into your note-book.

Effect on the muscle-curve of heating and cooling the muscle.—The same nerve-muscle preparation may be used, the apparatus being arranged exactly as in the last experiment. Mark on a new abscissa the point of stimulation. Then take the following curves on this abscissa :—

1. A simple muscle-curve at the room temperature.

2. A simple curve after warming the muscle by dropping Ringer's solution, warmed to about 25° C., upon the muscle.

3. A simple curve after cooling the muscle by dropping on it Ringer's solution cooled to 10° C.

4. A simple curve after cooling the muscle by dropping ice-cold Ringer upon it.

Finally, take a time tracing below the abscissa.

Determine the effect of heat and cold respectively upon the period of latency and upon the amount and duration of the contraction; tabulate and interpret your results. Note and account especially for the effects of different degrees of cooling upon the extent of contraction. (Compare your results with those of your neighbours.)

The apparent increase in *extent* of the contraction of the warmed muscle is largely an artefact and illustrates how easily false conclusions may be drawn from experiments in which the mechanical properties

of the recording system are not taken fully into account. Can you explain the reasons for the fallacious result in this instance ?

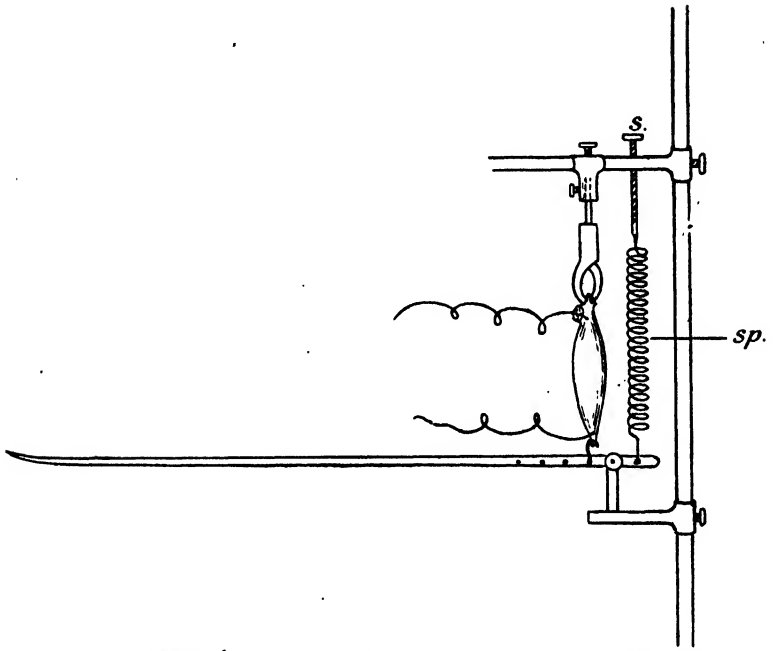

FIG. 31.—Method of studying isometric contraction. *sp*, spiral spring; *s*, screw for regulating its tension.

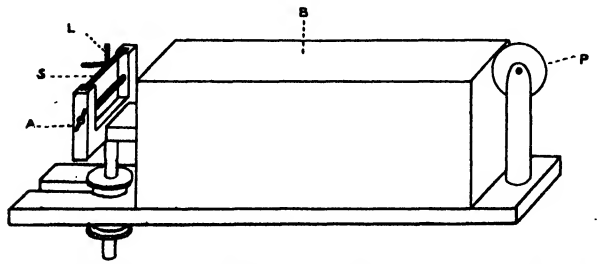

FIG. 32.—Isometric lever for class use. B, board to which muscle is fixed; S, flat spring soldered at ends to vertical supports and having attached to its mid-point the lever L; the muscle is connected by a thread to the vertical arm of the crank lever. The tension on the ends of the spring may be altered by the screw adjustment A. For the use of the pulley P, see p. 48.

Isometric contraction.—If the contracting muscle is prevented from shortening, or allowed only to shorten to so small an extent that it

remains practically of the same length throughout, the contraction is said to be *isometric*. Such a contraction is recorded by attaching the muscle very close to the fulcrum of the muscle lever, which is held down by a strong spiral spring (Fig. 31) instead of by a weight, or the lever may be of the crank pattern illustrated in Fig. 32, where a flat spring is employed to register the tension produced by the contraction. All the other arrangements of the experiment are the same as with the method already described, in which the muscle is free to shorten and raise a weight so that its tension remains constant throughout (*isotonic* contraction).

Repeat the previous experiments, using an isometric lever. Note any differences between the isometric and isotonic records.

Action of drugs on muscular contraction.—A hyoglossus preparation (see p. 27) is generally used for such experiments and the drugs are injected under the mucous membrane of the tongue.

If such muscles as the sartorius or gastrocnemius are to be investigated as to the action of drugs upon them they are immersed in Ringer; either in a Keith-Lucas muscle-bath or in the manner shown in Fig. 54. The drug is added to the Ringer and allowed to soak into thse muscle.

Mammalian skeletal muscles such as the diaphragm preparation or the œsophagus preparation (see p. 28) can also be used for drug experiments. For experiments on the actions of drugs on involuntary muscle, see Chapter XV.

CHAPTER VII

THE ACTION OF CURARI
THE CONTRACTION-WAVE IN MUSCLE

Action of curari. Bernard's experiment.—Cut through the skin and occipito-atlantoid ligament of a frog and destroy the brain by introducing a sharpened wooden match into the skull through the occipital foramen. Cut off the rest of the match. Make a longitudinal incision along the back of one of the thighs and expose and tie off the blood-vessels of the leg, care being taken to avoid injuring the accompanying sciatic nerve. A drop or two of 1 per cent. solution of curari is now injected under the skin of the back. (Tetramethyl ammonium iodide, or another quaternary ammonium salt, may be used if curari is not procurable.) After a short time the drug will have penetrated to all parts of the body except that leg the vessels of which are ligatured. The following observations and experiments may then be made :—

1. Notice that the whole body is paralysed except the ligatured limb.

2. On tapping any of the paralysed parts the foot on the ligatured side is moved—therefore not only are its muscles and nerve endings not paralysed but the conducting power of its nerves, afferent as well as efferent, and the reflex functions of the spinal cord, are not abolished.

3. Strip the skin off both legs and isolate both sets of sciatic nerves at the back of the abdomen. Tie their upper ends and cut them away from the vertebral column. Excite both sets of nerves high up, placing them upon the same electrodes, and observe the difference of effect. Excitation of the nerve of the limb which has been exposed to the poison produces no contraction of its muscles; excitation of the nerve of the ligatured limb produces the usual effect. Now stimulate the muscles of the two limbs, applying the electrodes directly to them. The muscles of the poisoned limb react like those of the normal limb, but the liminal stimulation[1] is greater. Determine at what distance of the secondary coil from the primary a response is obtained in each case.

The conclusion is that neither the nerve-fibres, sensory and motor, nor the nerve-centres, nor the muscle-fibres are paralysed, but that

[1] The stimulation which is only just effective, *i.e.*, the least stimulation which is responded to.

the poison has produced paralysis by blocking conduction between the motor nerve-fibres and the muscle-fibres.

The paralysing action of curari can also be shown by keeping a muscle-nerve preparation in Ringer's solution in which a little curari has been dissolved. It will be found that after a time the muscle will cease to respond to stimulation of its nerve, although it will contract readily if the muscle itself is stimulated. As a control, another preparation may be taken, and its nerve alone placed in the same solution during the same period, the muscle being supported above the level of the fluid. The preparation will respond to every stimulation of its nerve.

It is best to use a sartorius muscle-nerve preparation for this experiment on account of the length of time necessary for the curari to penetrate the gastrocnemius.

For the mode of preparing the sartorius—the nerve of which must be kept in continuity with the main trunk—see p. 27.

Engelmann's experiment.—The curarised sartorius is used to demonstrate polar excitation in muscle. (See p. 57.)

The wave of contraction in muscle. Aeby's experiment.—Separate from the remaining thigh muscles the adductor muscles (gracilis and semimembranosus; see Figs. 25, 26) of a frog which has been poisoned with curari to eliminate the intramuscular nerves. Leave the attachments to the tibia. Cut this bone through just below these attachments, and also sever the tibia from the femur at the knee-joint. It is then easy to complete the separation of the muscles up to their iliac attachments; a small fragment of the ilium may be cut away and removed along with them. Tie a thread to the tibial and another to the iliac attachment, stretch the muscular mass lightly between these threads, and fasten to the cork by a couple of pins at one end and a pair of pin-electrodes at the other end of the muscle. Allow two long light levers (which can be made of straws, working in simple brass holders capable of being pinned to the cork) to rest upon the muscle near each end close to their fulcra, and let the points of the levers write lightly on the drum, one exactly above the other (Fig. 33). When the muscle contracts, its swelling raises first the lever near to the electrodes, and later the one at the farther end. The movements of the levers are recorded upon the drum, and curves are obtained of the swelling of the muscle during its contraction in the same manner as the curves of shortening of the gastrocnemius were obtained in previous experiments. The drum must move at a fast rate, and the levers should be directed obliquely downwards—much more so than in the ordinary method.

Connect the pair of pin-electrodes with a du Bois-Reymond key (k^1) in the secondary circuit. Having adjusted the lever against the drum, *making use of the stop*, describe an abscissa, and mark the point of stimulation as in previous experiments by raising the end of each

lever by the hand when the two contacts on the drum just touch one another. Then take the two tracings of the contraction of the muscle, letting the drum revolve once only, and removing the levers the instant the curves are completed. The difference of latency of the two curves represents the time which it has taken for the wave of contraction to pass along the length of the fibres intervening between the two places on which the levers rest. Take a time tracing, and

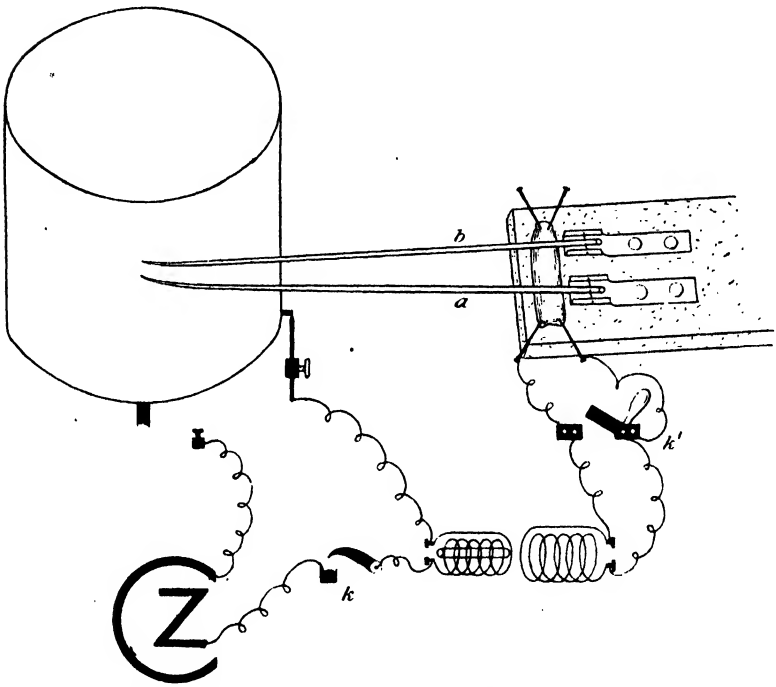

FIG. 33.—Diagram of Aeby's experiment for recording the muscle-wave. *a*, *b*, light straw levers resting on the muscle, which is curarised; *k*, *k'*, keys in primary and secondary circuits.

measure this difference, and from it and the length of muscle traversed by the wave calculate the rate of propagation of the muscle-wave. The measurements are made with compasses, after the tracing has been varnished and dried.

It is essential for the success of this experiment that the muscles used should have most of the fibres running longitudinally and parallel with one another. If very large frogs are obtainable the two sartorius muscles may be used with advantage instead of the adductor preparation described.

Another method of obtaining the curve of swelling of a contracting muscle, better adapted, however, for mammalian muscle than for the frog, is to use the *pince myographique* of Marey (Fig. 34). The muscle is grasped by this, and its contraction affects a tambour (T) which is

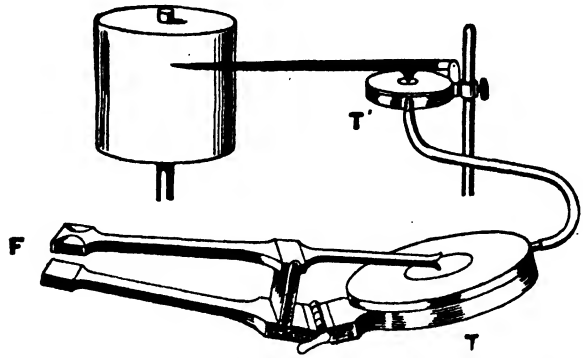

FIG. 34.—Myographic forceps of Marey. F, forceps for grasping the muscle, the contraction of which is to be recorded. The two blades of the forceps are drawn together by an india-rubber band. T, receiving tambour, the air in which is compressed by the swelling of the muscle, and from which the pressure is transmitted by an india-rubber tube to T', the recording tambour, the lever of which writes on a revolving drum.

connected by rubber tubing to another tambour (T'), writing upon the drum. The muscle is stimulated (1) at the point of application of the forceps, and (2) at some distance from the forceps. The difference of time between the two resulting curves is measured, and the rate of passage of the muscle-wave calculated therefrom.

CHAPTER VIII

EFFECTS OF SUCCESSIVE STIMULI UPON MUSCLE AND NERVE

1. **Summation of subminimal stimuli.**—If two stimuli, each of which by itself is ineffective in producing excitation, are applied to a nerve with a sufficiently short interval of time between them, the nerve is excited as indicated by a twitch of the attached muscle. The longest

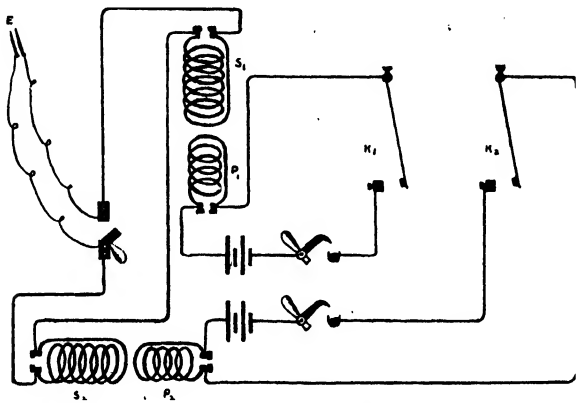

FIG. 35.—Arrangement of apparatus for investigating summation of subminimal stimuli and refractory period. K_1 and K_2 represent the keys of the Keith-Lucas pendulum. P_1 is the primary coil connected with K_1, P_2 the primary connected with K_2. The secondary coils, S_1 and S_2, are arranged in series with the electrodes E through a short-circuiting key. The mercury keys and pendulum keys in the primary circuits being closed, no current flows through the electrodes. If K_1 is now opened, the break-induced shock in S_1 flows through the tissue, S_2 acting as a resistance in series. Similarly the opening of K_2 causes the break-induced current in S_2 to flow through the tissue, S_1 acting as a resistance in series. The coils are arranged at right angles so that break of the circuit in P_1 will not cause induction in S_2 and *vice versa*.

time which can elapse between the application of two subminimal stimuli and excitation still occur is termed the "summation interval."

Determine the summation interval in the sciatic nerve of a frog using the contraction of the gastrocnemius, recorded on a stationary drum, as an indication of the success or failure of the stimuli. The

experiment is performed with the Keith-Lucas pendulum, the arrangement being that shown in Fig. 35, using two induction coils which must be at right angles to one another.

2. **Refractory period.**—If a single excitation is set up in a nerve or muscle (or in any excitable tissue) the excited part is incapable of being again excited until a certain interval of time has elapsed. The time during which a second stimulus, no matter how strong, will fail to produce excitation, is termed the "absolute refractory period." This is followed by a "relative refractory period," when the tissue will respond to a stronger stimulus than the first, and by a "supranormal phase" during which it will respond to a strength of stimulus which is less than would have occasioned excitation in the normal state.

Using the Keith-Lucas pendulum, determine the absolute refractory period of the sciatic nerve, using the contraction of the gastrocnemius muscle as an indication of the success or failure of the second stimulus.

Notice that when the pendulum keys are far apart, *i.e.*, when the stimuli fall on the nerve well outside the refractory period, the muscle gives a greater contraction than when the keys are opened simultaneously, *i.e.*, inside the refractory period. In the first instance there is summation of two contractions (see below).

3. **Summation of two muscle contractions.**—Arrange the muscle-nerve preparation on the myograph and connect the drum in the primary circuit in the manner employed to record a simple muscle-curve (Fig. 30). Place the secondary coil at such a distance from the primary that the excitation produced by a single contact projecting from the drum and striking the stationary contact in its revolution produces a maximal effect; describe a normal muscle-curve in the usual way. Mark the point of stimulation. Then arrange a second contact so that the excitation which it produces will affect the nerve at different intervals after the first excitation; viz., (*a*) during the rise of the first curve, (*b*) near the top of the first curve, (*c*) during the decline of the first curve. Record the results with the second contact in each of these positions, *remembering to mark the point of stimulation for the second stimulus immediately after each record and before moving the contact for the next observation.* All the curves may be recorded on the one abscissa, or each double curve may be made at a different level of the paper on its own abscissa. How can you reconcile your results with the "all or none" law for muscle?

4. **Summation of several successive contractions. Genesis of tetanus.**—For studying the effect on a muscle-nerve preparation of a rapid succession of stimuli a vibrating steel reed is used to make or break the primary circuit of the induction coil by allowing a wire attached to its end to dip into and out of a cup of mercury, the surface of which should

be clean and covered with dilute alcohol. The rate of vibration of the reed depends upon its length, which can be varied by clamping it at different places; it is marked at points for producing vibrations of five, ten, fifteen, twenty, and thirty per second (Fig. 36). The secondary coil should be placed at such a distance from the primary that only the break-shock is effective (see p. 34). The drum should revolve at moderate speed (one revolution in about twenty seconds).

Attach the muscle to the lever of the myograph in the usual way; place the nerve upon the electrodes; set the reed vibrating; set the

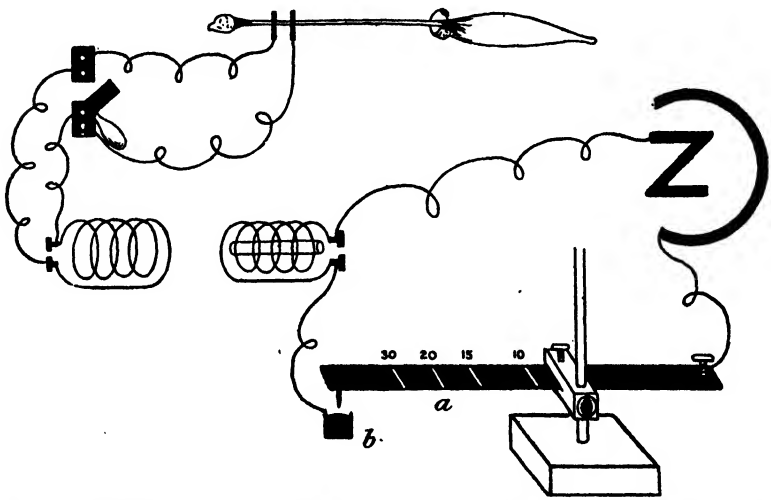

FIG. 36.—Experiment to investigate the genesis of tetanus. *a*, flat steel spring marked at intervals with the number of vibrations corresponding to certain lengths of the spring; *b*, mercury cup into and out of which a pin attached to the spring dips.

drum revolving and bring the lever point against it, *using the stop*; open the key in the secondary circuit for about a second and then close it; take the lever point away from the drum. A tracing is to be taken in this way at each of the above rates, each tracing on its own abscissa. If the vibrating reeds are not calibrated, a time tracing should be taken in order to determine the different frequencies of stimulation used.

How does the extent of contraction of the muscle vary with the frequency of stimulation? How do you account for the differences?

The response of a muscle to a succession of stimuli is known as a complete or an incomplete tetanus according to whether fusion of the individual contractions is complete or incomplete. Compare incomplete tetanus and clonus (see ankle-clonus, p. 158).

CHAPTER IX

WORK, EXTENSIBILITY, AND HEAT-PRODUCTION OF MUSCLE

THE experiments on work and extensibility are recorded upon a stationary drum, which is moved onwards by hand for about 5 millimeters after each observation. The stimulating circuit is arranged to give single induction shocks by making and breaking of the primary circuit by hand.

Make a muscle-nerve preparation and place it on a myograph in the usual way. The lever should have a light scale pan suspended from it. The scale pan is made to hang upon the long arm of the lever at the same distance from the fulcrum as the attachment of the thread which connects the muscle to the short arm.

1. **Effect of strength of stimulus on extent of contraction under isotonic conditions.**—A weight of about 30 grams is placed in the scale pan and the effect of gradually increasing the strength of stimulus from minimal to maximal is recorded. Note down on the curve the distances of the secondary coil at which each result is obtained (see also p. 33). Since the load is constant, different heights of contraction represent different amounts of work done (*cf.* below).

2. **Effect of load on work done by muscle.**—An isolated muscle can be made to perform work when it is either after-loaded or free-loaded. In the following experiments the performance of the muscle under these different conditions is compared. Note that while in the last experiment load was the constant factor and stimulus the variable one, here the load varies and the stimulus is constant.

(*a*) *When the muscle is after-loaded.*—In this case the lever is supported on the after-loading screw and the muscle only commences to raise the weight after the contraction has proceeded to a certain extent; the initial length of the fibres is thus the same, no matter what weight is applied to the lever. Determine the amount of work which the muscle performs in lifting different weights under these conditions. Beginning with the weight of the scale pan alone, a single maximal break stimulus is applied to the nerve. The drum is moved round 1 centimeter or so by hand; a further weight is added to the scale pan and the nerve again stimulated. The drum is again moved round, another weight is added and the nerve once more stimulated, and so on, until the muscle fails to raise the lever in response to stimulation.

Note down on the tracing the weight which corresponds with each ordinate. Measure the length of the lever and the distance from fulcrum to point of suspension of the weights. Note these distances on the tracing. The exact height to which the weight is raised at each observation is obtained by dividing the height of each ordinate by the magnifying extent of the lever.

Thus if L is the total length of the lever in centimeters, l the distance between the fulcrum and the point of suspension of the weight, and H the height of the ordinate, then h, the height to which the weight (w) is raised, is $\frac{Hl}{L}$. The work done by the muscle is then $wh = \frac{wHl}{L}$ gram-centimeters. (This, of course, is only true if the scale pan is hung as directed above.) The load with which the greatest amount of work is done by the muscle is termed the *optimal load*. Determine from your graph (see below) the optimal load for the muscle you are using.

(*b*) *When the muscle is free-loaded.*—In this case the lever is not supported by the after-loading screw, and each successive weight added increases the initial length of the muscle-fibres. Otherwise the experiment is performed as in (*a*). It is important to note that after recording the contraction for a given load the drum must be moved round 5 millimeters or so *before adding the next weight*. The same muscle, or the fellow-muscle of the same frog, should be used in making this experiment. (Measure the length of the muscle at the beginning of the experiment for use in connexion with the curve of extensibility described below.)

Since the lever point will be pulled lower and lower by the addition of successive weights it is desirable to have the lever pointing 30 degrees or so above the horizontal at the beginning. Otherwise the writing point may leave the drum half-way through the observations. Because of the large weight ultimately applied to the muscle in this experiment it is necessary to have the pin which fixes the preparation to the board very firmly in place. Similarly, the ligature round the tendo Achillis should be very tightly tied.

Before leaving the experiment obtain the curve of retraction (see below). Remember to measure the length of the lever from fulcrum to writing point and from fulcrum to point of suspension of the weights.

When you have worked out the results make a graph for each of the experiments (after-loaded and free-loaded) plotting work (ordinate) against load (abscissa). Draw both graphs on the same piece of paper. Interpret your results.

Extensibility of muscle.—The effect of gradually increasing weights in producing extension of muscle in the resting and contracted conditions respectively, is shown by the records obtained in 2 (*b*). For it is obvious that the lowermost point of any ordinate described by the muscle represents the length to which the resting muscle is extended by the particular weight, and the top of the ordinate the length to which the muscle when contracted is extended by the same weight. If the ordinates are at regular distances apart, a line joining their lowermost ends gives the curve of extension of the resting muscle, and a line joining the tops of the ordinates the curves of extension of the contracted muscle. Further, if the weights are removed in succession and ordinates are again described after each such removal, curves of recovery from extension—*i.e.*, of retraction—can be obtained. This experiment may be performed separately and the curves of extensibility and retractility of resting muscle may be compared with curves similarly obtained by substituting a rubber band for the muscle.

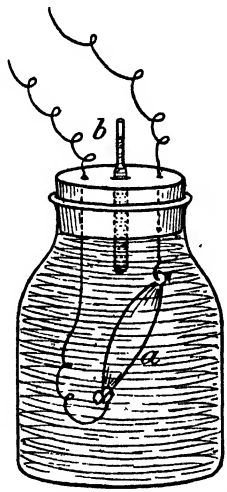

FIG. 37.—Experiment to determine if a muscle alters in volume during contraction. *a*, Muscle in Ringer's solution; *b*, capillary.

During contraction a muscle does not alter in volume.—Take a wide-mouthed bottle with well-fitting paraffined cork (Fig. 37). Through the cork are passed a glass tube drawn out above the cork to a capillary diameter, two copper wires of unequal length, the longer one coiled spirally and each ending below in a sharp hook: above the cork each wire ends in a loop. Fill the bottle to the brim with boiled and subsequently cooled frog-Ringer. Attach a fresh muscle by its two ends to the hooks, lower into the bottle, and press the cork in securely: the fluid should completely fill the bottle and capillary tube, to the exclusion of air-bubbles. Draw a little of the fluid out of the capillary by filter paper, and mark with ink the level at which the fluid then stands. Hook wires from the secondary coil to the loops above mentioned, and tetanise the muscle. If there were a diminution of volume the level of the water in the capillary would fall.

Isometric contraction.—In the foregoing experiments the muscle contraction has been "isotonic." As we have already seen (p. 36), a muscle can be set into activity under conditions where it is unable to shorten and perform external work. Under such conditions the chemical potential energy set free on stimulation is expressed in the

form of tension and is dissipated as heat; and the "contraction" is said to be "isometric."

Actually the muscle *is* allowed to shorten to a very small extent in order that the tension developed may be determined. In very accurate work the degree of shortening permitted is so small that optical methods are used to make this, and hence the tension developed, measurable.

Determine how the tension developed in an isometric contraction depends on the initial length of the muscle-fibres.

The isometric lever used is that shown in Fig. 32. It must be calibrated before the experiment commences. To do this fix the apparatus to the upright stand of the myograph, and in place of the muscle attach a long, strong thread to the short arm of the crank lever; allow the thread to pass over the pulley at the end of the muscle board. Starting with no weight in the pan, describe a horizontal line (abscissa) on the drum. Add gradually increasing weights to the scale pan, describing a horizontal line after each addition; in each case mark on the abscissa the weight added. Now remove the scale pan, and, without altering any of the adjustments of the apparatus, fix a muscle to the board and attach it to the short arm of the lever by a strong thread. Start with the thread slack, and with the point of the lever on the zero abscissa line. Measure with dividers the resting length of the muscle. Stimulate the muscle, either directly or through its nerve, with a single maximal break shock. If the muscle is sufficiently relaxed, *i.e.*, if the thread is sufficiently slack, there will be no tension developed. Now put a slight tension on the fibres by moving the muscle farther away from the lever. Again measure the length of the muscle, bring the point of the lever against the drum, and stimulate as before. Repeat the process until a series of ordinates has been described, moving the muscle slightly farther back each time and measuring the resting length of the muscle after each change. By plotting the tension developed in each contraction (*i.e.*, the difference between the "resting" tension and the tension developed[1] during contraction) against the length of the muscle, it will be seen that, up to a certain extent, the greater the initial length of fibre, the greater the tension developed in contraction. This relationship is important, and, as will be seen, holds for cardiac as well as for skeletal muscle.

[1] The *potential energy* developed in an isometric contraction of the frog's gastrocnemius is given approximately by the expression $\frac{Tl}{13}$, where T is the tension developed and l the length of the muscle. For the sartorius the value is $\frac{Tl}{6}$.

Heat production of muscle.—Make a double sartorius preparation from a large frog and fix it to a " Hill " thermopile in such a manner that it may be made to contract isometrically. Place the thermopile under a cover so that it will be unaffected by draughts, and arrange so that the muscle may work in an atmosphere of nitrogen or of air, at will. Connect the terminals of the thermopile with a sensitive mirror galvanometer of low resistance, using the same circuit as in Fig. 52, p. 72, so that the beam can be readily brought to zero by a compensating current. Allow nitrogen to bubble in a steady stream into the tube containing the thermopile, and let it continue to do so during the first part of the experiment. When there is no doubt that the muscle is in an atmosphere of pure nitrogen, and has been so for several minutes, faradise for a second or two. The muscle will contract despite the fact that it is under anaerobic conditions, and heat will be evolved, as evidenced by the large swing of the galvanometer. The swing represents the " initial " or *anaerobic* heat, associated with the anaerobic breakdown of phosphagen and glycogen which gives rise to the contractile process. When the galvanometer has come back to near its zero admit air (or oxygen) to the muscle. Immediately there is a further very considerable swing of the light beam in the same direction as before, the deflection taking some minutes to disappear. This represents the " delayed " or " recovery " heat, associated with the *aerobic* resynthesis of the substances broken down in the production of the contraction. A certain amount of recovery also takes place under anaerobic conditions. This gives rise to the " anaerobic recovery heat " which will prevent the galvanometer returning quite to the zero position for some time after the first observation.

CHAPTER X

FATIGUE OF MUSCLE AND NERVE

Effects of fatigue on muscle. (*a*) **On the form of the muscle-curve.**—A nerve-muscle preparation is fitted up as for recording a simple muscle-curve (Fig. 30). Make an abscissa, and mark, as usual, upon it the point of stimulation. Record a normal curve with the muscle free-weighted. Remove the writing point from the drum, which is then allowed to revolve continuously and to stimulate the muscle at every revolution. After twenty of such excitations without taking a record, apply the lever point again to the drum (making use, of course, of the stop), and let the muscle describe another curve at the same place as the first. Remove the writing point again for the duration of twenty excitations, and repeat the above procedure, and so on a number of times until the fatigue curves are pronounced. Notice the effects of fatigue upon muscle, in prolonging the latency period, diminishing the amount and slowing the course of its contraction, and greatly delaying, and at length even preventing, its relaxation.

A fatigue curve or series of curves can also be obtained by allowing the lever point to remain in contact with the cylinder during the whole of the experiment, and thus recording every contraction; but the individual curves in a tracing so obtained are very numerous, and tend to obscure one another.

(*b*) **On the extent of contraction.**—The effect of fatigue upon the *extent* of contraction is best recorded upon a very slowly moving drum: the extent of the contraction is shown by the ordinates described by the lever. If the slowest rate of movement of the drum (1 millimeter per second or less) is used, arrange rapidly to make and break the primary circuit about every half-second. This can be done either by closing and opening a key by the hand, or by allowing a metallic bridge, actuated mechanically (*e.g.*, by a metronome), to close and open a gap between two mercury cups in the circuit. Use maximal stimuli and ensure that only break-shocks are effective. Keep the point of the lever—which must be free-weighted—against the smoked paper, and record every contraction. In this way a *continuous fatigue curve* is obtained, exhibiting the effect of fatigue upon the amount of contraction, and also on the extensibility of the muscle both in rest and in contraction. Notice the "staircase" (gradual rise of the ordinates) at the beginning of the curve and the "contracture"

(permanent contraction remainder) near its termination. Carry the experiment to complete exhaustion—*i.e.*, until the stimuli produce no further perceptible effect through the nerve.

Application of the electrodes to the muscle itself will now be found to cause contraction—not that the nerve itself is fatigued, but that the junction of nerve and muscle is affected before the muscle substance itself. Stimulation of the muscle is best effected by placing, at the beginning of the experiment, a second pair of electrodes against the muscle, and arranging that the shocks from the induction coil can be switched over to these electrodes when desired.

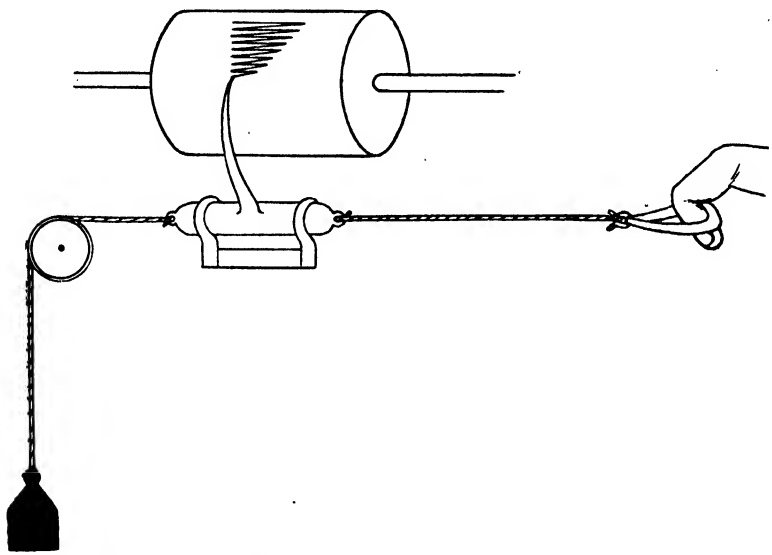

FIG. 38.—Diagram of Mosso's ergograph for the investigation of fatigue in the human subject.

Recovery.—Allow the preparation to rest, keeping it moist with frog-Ringer. After fifteen or twenty minutes again test the effect of a stimulus. Notice that there is a certain amount of recovery from the fatigue, even in a preparation such as this in which no blood is circulating. In muscle in which the circulation is maintained, fatigue not only comes on more slowly but is more rapidly recovered from, since the circulating blood removes the fatigue products.

Reaction of the fatigued muscle.—Cut across a muscle which is completely fatigued, and apply blue litmus or neutral red paper to it. Notice that the colour is changed, indicating the production of acid fatigue products. Compare the reaction with that of a piece of fresh.

unfatigued muscle. Muscle which has died and passed into rigor—whether natural or the effect of heat—is also acid.

Fatigue in voluntary contraction.—This is investigated by the *ergograph* (Fig. 38), the muscles of the fingers being fatigued by causing them repeatedly to raise a heavy weight or repeatedly deflect a strong spring. The extent of the contractions is recorded upon a very slowly revolving drum, and a fatigue curve or *ergogram*—which always shows individual peculiarities—is thereby produced in the same manner as with the frog muscle-nerve preparation.

This experiment can also conveniently be made with the adductor indicis muscle, employing the ordinary crank myograph, the lever of which is held down by a strong rubber band.

In the case of voluntary contractions the result is complicated by the fact that fatigue of nerve-cells in the central nervous system occurs before fatigue of the muscle itself or of the nerve-endings in the muscle. This fact can be shown by direct faradic stimulation of the median nerve (or of the long flexors of the fingers) after a fatigue curve produced by voluntary effort is completed. It is found that the muscles can still be made to contract by such peripherally applied stimuli.

CHAPTER XI

CONDUCTION IN NERVE

Conduction of nerve impulses may take place in both directions: Kühne's experiment.—Remove the gracilis with part of its entering nerve; lay it on a glass plate, with its inner surface uppermost. The nerve is seen to give branches upwards and downwards; as a matter of fact each nerve-fibre divides into two branches, one for the upper and the other for the lower part of the muscle, which has a tendinous intersection obliquely across its middle. The middle part of the muscle can be entirely cut through here without injuring these nerves, and the two parts of the muscle will then only be united by the forked nerves.

If the ends of the nerves in either of the pieces of the muscle are stimulated, whether electrically, osmotically (salt), or mechanically (by snipping with scissors), both pieces contract.

Rate of transmission of nerve impulse in frog-nerve.—Make a nerve-muscle preparation in the usual way; fix it upon the myograph, and lay the nerve out upon two pairs of electrodes, one placed as near the muscle as possible, the other close to the vertebral column. With the nerve of a large frog 5 centimeters (nearly 2 inches) will intervene between the two. Keep the nerve moist. Place a commutator without cross wires in the secondary circuit, and arrange so that by moving the bridge of the commutator the induction shocks can be switched on to one or other pair of electrodes. The drum is included in the primary circuit, and a short-circuit key in the secondary (Fig. 39). The lever should have a slightly greater dip than under ordinary circumstances, so as to ensure a sharp rise of the writing point.

Two muscle-curves are now successively taken with the fastest rate of cylinder and a maximal stimulus. The stimulus is applied to the nerve, first, close to the muscle, and second, close to the vertebral column. The muscle-curves are both taken in exactly the same way, and with exactly the same precautions as to the use of the stop, etc., detailed in Chapter VI.; both curves are to be traced upon the one abscissa, a time tracing being written above the abscissa, so as to intersect the rise of the curves. It will be found that the curves are not quite coincident, but that one succeeds the other by a very small interval. This interval represents the time occupied by the transmission of the nerve impulse along the length of nerve between the

two pairs of electrodes. Measure this length of nerve and, after the tracing has been varnished and dried, measure accurately the interval

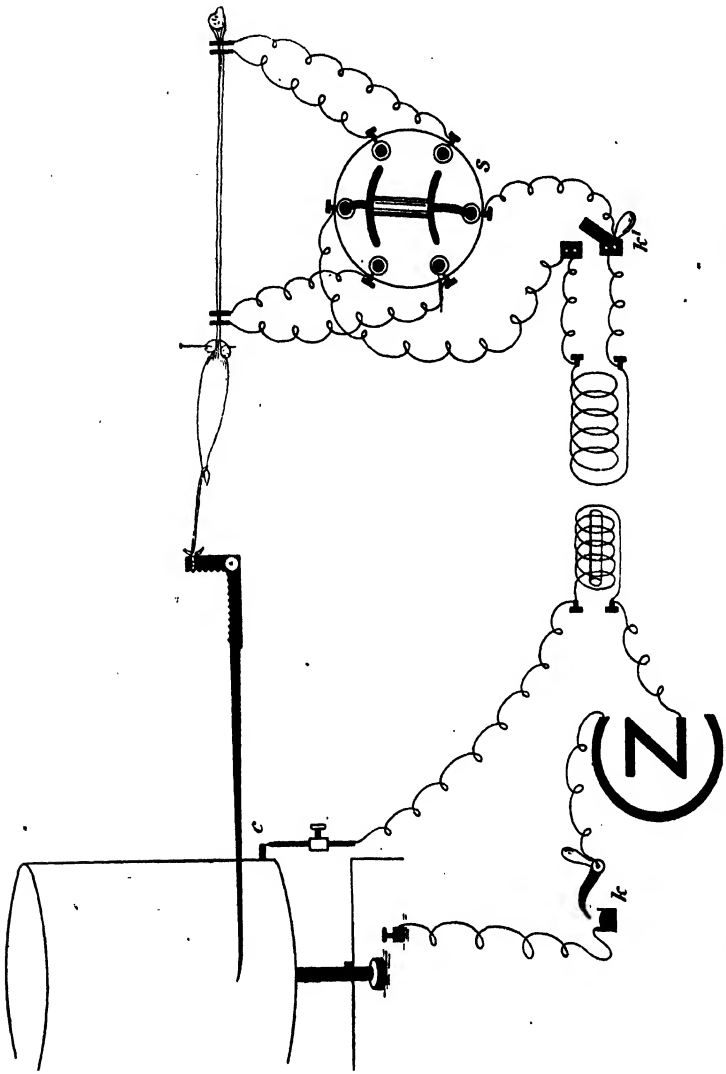

FIG. 39.—Experiment for recording the differences of latency of a nerve-muscle preparation when the nerve is stimulated (*a*) near the vertebral column, (*b*) near the muscle. *k*, mercury key in primary circuit; *c*, drum-contact in primary circuit; *k'*, key in secondary circuit; *s*, Pohl commutator used as switch.

between the two curves at the level where the time tracing intersects them. From the time tracing determine the time which this interval represents, and calculate from these data the rate of transmission of the nerve impulse.

The effect of temperature changes on rate of transmission.—(*a*) *Moderate cooling:* The effects of cooling the nerve are more easily investigated than the effects of warming. Obtain records exactly as in the last experiment, from which the rate of transmission of nerve impulses at room temperature may be determined. Without altering the apparatus in any way, cool the nerve by dropping on to it cooled Ringer solution, *taking care not to allow any of this cold fluid to affect the muscle.* Then take a muscle curve with the stimulus applied by the electrodes next the vertebral column. (*b*) *Extreme cooling:* The effects of extreme cooling of a portion of the nerve may be determined by laying the nerve over a flat copper rod which projects for a few centimeters beyond the edge of the muscle board. The rod is cooled by a spray of ethyl chloride directed on the part furthest from the nerve.

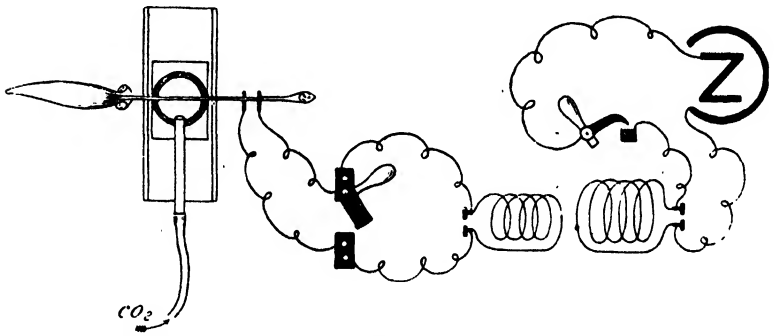

FIG. 40.—Effect of CO_2, etc., on conduction in nerve.

The above experiments may conveniently be carried out with the moist chamber described below.

Effect of cocaine on nerve conduction.—The effect of extreme cooling may be compared with the effect of painting a portion of the nerve with 5 per cent. cocaine solution. Stimulate the nerve at 30-second intervals.

Effect of various substances on nerve-conduction; carbonic acid; ether vapour; chloroform vapour.—Take a nerve-muscle preparation and lay the nerve across and partly sunk in a ring of plasticine placed upon a glass slide, to which a tube is cemented so that a current of air charged with gases or vapours can be conducted over the nerve. A cover-glass is placed upon the ring, which thus forms a moist chamber (see p. 7): the spinal end of the nerve projects beyond the chamber and rests upon a pair of electrodes (Fig. 40). The slide is placed upon the myograph cork and may be kept in position by plasticine, and the muscle is securely attached to the cork and connected to a crank lever in the usual way.

Find the minimal stimulus which will just produce a steady tetanus of the muscle, the drum being set to revolve slowly (one revolution in thirty seconds); then pass a current of CO_2 over the intervening nerve, and notice its effect in blocking the nerve impulse. Remove the CO_2 by a current of air, and observe the result.

Other experiments may be made with ether vapour and chloroform vapour instead of CO_2. It will be found that ether acts like CO_2, but more powerfully. Chloroform vapour is more powerful than ether; *after a short exposure to it the nerve does not recover its power of conduction on readmitting air*; it has, in fact, been killed.

Rate of transmission of nerve impulse in human nerve.—The rate of transmission of the nerve impulse in human nerve may be determined as follows:—

The thumb is arranged in such a way that contraction of its muscles will be registered on a rapidly moving surface by tambours or by the *pince myographique* (p. 41). The electrodes used consist of wash-leather pads soaked with strong salt solution. One electrode, large and flat, is placed against the skin of the upper part of the back, the other (smaller) one being applied, first, over the median nerve at the elbow and, second, over the brachial plexus above the clavicle; the interval between these points is usually about 12 inches. The other arrangements are the same as for the similar experiment on frog-nerve.

CHAPTER XII

POLAR EFFECTS OF GALVANIC CURRENT

THE passage of a galvanic current through a nerve or muscle produces secondary polarisation of these tissues, caused by the accumulation of positive and negative ions at or near the poles of the constant current. This polarisation is accompanied by certain physiological changes, the tissue being more excitable in the neighbourhood of the negative pole or kathode, and less excitable in the neighbourhood of the positive pole or anode. These effects—both physical and physiological—spread for some distance beyond the actual poles. And not only is the tissue rendered more excitable by the kathode, but this itself sets up excitation, which, in the case of a muscle, may cause its contraction, not only at the moment of closure, but during the whole time of passage of the current. On breaking the circuit the part of the nerve which was more excitable during the passage of the current becomes instantaneously less so than the rest (physiological rebound). On the other hand, the presence at the anode of a constant current not only renders the tissue less excitable whilst the current is passing, but on breaking the circuit there is again a rebound; the part which was the less excitable becoming the more excitable: this passage from less to greater excitability again acts as a stimulus. Hence, when a constant current is sent through a nerve or muscle, there is excitation at the kathode on making and at the anode on breaking the circuit. But the latter furnishes a rather weaker excitation than the former.

Polar excitation of muscle.—1. Engelmann's sartorius experiment. —A curarised sartorius is connected with a pair of non-polarisable electrodes which are joined up through a mercury key with a battery (Fig. 41). It will be observed that the twitch begins at the kathode when the circuit is closed; indeed, the muscle may remain more or less contracted at that end during the whole time of the passage of a strong current. On the other hand, on opening the circuit the twitch begins near the anode, and may again be followed by a prolonged contraction. These prolonged contractions show that the excitation is produced not only at the make and break, but also during the passage and for a short time after the cessation of a strong constant current.

2. An instructive variation of this experiment is to dissect out the rectus abdominis muscles of a curarised frog, and place the non-polaris-

able electrodes one in contact with the anterior, the other with the posterior end of the flat muscular mass (Fig. 42). The muscles are divided into several segments by tendinous septa, and it will be seen that during the passage of the constant current each of these segments has the part directed towards the kathode in a condition of contraction, and the part directed towards the anode in a condition of relaxation.

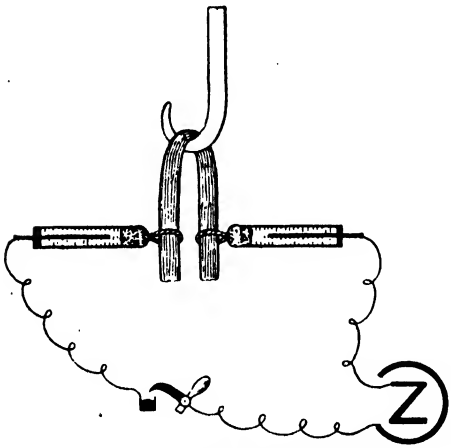

FIG. 41.—Polar effects of constant current upon curarised sartorius.

3. The effect of the poles of a constant current upon cardiac muscle can be exhibited on the frog's heart. The frog is killed by destroying the brain, and the heart is exposed *in situ*. Using non-polarisable electrodes and the whole E.M.F. of a Daniell cell or an accumulator, with a mercury key and a commutator in the circuit, place one electrode in the mouth or on any part of the body of the frog, and connect the other, by means of a short piece of cotton-wool wetted with Ringer's solution and drawn to a point, with the heart, so as to touch it near the base of the ventricle. If this electrode is the

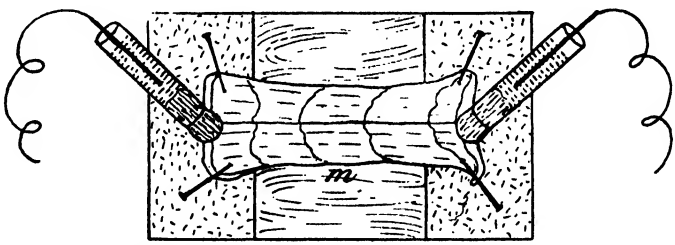

FIG. 42.—Polar stimulation of rectus abdominis. *m*, muscle, curarised and stretched between two pieces of cork.

anode, on closing the key it will be observed that the part of the ventricle underneath it does not participate in the contractions, but remains quiescent, and bulges during general systole: on opening the key this part passes into systole, even during general diastole (physiological rebound). If the current is reversed and the cotton-wool is made the kathode, the effects obtained are the reverse.

Polar excitation of nerve.—Take a muscle-nerve preparation with as long a nerve as possible and arrange it on the myograph (Fig. 43).

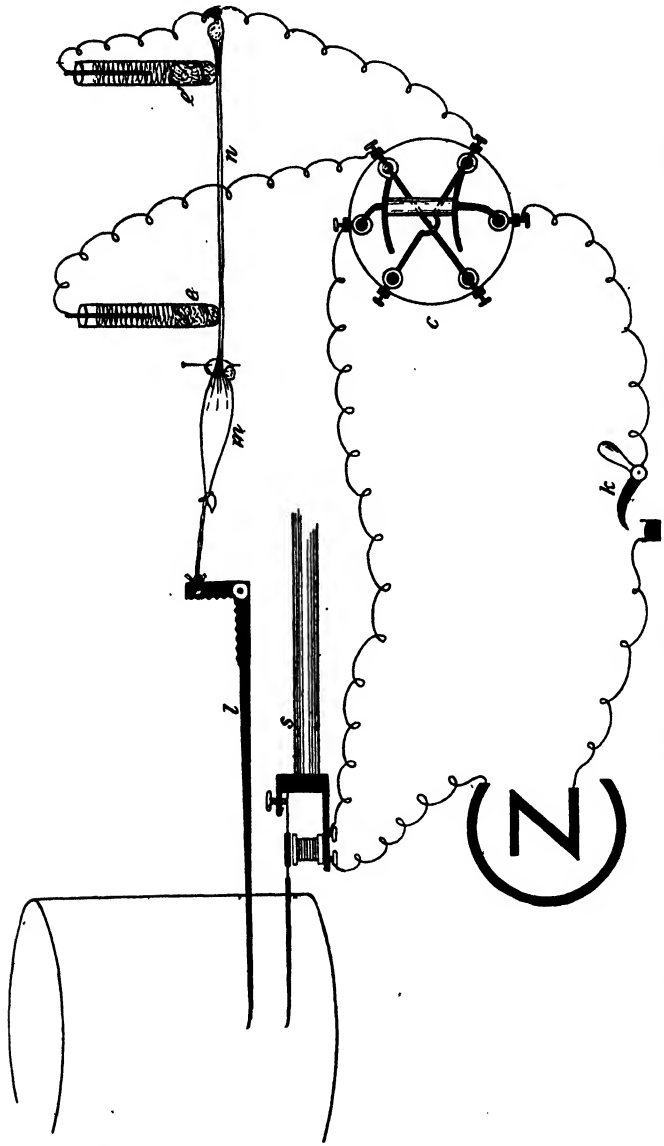

Fig. 43.—Experiment to show the polar stimulation of nerve. *m*, muscle; *n*, nerve; *l*, myograph lever; *e*, *e'*, non-polarisable electrodes; *c*, Pohl commutator; *k*, mercury key for closing or opening battery circuit; *s*, electro-magnetic signal.

Non-polarisable electrodes, connected with an accumulator through a mercury key, are placed—the anode in contact with the uppermost

end of the nerve, the kathode in contact with the lowermost end, *i.e.*, close to the muscle. Insert an electro-magnetic signal into the circuit and cause it to mark on the drum just below the myograph lever. Record two contractions, one produced by closing the mercury key, the other (on a different abscissa) by opening it. Make a time tracing below, and measure exactly the period of latency in each case, *i.e.*, the time elapsing between the movement of the electro-magnetic signal and the commencement of rise of the curve. Notice that it is slightly greater as the result of breaking the circuit than on making (by the time taken for the nerve impulse to traverse the length of nerve), since the excitation at *breaking* is at the anode, *i.e.*, at a point of the nerve farthest from the muscle, whereas on *making* the excitation was at the kathode, *i.e.*, close to the muscle. Note also that the "make" contraction is greater than the "break."

If, as represented in Fig. 43, an ascending current is used instead of a descending one, the result may be complicated by the blocking effect of the constant current on conduction (see p. 61). Thus, on *making* such an ascending current, if it were a strong one, the excitation being at the kathode, *i.e.*, at the uppermost end of the nerve, and the intermediate part of the nerve being at the same moment traversed by the current, this would block the passage of the nerve impulse generated at the kathode, and no contraction would result. Therefore, instead of obtaining a contraction at both make and break, only the break would produce a visible effect under these circumstances. On the other hand, if the constant current is weak, its removal may not be followed by contraction of the muscle, because the breaking of such a current furnishes a smaller excitation than its making.

Effects of constant current on excitability.—A polarising current, as already explained, produces changes of excitability not only at its poles but also in the adjacent parts of the nerve, and even some distance from them. This is due to the fact that owing to spread of current in the extra-polar regions changes of potential are manifested in those regions during the passage of the current between the poles, and are accompanied by physiological changes, viz., increased excitability near and beyond the kathode, and diminished excitability near and beyond the anode. Such a condition is known as *electrotonus*; that produced by the kathode being termed *katelectrotonus*; that produced by the anode, *anelectrotonus*.

Take a pair of non-polarisable electrodes and connect with a battery of at least two cells, inserting a rheochord, a commutator, and a mercury key into the circuit (*polarising circuit*) (Fig. 44, lower part). Another circuit (Fig. 44, upper part) is also prepared (*exciting circuit*), including cell, induction coil, and mercury key in connexion with the primary coil; the secondary coil is furnished as usual with a short-circuit key,

with which a pair of ordinary metallic electrodes are connected; these electrodes are brought in contact with the nerve of a muscle-nerve preparation *near the muscle*. Be careful to keep the nerve moist. The non-polarisable electrodes, which may be of the boot pattern, are slightly raised above the myograph cork: they must not be allowed to be short-circuited by the Ringer solution used to keep the preparation moist; the upper part of the nerve is laid upon them (Fig. 44). The record of the muscular contractions obtained is made on a stationary drum.

Place the secondary coil at such a distance from the primary coil that faradisation (Helmholtz modification) just produces a small contraction. Now put in the polarising current (1) in an ascending and (2) in a descending direction, and determine the effect of its poles in

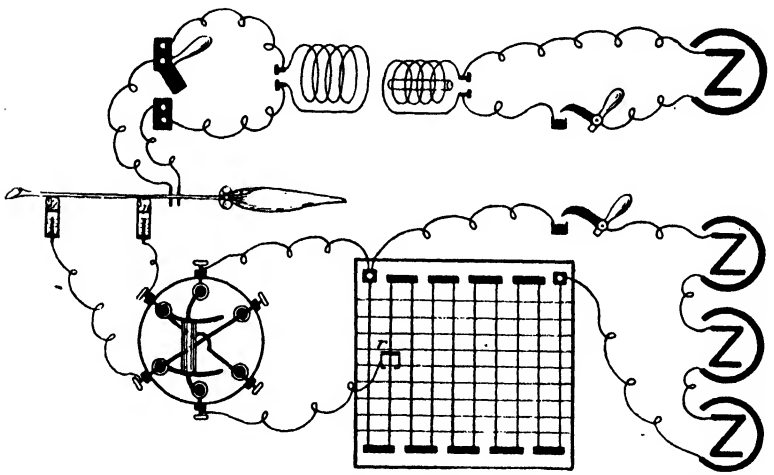

FIG. 44.—To test the polar effects of a constant current on nerve excitability.

diminishing or increasing the excitability of the nerve as tested by the height of the ordinates described by the myograph lever.

This experiment can be performed without taking a graphic record by noting at what distance the secondary coil must be placed in order just to produce a contraction. In this way the varying conditions of excitability produced by the polarising current may be recorded numerically.

A variation of the experiment is to replace the exciting circuit by a few crystals of salt and wait until the penetration of this begins to excite the nerve-fibres.

The rheochord may be dispensed with in the polarising circuit.

Effects of constant current on conductivity.—Bernstein's experiment. —A galvanic current, in addition to producing changes in excitability at and in the region of the poles, produces also changes in conductivity.

If the current is sufficiently strong the effect may be so pronounced as to offer a complete block to the passage of the nerve impulse.

To exhibit the blocking effect of a galvanic current upon nerve, take a muscle-nerve preparation with long nerve and attach the muscle to the myograph lever in the usual manner, so that its contractions may be recorded upon a slowly moving drum (one revolution in thirty seconds). Apply stimulating electrodes from the secondary coil to the part of the nerve near the vertebral column, using the Neef's hammer (with Helmholtz wire) for tetanisation, and a strength of stimulus just sufficient to produce a maximal contraction. Apply a pair of non-polarisable electrodes, connected through a mercury key with a battery of three Daniell cells or two accumulators, arranged so that the current can be passed up the nerve [1] (polarising circuit).

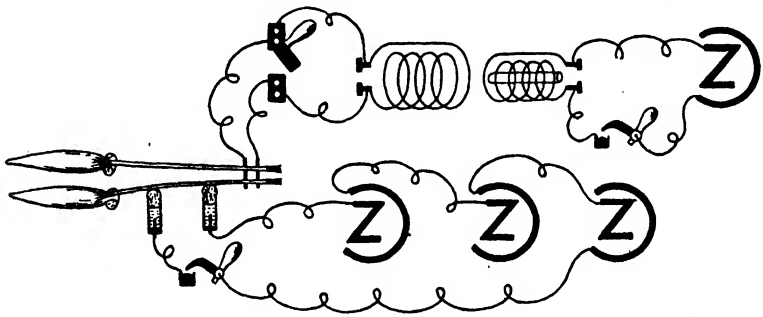

FIG. 45.—Diagram to illustrate the blocking effect of a constant current upon conduction of nerve impulses.

Take a tracing of the muscle during tetanisation, and whilst this is progressing close the polarising circuit. The tetanus at once ceases, to be renewed on again opening that circuit, and so on repeatedly.

This experiment can be varied in the manner shown in Fig. 45; using two nerve-muscle preparations, and merely observing the contraction of the muscles, without recording it.

Pflüger's experiment.—The varying effects of opening and closing a galvanic current (misnamed " the law of contraction ") are illustrated by an experiment devised by Pflüger. The nerve of a nerve-muscle preparation is placed on non-polarisable electrodes, which are connected with a battery of at least three Daniell cells or two accumulators, through a commutator and rheochord: a mercury key is introduced into the circuit (Fig. 46). Beginning with a *very weak current*, the rider of the rheochord being brought near to the end *a* of the rheochord

[1] The polarising current will act as a block to the conduction of the excitation equally well if passed downwards; but in that case it is itself liable to produce stimulation should the preparation be very excitable (see p. 63).

wire (see Figs. 17, 18), determine the effect upon the nerve, as indicated by the contraction of the muscle, of making and breaking the current when it is (1) ascending and (2) descending. Repeat the experiment, using a *moderate strength of polarising current—i.e.*, with the rider of the rheochord near the end *b* of the wire. Finally the effect of a *strong current* is to be studied by eliminating the rheochord altogether. Note down in tabular form all the results obtained. The contractions of the muscle need not be recorded graphically.

If the nerve is very excitable [1] the muscle may remain in contraction during the whole time of the passage of a strong descending current (*closing tetanus*), and may also remain contracted for a considerable time after the removal of a strong ascending current (*Ritter's opening tetanus*).

Galvanic stimulation in man.—Similar results to those obtained upon the frog's muscle-nerve preparation are obtained on closing or

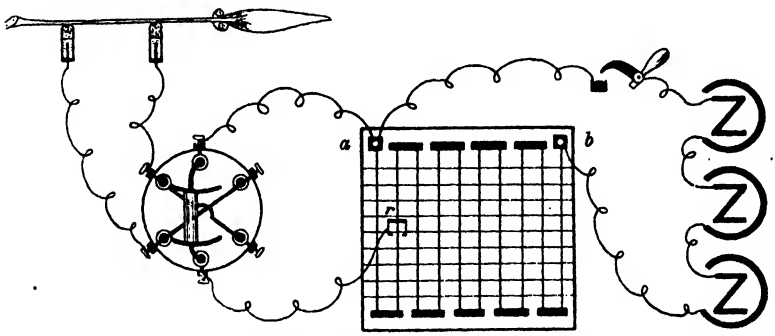

FIG. 46.—To test Pflüger's "Law of Contraction."

opening galvanic currents in the human limbs, although they are somewhat modified by the fact that the nerves to be tested are embedded in the tissues and that the human body offers great resistance to the passage of the current, so that it is necessary for obtaining some of the results to use a battery of as many as 30 or 40 cells. It is usual to employ one large-surfaced flat electrode, which generally takes the form of a zinc disc covered with flannel, soaked in strong solution of salt, applied on the neck or sternum, and a smaller rounded electrode similarly covered and applied over the nerve or muscle to be tested. This (active) electrode is either the kathode or the anode according to the direction of the current, which may be altered at will by a commutator. When it is a kathode, and contraction of the muscle is obtained on closing the current, the result is described as a kathodic

[1] The excitability of a muscle-nerve is much increased when the preparation is made from a frog which has been in a cold place or in contact with ice, and then kept for an hour at the ordinary room temperature before being killed.

closing contraction (KCC); when it is an anode, and contraction results from closure, it is an anodic closing contraction (ACC). Similarly the terms kathodic opening contraction (KOC) and anodic opening contraction (AOC) are employed to express corresponding results. It should be noted, however, that this terminology is merely one of convenience, and that there is no such thing as an ACC or a KOC strictly so called. What is termed ACC is actually a peripolar KCC; similarly KOC is a peripolar AOC. The current is measured in milliampères.

Reaction of degeneration.—The character and sequence of the anodal and kathodal muscular response as the current is strengthened is of diagnostic value in conditions of nerve-degeneration from disease. Thus while in health the sequence is KCC > ACC > AOC > KOC, at a certain stage of degeneration KCC may no longer be the most easily elicited, and the responses generally may be sluggish. Degenerative changes can be demonstrated by the chronaximetric method at a much earlier stage than by this experiment.

R. S. Lillie's wire model.—Ordinary iron wire, if placed in dilute nitric acid, will soon dissolve. But if first immersed in concentrated nitric acid it becomes passive, by the formation of a surface film of oxide, which does not dissolve spontaneously in dilute acid. If, however, touched with a piece of zinc while in the dilute acid, it will immediately react at the point touched, and a wave of chemical change will pass to the ends of the wire.

The phenomena associated with the genesis and propagation of this wave have much in common with the excitation of a nerve. Thus the wire may be activated mechanically, chemically, electrically; the impulse has a definite velocity (determined by the conditions of the experiment), has associated with its passage electrical changes (action potential), and renders all parts of the wire refractory to a second activation for a definite period after the passage of the first.

No. 20 piano wire is used for these experiments. Pieces of suitable length are cut, one end being bent over for manipulation. Place a length in concentrated HNO_3 (sp. gr. 1·42) for a few seconds. Then transfer it with the aid of a glass hook to a dish containing dilute acid (s.g. 1·20). Activate by touching with *another* metal, *e.g.*, zinc. The reaction will continue until the iron is dissolved. With stronger acid solutions (s.g. 1·25) this may not happen; the reaction may be temporary, the metal returning spontaneously to the passive condition. By using graded strengths of acid, graded effects will be obtained.

CHAPTER XIII

THE EXCITABILITY OF TISSUES

Measurement of excitability: Excitation-time.—We have already seen (p. 29) that a measure of the excitability of a nerve or muscle is afforded by the greatest distance to which the secondary coil of an inductorium can be removed from the primary and still produce excitation in the tissue on make or on break of the primary circuit.

A more accurate measure of the excitability of a tissue is afforded by what is called the *excitation-time* or *chronaxie* of the tissue. This depends on the fact that a galvanic current must, in order to produce excitation, not only be of sufficient *strength*, but must also be allowed to act for a sufficient *time* upon the preparation which is to be stimulated. The time during which a voltage of twice the threshold strength must act on a tissue in order to produce excitation is generally measured, and to this the term "excitation-time" is applied.

The principle on which this method of measuring excitability rests may be made clearer by the following: If the times during which currents of different voltage must act to produce excitation in a given tissue are determined (this can be done by the methods about to be described), and if these times are plotted as abscissæ against the voltages as ordinates, the resulting curve (voltage-duration curve) has the form of a rectangular hyperbola. This curve gives, in fact, the complete expression for the excitability of the particular tissue used. The horizontal asymptote gives the voltage which theoretically would require to be infinitely prolonged to produce excitation. This voltage is termed the *rheobase* by Lapicque. The excitation-time is given by the abscissa of that point on the curve whose ordinate is twice the rheobase value and lies towards the vertical asymptote of the curve. It may be considered that a change in the resistance of the tissue, etc., leads simply to a movement upwards or downwards of the voltage-duration curve, and hence to no appreciable change in the excitation-time; whereas a true change in excitability causes a lateral movement of the whole curve. Thus by using the excitation-time as a measure of excitability, apparent changes in excitability due to changes in the physical condition of the experiment are

largely obviated. The form of the voltage-duration curve is, however, affected by the type of electrode used and, more particularly, by the size and distance apart of the points of the electrodes. In experiments on muscle and nerve electrodes not less than 1 centimeter apart should be used.

It may be noted in passing that the term *excitation-time* is employed here in preference to *chronaxie*; the chronaxie of a tissue being only equivalent to the excitation-time when the strength-duration curve of the tissue is of a particular form.

The excitation-time is determined by first finding the smallest voltage which will just excite when its duration is indefinitely prolonged (a duration of the order of $\frac{1}{100}$ second is sufficient for nerve). This voltage (rheobase) is doubled, and then, by means of suitable methods detailed below, the time during which this current must act on the tissue to produce excitation is determined (excitation-time).

Determination of excitation-time by Keith-Lucas pendulum.—The circuit depicted in Fig. 47 shows the arrangement used. A battery and rheocord are connected with a Pohl commutator P, from which the cross-wires have been removed, so that it is used as a switch. K_1 and K_2 represent the keys of a Keith-Lucas pendulum. These are wired as shown and connected with the switch, so that the battery current can be sent through the pendulum or through a voltmeter V as desired. Between the pendulum and the non-polarisable electrodes E is interposed a box of non-inductive resistances, R_1, R_2, and r, of 10,000ω, 7,000ω, and 3,000ω respectively, and which can be combined in different ways by means of the plugs A, B, and C. These resistances serve a double purpose. They avoid the use of very small potentials on the potentiometer and at the same time permit the stimulation, with approximately similar potentials, of tissues with widely different resistances of their own. Freshly coated Ag-AgCl electrodes (see p. 12) fixed 1 centimeter apart may be used for the determination. It is important that the size and distance apart of the electrodes shall be standardised for comparative work. If the electrodes are less than a centimeter apart irregular results are obtained.

It will be seen from the diagram that when K_1 and K_2 are both closed no current flows through the preparation, the circuit being "shorted" through K_1, via the 10,000ω resistance R_1. When K_1 is opened the current flows through the resistances, electrodes, and tissues, to cease at the moment the circuit is broken by the opening of K_2. By varying the distance between K_1 and K_2 the time of passage of the current can be varied at will.

The resistances are used as follows: If it is desired to measure the

excitation-time of a muscle, no plugs are inserted. Now the resistance of a muscle is of the order of 3,000ω for electrodes 1 centimeter apart, and thus the total resistance of the circuit in this case is 10,000ω. If, on the other hand, it is desired to measure the excitation-time of a nerve, plug C is inserted. The resistance of a centimeter of nerve may be anything between 50,000ω and 100,000ω, so that by placing the resistance r in parallel with the nerve, the resistance of the circuit is not appreciably different from what it was in the case stated above for muscle. This is of particular importance when excitation-times are being determined by the condenser method which will be described later.

In determining the rheobase it is more convenient to open K_1 and make and break the circuit by means of the mercury key, rather than use the pendulum with the keys set wide apart. Or K_1 may be opened and closed by hand, the mercury key and K_2 being closed.

The rheobase having been determined and doubled the *excitation-time* is determined by (*a*) finding the closest approximation of the keys which will just give excitation as they are opened in succession by the pendulum; (*b*) reading the angle of deflection (the zero having been previously determined); and (*c*) translating this angle into time by means of the factor for the particular instrument in use. After each determination the rheobase should

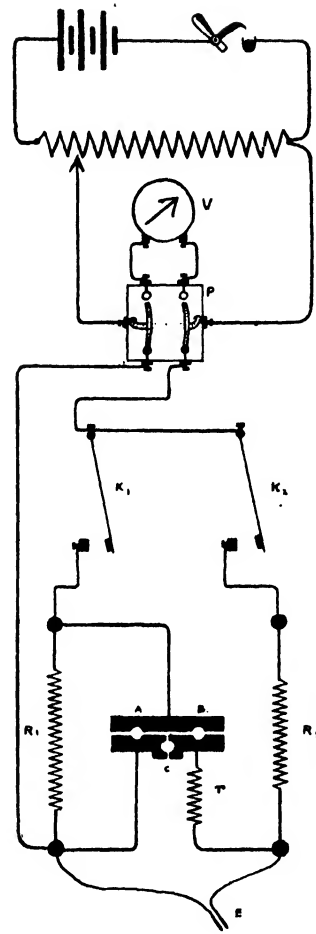

FIG. 47.—Diagram of circuit for determination of excitation-time by Keith-Lucas pendulum. K_1 and K_2 represent the keys of the pendulum; R_1, R_2, and r are non-inductively wound resistances of 10,000ω, 7,000ω, and 3,000ω respectively; by means of one or more plugs in the holes at A, B, or C, the resistances may be combined in different ways. The electrodes E are non-polarisable (Ag-AgCl).

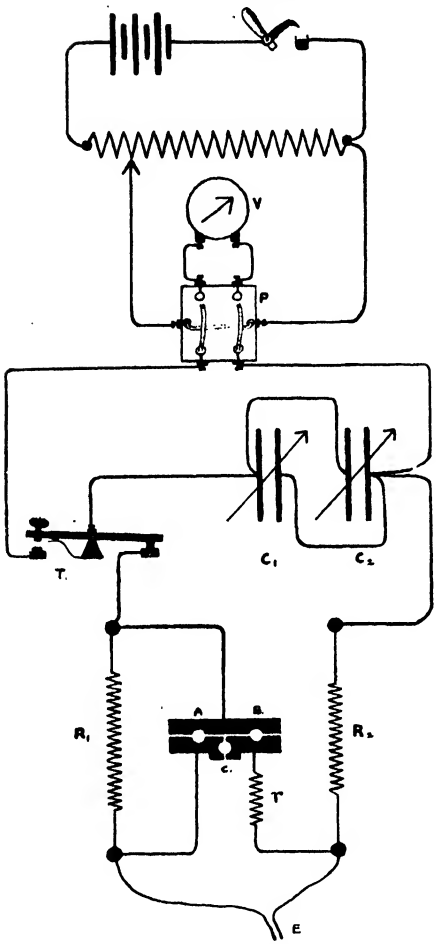

FIG. 48.—Diagram of circuit for determination of excitation-time by condensers. C_1 is a condenser of 5 μF capacity, variable in steps of 1 μF, for the determination of the rheobase. C_2 is a condenser of 1 μF total capacity, variable in steps of 0·001 μF, for the determination of the excitation-time. The condensers are charged and discharged by means of the key T. The resistances have the same values as in Fig. 47.

be redetermined, and the excitation-time accepted only if the rheobase has not altered.

Determination of excitation-time by condenser method.—Another method of measuring the excitation-time is to employ condensers of varying capacity. For, assuming that the resistance in the circuit remains unaltered, the duration of the current on discharge is directly proportional to the capacity of the condenser.

A condenser of 3 microfarads capacity is charged to increasing potentials until it just excites the tissue on discharge.[1] This furnishes the rheobase. The E.M.F. for charging is now doubled, and this is employed to charge a series of condensers, arranged in parallel and having a total capacity of 1 microfarad or thereabouts, variable in steps of 0·001 microfarad. The lowest capacity which just serves to stimulate the preparation on discharge is found. The excitation-time, in seconds, of the tissue is obtained from the following formula :—

$$0{\cdot}37 \times R \times C$$

where R is the resistance of the circuit in ohms, C the capacity of the condenser in farads, while the number

[1] The discharge of such a condenser will occupy about $\frac{1}{100}$ second under the conditions of the experiment.

0·37 is an empirical factor determined by Lapicque, by which the time given by R×C is reduced to the excitation-time as found by rheotome methods.

The circuit employed in determining excitability by this method is shown in Fig. 48. C_1 is a condenser of 5 μF capacity used in determining the rheobase, while C_2 is the condenser of 1 μF capacity, variable in steps of 0·001 μF. T is a Morse key which when depressed charges the condenser, and when released discharges the condenser. The resistances are the same as in the other case and are used in the same way. Note, however, that R_1 is here in series with the tissue. Thus, with the electrodes on a muscle the total resistance with no plugs inserted would be 20,000ω, and with plug A inserted 10,000ω. For nerve, a total resistance of 20,000ω is obtained by inserting plug C; 10,000ω by inserting plug B.

Finally, it is of importance that the circuits used in excitability measurements should be free from self-inductance and capacity, therefore all resistances must be non-inductively wound or be made from graphite, and all wires uncoiled and cross at right angles.

Experiments.—1. On a muscle-nerve preparation determine the excitation-time of the nerve. The success or failure of the current to produce excitation in the nerve is indicated by the muscular response. A just perceptible response of the muscle is taken to indicate that an excitation is set up in the nerve. Or the presence or absence of an action potential may be used as an index. The response taken as the excitation-time response should be similar to the rheobasic response, and the rheobase should be checked after each determination of the excitation-time.

2. Determine the effect of temperature changes on the excitation-time of the nerve.

3. Determine the excitation-time of the frog or tortoise heart-muscle *in situ*. One electrode (anode) should be large and placed with a swab of wet wool in the animal's mouth (indifferent electrode); the other (kathode, Ag coated with AgCl) should be in the form of a fine hook fastened into some part of the heart, the movements of which are recorded by a lever. The stimulus is sent into the heart before an ordinary systole is due, and not immediately after a previous systole. The success or failure of a stimulus to produce excitation is indicated by the presence or absence of an extrasystole following it.

4. Determine the effect of vagus stimulation upon the excitability of the heart.[1]

[1] The experiments upon the frog-heart (3 and 4) may be deferred until those described in Chapters XVI. and XVIII. have been performed.

CHAPTER XIV

THE ELECTRICAL PHENOMENA EXHIBITED BY MUSCLE AND NERVE

A GALVANOMETER or oscillograph is used to study quantitatively the electrical conditions of muscle and nerve, but certain facts can be demonstrated qualitatively without special apparatus.

Demarcation potential of muscle: Contraction without metals.—By means of a glass rod, loop up the nerve of a nerve-muscle preparation and allow its cut end to come in contact either with another part of the surface of its own muscle (Fig. 49) or with other muscles (preferably with an injured part). There will be a contraction of its muscle each time that the contact is made or broken. The excitation is caused by the passage through the nerve of the so-called "demarcation current" of the injured muscle.

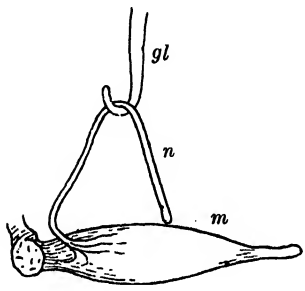

FIG. 49.—Experiment of the contraction without metals. *gl*, bent glass rod; *n*, nerve; *m*, muscle.

The result can sometimes be obtained if the cut end of the nerve is allowed to touch another part of the nerve: in this case it is the demarcation potential of the nerve which stimulates its own fibres.

This experiment is only likely to succeed if a very excitable preparation, such as is obtained from a cooled frog (see footnote, p. 63), is employed.

Action-potential of muscle; Secondary contraction.—Take a nerve-muscle preparation, and lay its nerve over another nerve-muscle preparation, the nerve of which is placed upon electrodes (Fig. 50). Tetanise this nerve; the nerve of the first-named preparation will be stimulated by the electrical variations which accompany the excitation of the tetanised muscle. A nerve-muscle preparation thus used in place of a galvanometer to indicate electrical variations is known as a *rheoscopic frog preparation.*

The result can also be obtained with single excitations.

Secondary contraction from the heart.—Lay the nerve of a muscle-nerve preparation upon the beating heart of the frog. If the prepara-

tion is very excitable the muscle will twitch with each beat of the ventricle.

A similar experiment may be performed on the anæsthetised or spinal mammal by cutting the left phrenic nerve high up in the thorax and laying the cut end on the heart. The left half of the diaphragm will contract with each heart-beat.

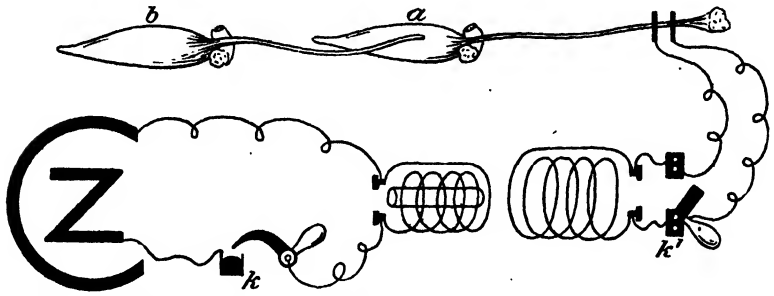

FIG. 50.—Experiment to show secondary contraction. *k*, mercury key in primary circuit; *k'*, short-circuit key in secondary circuit; *a*, first muscle; *b*, second muscle with its nerve laid over the first.

String galvanometer of Einthoven.—This consists of a microscopically fine thread of silvered quartz or of wire stretched between the poles of a powerful electro-magnet (Fig. 51). When a potential difference is applied to its ends the thread ("string") is deflected to one side or the other to an extent varying with the E.M.F. —the tension of the thread being supposed constant. The movement is observed with a microscope, or the magnified image of the string is photographed on a moving sensitised surface. This usually takes the form of highly sensitive film or paper placed on a drum within a photographic camera, and moved vertically by clockwork or otherwise behind a narrow horizontal slit. Movements of the string then appear, after development, as lines on the paper. A time-marker operates by casting a shadow on the slit at the required interval (usually $\frac{1}{5}$ second).

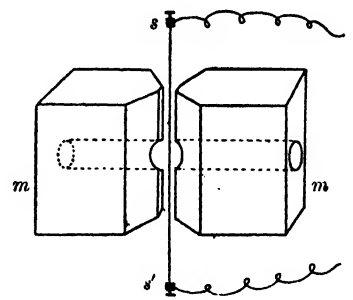

FIG. 51.—Diagram of Einthoven string galvanometer. *s*, *s'*, wire or silvered quartz thread, stretched between the two poles of a powerful electro-magnet, *m*, *m*, which are perforated to allow a microscope to bear upon the thread.

For certain investigations, especially those relating to the electrical conditions accompanying the action of the heart, the string galvano-

meter is more convenient than the capillary electrometer. It has been largely adopted by clinicians, since the heart-records obtained by it (electro-cardiograms) furnish valuable indications as to the nature of cardiac affections which might be otherwise difficult to diagnose (see p. 111).

The kathode-ray oscillograph.—While the capillary electrometer and the string galvanometer are suitable for many types of investigation each instrument has defects, and for very accurate work on action potentials in tissues, some form of high-frequency oscillograph is

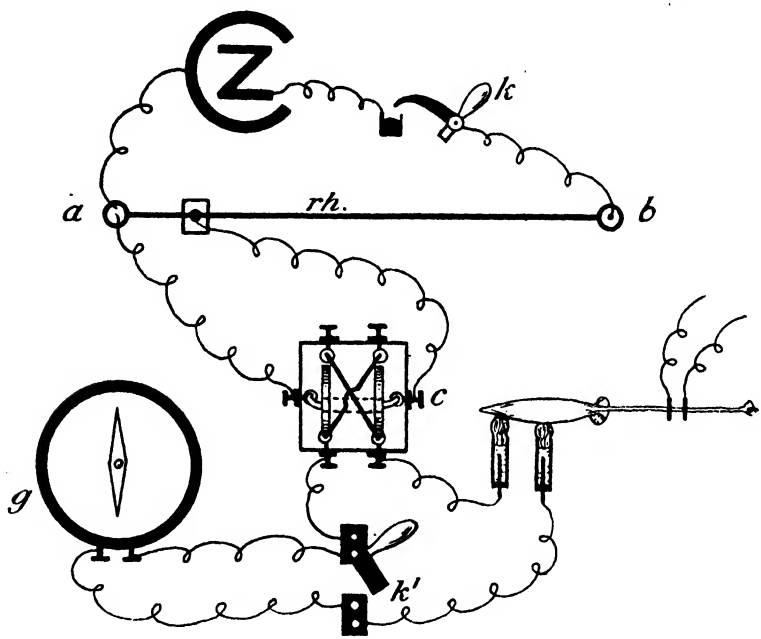

FIG. 52.—Diagram of arrangement of apparatus for studying muscle potentials with galvanometer.

necessary. The kathode-ray oscillograph has recently been brought into use for this purpose. In this the moving part is a stream of electrons, and is practically free from inertia. Such an instrument is shown diagrammatically in Fig. 53. The tube T is of glass and is filled with argon at low pressure. The kathode C is maintained at red heat by a current of about one ampere from a two-volt cell. The anode A is perforated by a circular hole at its centre and is maintained at a positive potential of from 1,000 to 3,000 volts. Under these circumstances a stream of electrons issues from the kathode, and passing, through the hole in the anode, appears as an irregular patch of light

on a fluorescent screen (FS) covering the larger end of the glass tube. The electron stream or kathode ray is focused on to the fluorescent screen by suitably adjusting the negative potential (—200 to —400 volts) on the focusing cylinder S, which surrounds the beam near its origin. The application of a potential difference between the parallel plates P causes a deflection of the beam, and hence of the luminous spot on the fluorescent screen, from the more negatively towards the

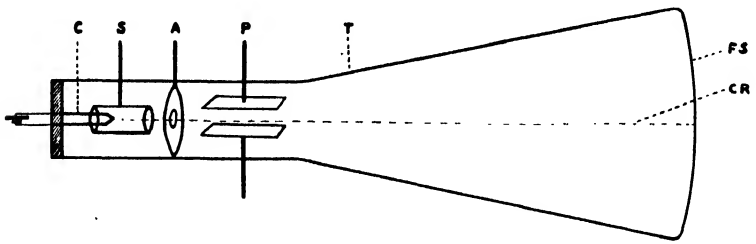

FIG. 53.—Diagram of kathode-ray oscillograph (Von Ardenne type). T, clear glass tube containing argon at low pressure; C, kathode; A, anode; S, screen for focusing ray; CR, kathode ray; FS, fluorescent screen covering end of tube; P, parallel plates to which action potentials, etc., are led, thus deflecting the ray.

more positively charged plate. The action potentials are led from the tissue to be investigated to these plates after suitable amplification. By photographing the end of the tube with the aid of a wide aperture lens, the direction of travel of the film being at right angles to the direction of movement of the ray, continuous records of action-potentials can be obtained. Various movements, such as those of respiration and of the heart, can be recorded mechanically on the same film, along with the variations in potential which accompany them.

CHAPTER XV

INVOLUNTARY MUSCLE

Mammalian Intestine and Uterus. Dorsal Muscle of Leech Frog-bladder

Mammalian intestine.—The reactions of mammalian isolated intestine to changes of temperature, to drugs and autacoids, and to the stimulation of nerves can readily be investigated if the muscle is suspended in a bath of Locke fluid maintained at body temperature. If the isolated piece of intestine is undistended by internal pressure, the movements which it executes are chiefly pendular, *i.e.*, contractions of the longitudinal muscle; whereas if the lumen is distended peristaltic movements may also occur.

Magnus method: Pendular movements.—The following method (modified from Magnus) is the most convenient with which to study the actions of drugs, etc., on intestinal muscle. The arrangement of the apparatus is evident from Fig. 54.

The experimental tube, filled with Locke's solution, is immersed in a beaker of water maintained at 35° C. A longitudinal piece of rabbit's small intestine is emptied of its contents and tied off at each end with a thread. One thread is attached to a leaden or brass weight which is then dropped into the experimental tube; the other is fixed to the recording lever with plasticine.

Several test-tubes filled with normal Locke solution are placed in the warm bath so that the fluid within them maintains the same temperature as that in the experimental tube. Substances to be tested are added to the Locke in one of the tubes, and, when a normal record has been made, the fluid in the intestine bath is replaced by the test solution by pouring this into the funnel F and thus displacing the normal Locke. Normal Locke is subsequently added to the funnel to displace the test solution.

Though desirable, it is not necessary to have oxygen bubbling through the Locke in the experimental tube, provided that the Locke has been thoroughly saturated with oxygen or air before adding it to the bath. If the intestine does not start to contract a few minutes after being set up, thoroughly aerate the Locke in one of the test-tubes by shaking violently, and replace the original solution with this.

Action of drugs on intestine.—With the above preparation determine

the effects upon the rate, character, and amplitude of the contractions, and upon the tone of the muscle, of (*a*) adrenaline, (*b*) acetylcholine, and (*c*) atropine. Take a record of the normal contractions. Then displace the normal Locke with that containing adrenaline, 1 in 100

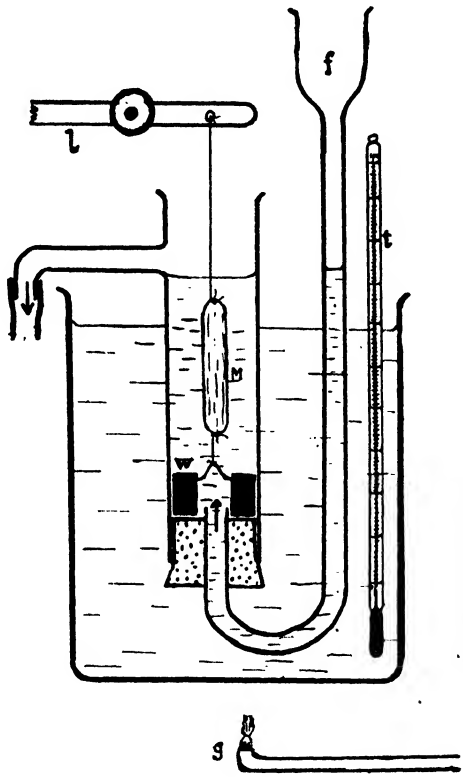

FIG. 54.—Diagram of apparatus for investigating the contractions of mammalian plain muscle and the action of drugs and extracts upon it. M, muscle within experimental tube filled with Locke's solution. The strip of muscle is attached below to a leaden weight W, and above to a lever *l*. A bent glass tube terminating in a funnel *f* serves to introduce fresh Locke, containing a known percentage of the drug, into the tube, displacing the normal Locke. *g*, gas-jet; *t*, thermometer.

millions (1 in 10^8). When sufficient record of the action has been obtained, wash out the adrenaline solution with fresh Locke. When conditions have returned to normal investigate the action of acetylcholine, 1 in 10^8, in the same way. The observations may be repeated using different final concentrations of the drug, *e.g.*, 2 in 10^8 and 1 in 10^7.

Always allow conditions to return to normal before each successive experimental observation. Now add Locke containing atropine sulphate, 1 in 10^5. Allow this to remain in the bath for a few minutes and then repeat the observation with acetylcholine, 1 in 10^7.

When the actions of unstable substances such as adrenaline and acetylcholine are being investigated, an amount of a stable stock solution, to give the desired final concentration, is added to one of the test-tubes in the warm bath immediately before use. (For stable stock solutions, see Appendix.)

Trendelenburg method: Peristaltic and pendular movements.—This method is illustrated in Fig. 55. The warming bath of the last method forms the actual intestine bath in the present. Oxygen is bubbled through the fluid from tube A. The longitudinal piece of rabbit (or guinea-pig) intestine is tied off at its oral end. Into the aboral end is fixed the lower end of the glass tube B. Tube B is fixed to a stand and is connected with rubber tubing to a reservoir (T) consisting of a burette tube calibrated in milliliters and which is fixed to an upright in such a way that it may be moved smoothly upwards or downwards as desired. The lumen of the intestinal strip and the tubes connecting it to T are filled with fluid, as indicated in the diagram. By raising or lowering tube T the pressure of fluid within the lumen of the intestine (measured by the distance between the surfaces of the fluid in the beaker and in the burette) can be varied at will.

The following observations are permitted by this method: (1) The lever attached to the oral end of the intestine records chiefly the pendulum movements and changes of tone of the longitudinal muscle, as in the method last described. (2) The presence or absence of peristaltic movements is determined by direct observations of the gut. The extent of these movements is measured roughly by the fluid they displace, *i.e.*, the changes in level at the fluid surface of tube T.

The peristaltic movements may be recorded by having a T-tube attached to the upper end of tube T. One limb of this is attached to a Marey tambour (see p. 41). The other is opened to the air when adjustments of tube T are being made, and is kept closed at other times. The lever of the Marey tambour is made to write exactly above that recording the longitudinal movements.

(3) The hydrostatic pressure applied to the gut is given by the distance between fluid levels in beaker and burette. (4) The increase of volume of the gut produced by increased pressure is given by the difference in burette readings at the original and at the new pressure. This gives a rough indication of the tonus of the gut.

Effects of pressure changes on movements of gut.—Starting with the pressure at zero, determine the effects upon the gut movements of slowly raising the pressure (1 millimeter per second) to about 5 centimeters. Note the point at which peristaltic contractions start and observe the direction of these movements. (The points at which

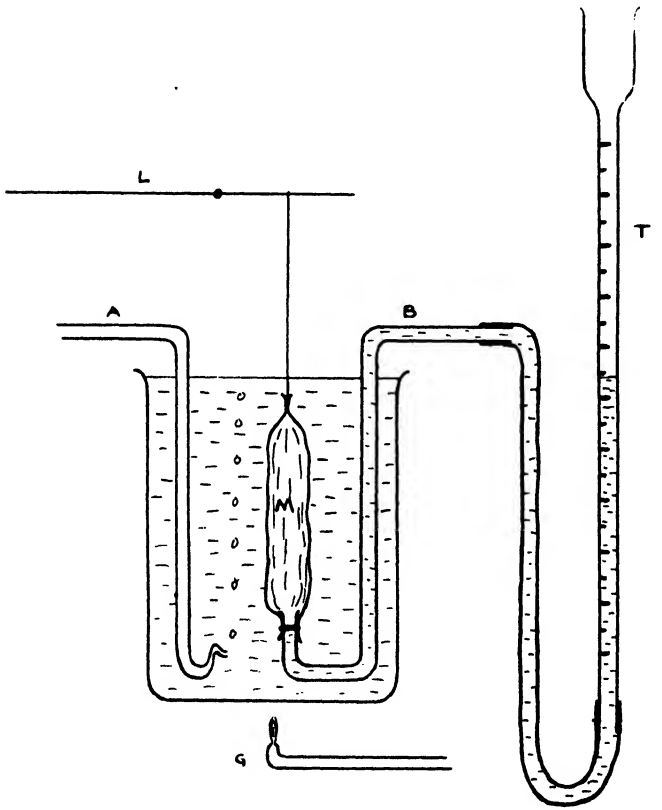

FIG. 55.—Diagram of apparatus for investigating peristaltic and pendular movements in isolated mammalian intestine (Trendelenburg method). For description, see text.

each centimeter rise in pressure is reached should be marked on the drum underneath the tracing.) Lower the pressure to zero, and, when activity of the gut is once more normal, raise the pressure again, but this time much more rapidly. What difference do you notice between the effects produced by slow and by quick changes of pressure ?

Repeat the slow raising of pressure and obtain quantitative

measurements of the relation between pressure, filling and extent of gut movements (pendular and peristaltic). Tabulate the results.

Effects of temperature changes.—These are investigated by altering the temperature of the fluid in the intestine bath, either by suitable adjustment of the heating arrangements or by adding warmed or cooled Locke to the bath until the desired temperature is reached. Take records at 35°, 30°, and 25°. What is the ratio between the frequency at T and at T + 10 ? Has lowering of temperature any effect on the tonus of the gut muscle ?

Effects of nerves on gut movements.—The effects of the sympathetic nerve supply to the gut can be readily demonstrated in the following manner. A 6-10 centimeter length of rabbit small intestine, together with the mesentery and its contained blood-vessels, is suspended in a bath similar to that of Fig. 55. The upper end is attached to a lever and the lower may either be connected with a reservoir of fluid, as in the Trendelenburg method, or may be tied to the oxygen inflow tube A. That part of the mesentery containing the most obvious blood-vessels is placed on electrodes. A normal record is first obtained and the tissues on the electrodes are then stimulated with tetanising shocks. Mark on the drum the point where stimuli are applied and where they cease. The effects upon the movements and tone of the intestine are due to stimulation of the sympathetic nerves which reach the intestine via the arteries (the so-called peri-arterial nerves). Compare the effects with those of adrenaline. Note that the effects of sympathetic stimulation on plain muscle are not always inhibitory (*e.g.*, action on sphincters of gut and on splanchnic arterioles).

Uterine muscle. Action of drugs on uterus.—The movements of the uterus are recorded in the same way as the pendular movements of the intestine, by the method of Magnus. Fix one of the uterine cornua of a guinea-pig or rabbit in the experimental tube, which should be filled with well-oxygenated Locke. Spontaneous movements will usually occur. Investigate the action of the following drugs upon the movements and tone of the uterus :—

(*a*) Pituitary (posterior lobe) extract, 0·02 units.
(*b*) Adrenaline, 1 in 10^8 ; 1 in 10^7.
(*c*) Ergotoxine ethanesulphonate, 2 in 10^5. Leave this in the bath for 5 to 10 minutes.
(*d*) Repeat (*b*) and, finally, (*a*). Has ergotoxine affected either response ?

The reactions of the uterus to adrenaline depend on the species and sometimes also on whether the animal is pregnant or not. The guinea-pig uterus relaxes with adrenaline and the rabbit uterus contracts; the pregnant cat uterus contracts, but the non-pregnant

cat uterus relaxes. Ergotoxine causes contraction of uterine muscle and other plain muscle supplied by motor adrenergic nerves, and ultimately prevents the effects of motor adrenergic nerves upon tissues while leaving inhibitory effects of adrenergic nerves unimpaired. Similarly it prevents motor actions of adrenaline, but not inhibitory effects. Ergotoxine thus does not affect the action of adrenaline upon the guinea-pig uterus, but inhibits or reduces the effect upon the rabbit uterus. Ergotamine acts in the same way as ergotoxine. Ergometrine, on the other hand, while causing contractions of the uterus and, in larger doses, of other plain muscle with a motor adrenergic innervation, does not readily paralyse the effects of motor adrenergic activity, or the motor actions of adrenaline.

Bio-assay of oxytocic activity.—If a virgin guinea-pig is used for such experiments it will be found that, after the first few responses to pituitary extract, the responses to different submaximal doses become almost constant for each particular dose. This being so, the virgin uterus forms a suitable tissue on which to assay the oxytocic (or uterine stimulating) properties of posterior pituitary extracts: *i.e.*, the activity of a preparation of unknown potency can be compared with the activity of a preparation of known potency (standard preparation) and the activity of the former preparation expressed as a percentage of that of the standard. Such quantitative determinations of the activity of drugs, etc., are known as bio-assays. All bio-assay involves the comparison of a preparation of unknown potency with a preparation of known potency. The preparation of known potency is known as the standard preparation. In the case of pituitary (posterior lobe) extract and a number of other official substances, this is an arbitrarily chosen batch of extract which has received international recognition as an "International Standard." The International Unit is the activity of a certain weight (0·5 mg. in the case of pituitary) of the International Standard Preparation.

The principle involved in the bio-assay of posterior pituitary extract can be demonstrated with the Magnus apparatus described above. In practice, more complex apparatus is used.

In carrying out an assay a convenient drum speed is 0·5 centimeters per minute. The following points are important: It is essential to use only submaximal doses. The effects of successive doses should not differ greatly. The doses should be applied at regular intervals (10 minutes). The drug should be washed out as soon as the lever begins to fall after reaching the height of its rise: the drum may then be stopped until the muscle has fully relaxed and the next dose is almost due. Since the uterus often tends to show a "rhythm" in its response, *i.e.*, to give alternate large and small contractions irrespective of the dose of extract applied, it is important in carrying out an assay

to nullify possible effects of such behaviour by giving successive identical doses of standard or unknown throughout the assay. According to Burn a result should depend on at least two determinations, each of four successive doses, of which one shows that the test solution contains slightly more and the other that it contains slightly less than a certain number of units per milliliter.

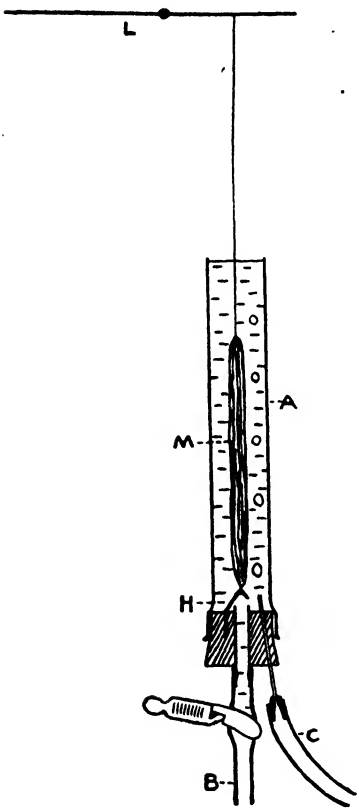

FIG. 56.—Diagram of muscle bath for assay of acetylcholine on eserinised dorsal muscle of the leech. A, glass tube (capacity, 2 to 5 milliliters) with rubber bung at lower end carrying B, outflow tube; C, air inflow tube (stainless-steel hypodermic needle); H, silver hook for attachment to lower end of muscle M. The outflow tube is closed by a spring clip.

Dorsal muscle of leech. Bio-assay of acetylcholine.—The muscle of the isolated dorsal body wall of the leech (*Hirudo medicinalis*) when treated with eserine reacts almost specifically to acetylcholine. Under good conditions it will give an appreciable contraction with 1 part in 1,000 millions of acetylcholine. Submaximal responses vary in a regular manner with the dose. The apparatus used is shown in Fig. 56. The bath may have a capacity of from 2 to 5 or more milliliters. The fluid used is a dilute Locke solution: Ringer-Locke, 100 parts diluted to 135 parts with distilled water. For eserinisation of the muscle before each observation, the same solution is used but with the addition of eserine sulphate, 1 part in 200,000.

The muscle is prepared in the following way: A small or medium-sized leech is fixed on its back on a piece of cork by a pin at each extremity. The skin is slit up either side along the junction between the pale (ventral) and the darker (dorsal) surfaces. The whole of the ventral skin, and the underlying viscera, are removed. All the tissues adhering to the dorsal body-wall are cleared away by careful dissection —preferably with curved scissors—until nothing but the dark-coloured sheet of muscle is left with the dorsal blood-sinus running down the

middle. The preparation is split into two along the mid-line with a sharp scalpel after fine silk ligatures have been threaded through the ends of the two halves. Two muscle preparations are thus provided from each leech. One ligature is attached to the hook in the rubber stopper and the other to the lever, which should be of the gimbal pattern.

The correct loading of the lever is found by trial. After the preparation is set up the bath is filled with dilute Locke solution and a stream of air sent continuously through the fluid. The muscle may contract up at first and should be left for at least an hour or so before beginning observations. By this time it will probably have relaxed.

The lever should write on a very slowly moving drum (say, about 1 centimeter in 3 minutes). A few minutes before a solution is to be tested the normal fluid is run out and is replaced by that containing eserine, 1 in 200,000. Fluids are added to the bath by pouring them into the top end of the tube. Test fluids are allowed to remain in the bath for a definite period (2 or 3 minutes), timed by a stop watch, and are then washed out with dilute Locke. If the muscle has contracted in response to the test solution, the relaxation may take 20 minutes or more, depending on the extent of the contraction. Thus positive test solutions can be applied only at infrequent intervals. In quantitative work, test solutions and standard solutions are applied alternately. Dilution and mixing of test and standard solutions of acetylcholine should always be done in stoppered vessels: contact of such fluids with human skin confers upon them acetylcholine-like properties. It must never be assumed that preparations from the same leech have the same sensitivity to acetylcholine.

Suitable concentrations of acetylcholine for work on the leech range from 1 in 1,000,000,000 to 1 in 50,000,000 approximately. The necessary dilutions for testing are made from stable stock solutions (see Appendix).

Use the leech preparation to determine whether acetylcholine is liberated (*a*) by stimulation of vagus to frog's-heart (p. 98), (*b*) by stimulation of vasodilator nerves to tongue (p. 129).

The isolated eserinised rectus abdominis muscle of the frog may be used in the same way as leech muscle for the assay of acetylcholine, but it is much less sensitive than the leech.

Stomach or bladder of frog.—Take either a transverse strip from the stomach—the frog should have been recently fed—or the whole urinary bladder, fastening a thread to each end. For recording the contractions, which may be spontaneous and rhythmic, or may need to be elicited by excitation, the apparatus shown in Fig. 57 is employed. This consists of a glass tube of the size and construction shown in the diagram. One end of the preparation is tied securely

to a hooked wire passing through a cork, which closes the lower end of the tube (this wire serves as one electrode), whilst the other end is attached by a thread, passing through the open upper end of the tube, to the short arm of a very light lever. The tube has three lateral openings; one, near the bottom for inflow of fluid, a second near the top for outflow, and a third, closed by a cork, through which a wire is passed to serve as a second electrode. The glass tube is filled with Ringer's solution. Bring fine wires from a battery of two or three Daniell cells or accumulators, placing one wire in contact with the lower, the other with the upper electrode. This upper electrode need not touch the tissue. Use a very slow drum. Stimulate by making and breaking the circuit.

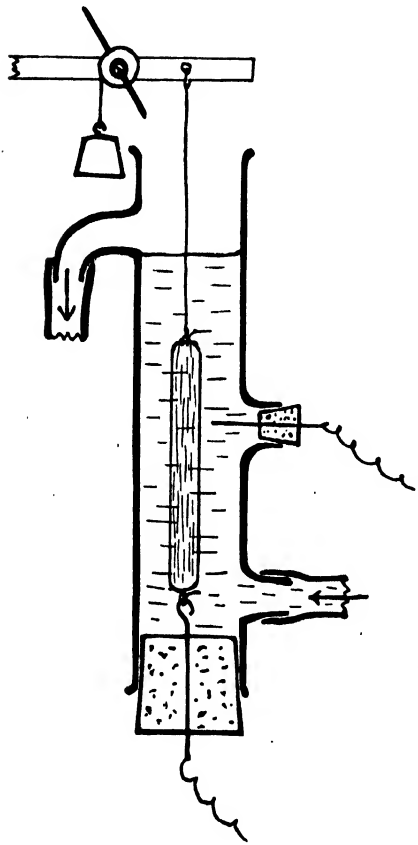

FIG. 57.—Diagram of apparatus for recording the contractions of a strip of frog's plain muscle immersed in Ringer's solution, and for investigating the action of drugs and animal extracts upon the tissue.

If induced currents are employed there is usually no response with a single make or break of the primary circuit owing to the short duration of the induction shock, but by repeating the stimulus the tissue will contract and the lever will describe a simple prolonged muscle curve on the drum. The contraction may also be obtained by a rapid succession of induced shocks, using the Neef's hammer, but it is always a simple contraction, not a tetanus.

CHAPTER XVI

THE FROG-HEART: AUTOMATICITY AND CONDUCTIVITY

Dissection.—Make a special dissection of a large frog to show the situation and connexions of the heart, its several cavities, and the blood-vessels leading to and from it. It is advantageous to distend the cavities with gelatin solution and allow this to set. Notice a small nerve entering the heart on each side along the superior vena cava; this is the cardiac nerve, and is given off from the vagus; it contains also fibres from the sympathetic which reach the vagus near the skull. Cut out a piece of the interauricular septum; place it in

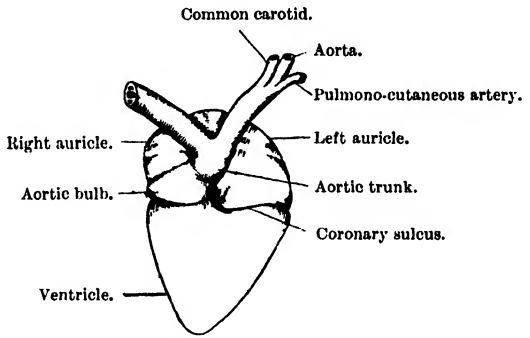

FIG. 58.—Heart of frog; ventral aspect (Gaupp). ½.

very dilute methylene blue for five minutes; wash with water, and examine in water under the microscope for nerve-fibres and groups of nerve-cells. The dissection of the heart may be made beforehand and kept for reference in dilute formalin.

Experiments on Automaticity and Conductivity in the Heart

1. **Inspection of the beating heart** *in situ*.—In a frog, the brain and spinal cord of which have been destroyed, cut away the skin over the sternum and for about 2 centimeters below this. Lift up the ensiform cartilage with a pair of forceps and cut through its lower end. Lift that part attached to the sternum and cut a V-shaped flap towards the sternum, taking care to avoid damaging the heart which will now

come into view. Cut through the sternum on each side—as near to the shoulders as possible—and remove the bone. Carefully cut through the pericardium so as to expose the heart. Very gently raise the tip of the ventricle with a blunt instrument, and sever the pericardial

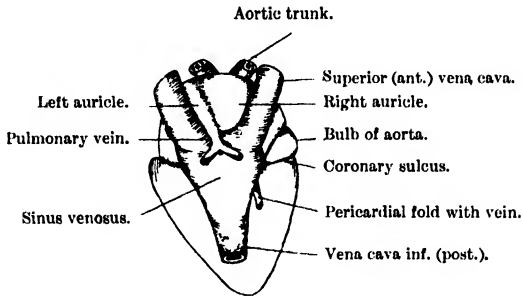

FIG. 59.—Heart of frog from dorsal aspect (Gaupp). ⅔.

ligament which binds the ventricle to the back of the pericardium. *Do not grasp the heart with forceps or injure it in any way by manipulation.*

On raising the ventricle the sinus venosus comes into view, receiving the two venæ cavæ superiores and the vena cava inferior.

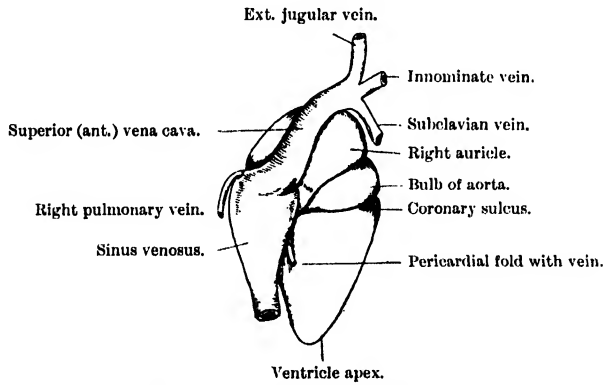

FIG. 60.—Heart of frog, seen from the right side (Gaupp). ⅔.

Above, the sinus is continuous with, but marked off by a whitish line (sinus-auricle junction) from, the auricle, which is double and receives on the left side the pulmonary vein; the two auricles open into a single ventricle. On the front the bulbus aortæ is seen leaving the ventricle and dividing into two trunks, the right and left aortæ, each of which again soon divides into three branches.

Notice that with each systole the venous part of the heart (sinus

venosus) contracts first; its contraction is followed by that of the auricles, which contract together, and this by that of the ventricle. There is actually a short but distinct pause between the contractions of the different parts of the heart. In the case of auricle and ventricle this pause is known as the auriculo-ventricular interval. In a frog

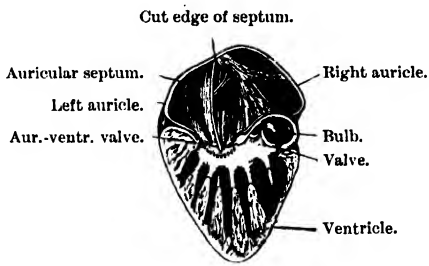

FIG. 61.—Section through heart of frog. Front half seen from behind (Gaupp). The spongy structure of the ventricle is shown. ⅔.

the spinal cord of which has been destroyed, there is usually little or no blood passing through the heart. But if blood is being pumped through, notice the sudden distension (diastole) of each cavity which immediately succeeds its contraction (systole). If the finger is very

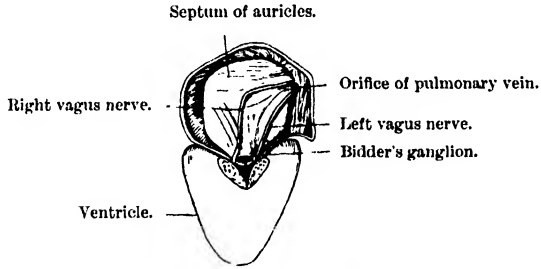

FIG. 62.—Heart of frog with left auricle cut open to show the vagus nerves in the auricular septum (Gaupp). ⅔.

lightly placed on the ventricle the hardening which accompanies systole may be felt (cardiac impulse).

Recording of normal heart-beat.—The contractions of the frog-heart are recorded in the manner indicated in Fig. 63. If the long arm of the cardiograph lever is much heavier than the short arm, plasticine should be fixed to the short arm so that the lever is almost balanced. Attach a thread to a small pin bent in the form of a hook. Carefully insert the hook through the apex of the ventricle. Fix the free end of the thread to the short arm of the cardiograph lever by means of a small

piece of plasticine. Pass a pin through the mediastinum into the cork board so as to fix the preparation. If the lever is nearly balanced, the contractions of the heart will cause it to execute large movements, but if the writing point is now placed against the drum it is probable that the movements of the lever will either cease so that no tracing is obtainable, or will be diminished and record irregularly. Gradually remove plasticine from the short arm of the lever until good recording is obtained. By suitable adjustment of the load on the heart-muscle, the optimal conditions are found. Now set the drum in motion at such a speed that the record of each heart-beat is spread over at least 5 millimeters, and thus obtain a tracing of several successive beats. Take a time record in second or 5-second intervals underneath this

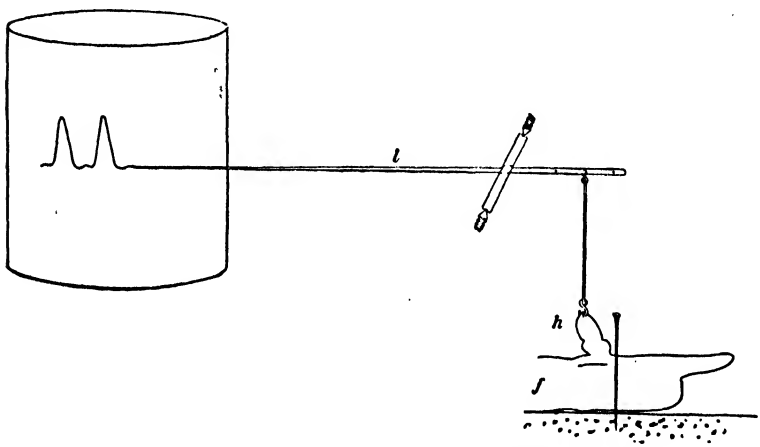

FIG. 63.—Frog cardiograph. *f*, frog, *h*, heart; *l*, lever.

record. This is preferably done at the same time as the observations, but may be recorded subsequently with the drum running at the same speed as when the records were made.

Under ordinary conditions the record of each cardiac cycle will show contractions due to auricles and ventricle. Under better conditions the beats of the sinus venosus will also show. Often the contractions of the bulbus aortæ will be evident in addition. *The student should determine, by careful correlation of what is happening in the heart and in the record, to which parts of the heart the different components of the record are due.*

2. **Effect of heat and cold on rate of beat.**—Take a record of the normal heart-beat and a time record in second or 5-second intervals. Stop the drum and apply an ice-cold copper rod to the surface of the sinus venosus. Remove the rod and immediately record a few cardiac

contractions. When the beat has returned to normal, repeat the observation but this time cooling the auricles. Finally, obtain a record after cooling of the ventricle. In the same way investigate the effects of warming the different chambers of the heart with a warmed copper rod. Note whether the heart you are using is pumping blood or is empty. Compare your results with those of your neighbours. Do any of the effects observed differ according to whether the heart is empty or not ? How do you account for any such difference ?

3. **Heart-block.**—If the conductivity between the auricles and ventricles is *impaired*, without being completely abolished, not every auricular contraction will be followed by a ventricular one. Such incomplete dissociation of auricular and ventricular contractions is known as *incomplete* or *partial heart-block*. In the following experiment attempts will be made to establish various grades of partial A-V block.

Take a piece of 5-ampere lead fuse wire and place it round the auriculo-ventricular groove in the form of a loop. Cause the loop to exert pressure round the groove by twisting the free ends together. Take records of the heart-beats with different degrees of compression, and note the different grades of heart-block produced. The ventricle may respond to every other auricle contraction (2 : 1 block), to 2 out of 3 auricle contractions (3 : 2 block), to 1 in 4 (4 : 1 block), and so on. (Note that in describing the degree of block the auricle rate is named first.)

In addition to the dissociation of auricular and ventricular beats in this experiment, note that on exerting pressure round the groove the A-V interval may be prolonged before actual dissociation of beats occurs. Similarly on removing the wire the A-V interval will usually be longer than normal for some time after recovery from the block. *It is important in this experiment accurately to correlate what is happening in the tracing with what is happening in the heart.* Note any changes in the *force* of the contractions of the auricles and/or ventricle in the course of the experiment. How may they be explained ?

4. **Stannius' experiment.**—In the experiment just described, conduction between auricles and ventricle was impaired, thus giving rise to a condition where *some* of the auricular impulses failed to reach the ventricle. In the present experiment functional continuity, and therefore conductivity, between the different chambers of the heart will be completely abolished. The resulting condition is known as *complete heart-block.*

Attach a heart to the cardiograph in the usual way, but try to adjust the loading of the lever so that the beats of the sinus venosus will record. Take a record of the contractions. Stop the drum. Now pass a thread under the sinus and tighten it round the sino-auricular junction (first *Stannius ligature*). The sinus venosus continues to beat as before

(take a record or count the rate), but, because the impulses arising in the sinus are now no longer able to reach them, the auricles and ventricle usually come to a standstill in diastole. Such a heart is termed a *Stannius heart*, and the condition set up is that of complete sino-auricular heart-block.

Gently prick auricle or ventricle, and notice that each stimulation is followed by a contraction starting from the point of stimulation and spreading to all parts of auricles and ventricle. After a variable period the auricles and ventricle will commence to beat again, but at a slower rate than before, the auricles having now taken up the function of pacemaker.

[*While waiting for the auricles and ventricle to resume beating, experiments* 5, 6, 7, and 8 *below may be performed.*]

After the auricles have started beating, again take a record. Then stop the drum and pass a second ligature *behind* the aortæ and secure it round the auriculo-ventricular junction (second *Stannius ligature*). The ventricle usually gives three or four beats, and then both it and the auricles again come to a standstill. Either can, however, be made to beat by artificial stimulus (prick, electric shock).

After a certain lapse of time the auricles and ventricle may recommence beating regularly and rhythmically. When this happens take a further record. It will be found that the auricles are beating at the same rate as they assumed after tying the first ligature, while the ventricle is beating with a still slower rhythm (idioventricular beat). In other words, while all three parts may ultimately be found beating spontaneously, it will always be noticed that the rate of the sinus is the fastest, that of the auricles next, and that of the ventricle the slowest. (The bulbus aortæ is also spontaneously contractile; even small pieces can be observed to beat rhythmically.)

Further properties of cardiac muscle.—Apart from its regular spontaneous rhythm the heart-muscle shows certain phenomena, which are not peculiar to it, but are more strikingly exhibited than by skeletal muscle. To investigate these the heart is attached to the cardiograph in the usual way and a first Stannius ligature is tied. (If the ventricle does not become quiescent it is probable that part of the sinus is still in functional continuity with the auricles and another first Stannius ligature should be tied somewhat farther forward than the other.)

Place a pair of electrodes, connected with an induction coil, in contact with the quiescent ventricle. They must be fixed in position with plasticine, or otherwise, not held in the hand. The following experiments may now be made:—

5. **Latent period.**—Arrange the apparatus in the same way as for the recording of the simple muscle curve, *i.e.*, with the primary circuit

connected through the drum. Take a record of the contraction of the ventricle on a moderately fast drum; mark the point of stimulation. Take a time tracing in 100ths of a second and thus determine the latent period of ventricular muscle.

6. **Refractory period.**—For this experiment the drum must move at a rate fast enough to spread out the curve of the ventricular contraction to at least 1 centimeter. An electromagnetic signal should be placed in series with the primary circuit and its writing point made to record immediately below the cardiograph writing point. The primary circuit is made and broken by hand.

A first stimulus is applied to the ventricle, and, during some part of the resulting ventricular cycle, a second stimulus is made to follow it. (If a signal is not incorporated in the circuit, mark the moment of putting in the second stimulus by a dot on the curve.) Repeat the observation sufficiently often for the second stimulus to have fallen at, say, a dozen different points in the ventricular cycle. From your results construct a diagram showing the exact phase of this cycle in which you found the muscle refractory to stimulation.

If the second stimulus comes soon after the first, so as to reach the heart while it is still in process of contraction, no additional effect is produced; there is *no summation* (compare with skeletal muscle, p. 43). In other words, whilst the contraction produced by the first stimulus is actually proceeding, cardiac muscle is entirely *refractory* to a second stimulus. This refractory condition is continued in a modified degree during the period of relaxation of the muscle. A consequence of the long refractory phase and of the long latent period is that *cardiac muscle never shows a true tetanus.*

The existence of a refractory period can also be determined by exciting the exposed frog-heart whilst beating normally *in situ.*

7. **Staircase phenomenon** (see p. 50).—Use the above "Stannius" preparation and a very slowly moving drum; or have the drum stationary during the record, and move it about 5 millimeters by hand between the stimulations.

After a period of rest, put in single maximal stimuli at intervals of about two seconds, and record each contraction. Notice that there is a slight increase in the extent of the first few successive contractions, the second ordinate being a little higher than the first, the third than the second, and so on, but they soon become of exactly the same height.

8. **"All or none" contraction.**—Put the secondary coil far from the primary, and excite the preparation (Stannius heart) by breaking the primary circuit. Determine the excitability by ascertaining to what division of the scale the secondary coil must approach the primary before a contraction is produced. This represents the *liminal stimulus.* Now bring the secondary nearer the primary and again stimulate.

The contraction is not appreciably larger. Repeat with stronger and stronger stimuli: lastly, repeat with diminishing strength of stimulus. Apart from the "staircase" effect already studied—and which will only be seen if adequate stimuli follow one another with a sufficiently short interval of time between—in every case the extent of contraction, if any, is the same. This is not so with skeletal muscle. The difference is connected with the fact that the individual fibres of skeletal muscle contract independently of one another, and with the stronger stimuli a greater number are thrown into contraction, whereas in the heart all the muscle-fibres are in functional continuity so that the whole ventricle behaves like a single skeletal muscle-fibre.

CHAPTER XVII

PERFUSION OF FROG-HEART

Action of Ions and Autacoids: Law of the Heart

Perfusion of the whole heart.—For this purpose a wide glass tube (Fig. 64, v), open at the upper end and terminating below in a cannula, is used: there is a short lateral tube (L) near this end. The cannula (which should contain Ringer's fluid to prevent blood clotting in it) is introduced into the sinus venosus and tied in. The heart is

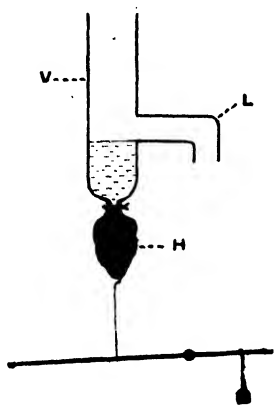

FIG. 64.—V, vertical limb of cannula into which projects a rubber tube from a reservoir of Ringer (not shown in diagram). The outflow from the reservoir is adjusted by means of a screw clip so that there is a constant drip from the overflow limb (L) of the cannula. The perfusion pressure is thus maintained constant under all conditions of the heart's activity. Drugs, etc., are added to the fluid in the cannula by means of a pipette: or the perfusion fluid in the cannula is completely displaced by Ringer containing the substance to be investigated.

then completely cut out from the body, and the tube is fixed vertically to a stand, the apex of the ventricle being attached by a hook and thread to the long arm of a light lever. Ringer's fluid is led into the cannula from a reservoir (see Fig. 65) by means of rubber tubing, arranged so that the fluid drops slowly down the inside surface of the glass tube. The lateral limb serves as an overflow, so that the perfusion

pressure remains constant throughout: it also permits of the rapid washing-out of an experimental solution.

Another type of perfusion apparatus is shown in Fig. 65. In both types the heart is perfused with the Ringer solution contained in the reservoir R, which is provided with a Mariotte tube to keep the pressure constant. The solution escapes from the cut aorta and drops into a suitable receptacle (not shown in the diagram) placed below.

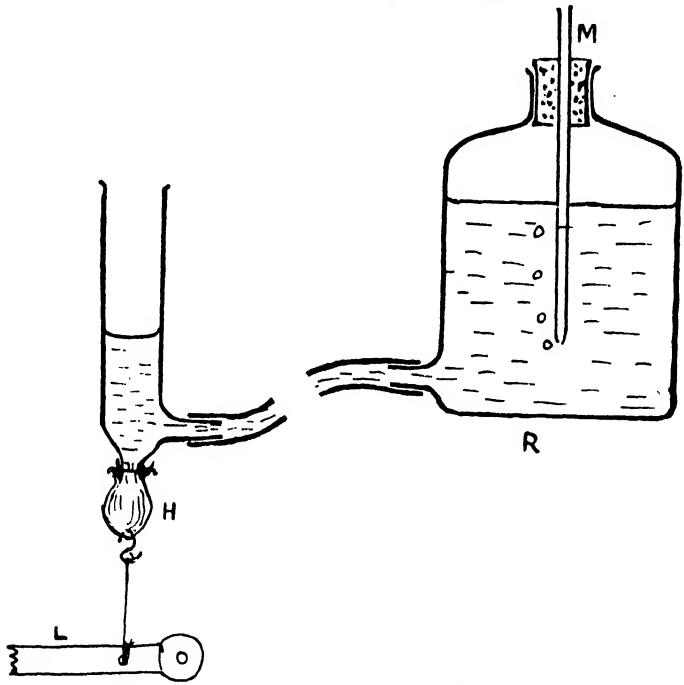

FIG. 65.—R, reservoir of Ringer's solution, connected with heart cannula by india-rubber tubing provided with a screw-clip (not shown in the diagram); M, Mariotte tube for maintaining a constant pressure; H, heart tied to cannula and connected by fine hook and thread to lever L.

For certain experiments (*e.g.*, Ringer's—see below) it is convenient to have two reservoirs connected by a T-piece to the inflow tube of the cannula. While perfusion is being carried out with the fluid from one reservoir, the tube from the second is kept closed by means of a bulldog clip. When perfusion is started from the second reservoir, the first can be replenished with a third fluid, and so on. When two reservoirs are not provided, drugs and other substances are applied to the heart by injecting them into the vertical tube of the cannula by a syringe or fine glass pipette.

Experiments on the perfused heart.

1. **Action of ions.**—(*a*) *Ringer's experiment.* For this experiment it is convenient to have the perfusion cannula connected with a double reservoir, but the observations can also be made by introducing the fluids directly to the vertical tube of the cannula by means of a 10 milliliter pipette, the inflow of normal Ringer being stopped meanwhile.

Flush the heart with a solution containing six parts of pure NaCl to 1,000 of distilled water: the organ will soon cease to contract and to respond to excitations. Now replace this solution with another of the same composition, but to which 1 milliliter of a 1 per cent. solution of calcium chloride has been added to each 100 milliliters, and flush the heart with the mixture. Contractions will be resumed, but each one will be too prolonged, and the heart will again stop: this time in systole. Next add 0·75 milliliter of a 1 per cent. solution of potassium chloride to 100 milliliters of the second solution, which contains the calcium, mix thoroughly, and flush the heart with the mixture. The beats will recommence, either spontaneously or in response to stimulation, their normal character being resumed. If the potassium salt is added in excess the heart will be arrested in diastole. Indicate in your notebook the significance of these observations.

(*b*) The effects of ions upon the frog-heart can also be demonstrated by adding to the normal Ringer in the cannula an excess of NaCl, $CaCl_2$, and KCl respectively.

(*c*) Determine the effects of changes in the pH of normal frog-Ringer upon the character and sequence of the beats.

2. **Action of drugs.**—Determine the effects upon the heart of adding to the fluid in the cannula a few drops of a solution of (*a*) adrenaline, 1 in 10,000,000; (*b*) (i) acetylcholine, 1 in 100,000,000, (ii) 1 in 10,000,000, and (iii) 1 in 1,000,000; (*c*) repeat (*b*) (i) or (*b*) (ii) a few minutes after addition to the fluid in the reservoir of sufficient eserine sulphate to give a final concentration of approximately 1 in 250,000; (*d*) repeat (*b*) (iii) after adding a few drops of 0·1 per cent. atropine sulphate solution to the cannula.

Experiments on the action of drugs can also be carried out using the "closed circuit" perfusion method described below.

"**Law of the heart.**"—A complete demonstration of the factors affecting the output of the heart is made on the heart-lung preparation of the dog (see Chapter XX.). But an effective demonstration of the "law of the heart" is afforded by the following very much simpler preparation of the frog's heart. The arrangement of the experiment is shown in Fig. 66.

The heart of a large frog is exposed. The two superior venæ cavæ

and the right branch of the aorta are tied off. Loose ligatures are placed round the left branch of the aorta and round the sinus venosus. A wide cannula (Fig. 66, A) filled with Clark-Ringer is inserted into the sinus venosus and tied in place. When the heart has been washed free from blood a snip is made in the left aorta and a small straight cannula B, attached by rubber drainage tubing to a piece of glass tube C, bent at one end, is inserted and tied in place. The two tubes, C and A, are secured together by a rubber band and the heart is freely dissected from the body, together with the lungs and part of the liver. The wide cannula is fixed to a stand by a spring clip (not shown in diagram) and the relative positions of the two cannulæ adjusted so that there is a free flow into and from the heart. The lungs are ligatured near their base. Superfluous tissues are carefully removed, so as to expose the heart as fully as possible. The following observations are then made.

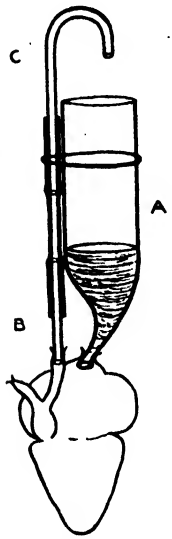

FIG. 66.—Arrangement of cannulæ for continuous perfusion of frog-heart. For description, see text.

Starting with a very small venous inflow, note the rate and output per beat of the heart; note also the relative systolic and diastolic volumes. Increase the rate of inflow by adding some fluid to the venous cannula with a pipette. Again note the rate, output per beat, diastolic volume, etc. Repeat with different inflows (*i.e.*, different heads of pressure in the venous cannula). How may the effects you observe be explained?

Instead of the fluid circulating continuously as in the above experiment, the inflow cannula can be fed from a Mariotte bottle and the outflow tube made to empty into a measuring cylinder. The inflow can be measured by counting the rate at which bubbles issue from the Mariotte tube the lower aperture of which should be very small.

Devise experiments, using this preparation, to demonstrate (1) the effects of changes in rate of the heart upon the output, and (2) the effect of altered resistance to outflow upon output.

Mode of action of cardiac nerves.—An experiment, on the perfused frog-heart, to demonstrate the mode of action of the cardiac vagus is described in the following chapter.

CHAPTER XVIII

CARDIAC NERVES OF FROG

DESTROY by a wire the spinal cord of a frog, and also remove the cerebral hemispheres; this can be done without special dissection by cutting away with strong scissors the upper jaw and anterior part of the skull at the level of the front of the tympana (see Fig. 91, p. 151). The posterior part of the brain with the medulla oblongata must not be injured. Arrange for tetanisation of the medulla oblongata—using the Helmholtz method (p. 19).

Lay the frog upon its back on the frog-cork, and fix it securely by strong pins; expose the heart and the chief nerves which are proceeding from the base of the skull to the hyoid region (vagus, glosso-pharyngeal, and hypoglossal (see Fig. 67). The vagus gives off a small branch on each side, which runs close along the superior vena cava to the sinus venosus. Place one vagus trunk upon a fine pair of wire electrodes (which must be fixed by plasticine to the frog-cork, not held in the hand).

Fix a pair of pin-electrodes into the cut end of the skull, so that the points lie between bone and tissue, on the surface of the medulla oblongata. Fix these electrodes to the board with plasticine. Try to avoid damage to the substance of the medulla. Connect both pairs of electrodes to a commutator without cross wires, so that the faradising shocks can be sent to one or other pair as may be desired. Attach the apex of the ventricle to the short arm of the lever in the usual way (Fig. 63). Record the contractions of the heart upon a slowly moving drum (one revolution per minute). Use the "stop" for adjusting the lever and for readjusting it after removal from the drum, so that the pressure of the lever point is always exactly the same. Without this precaution the strength of the contractions at different times may be wrongly judged.

It is important in the following experiments to have a time record, in 5 or 10 second intervals, inscribed on the drum. If electromagnetic markers are not available it is easy, either before beginning the experiment, or at the end, to fix the cardiograph lever so that it traces a straight abscissa; if the stand to which the lever is attached is now gently tapped at the required intervals, as timed by a watch, a time record will result. This time tracing is to serve for determining the exact rate of the heart-beat under the different circumstances of the experiment. Make the following sets of observations in the order given.

1. **Stimulation of vagus centre in medulla oblongata.**—Record a normal tracing of the heart-beat. While this record is proceeding, stimulate the medulla oblongata, allowing the result to be traced continuously. (The excitations must in no case be so strong as to escape to other parts of the preparation.) Mark on the drum with a needle the exact points between which stimulation was carried out.

Try to obtain graded responses by repeating the observations several times with different strengths of stimulus. Always allow the

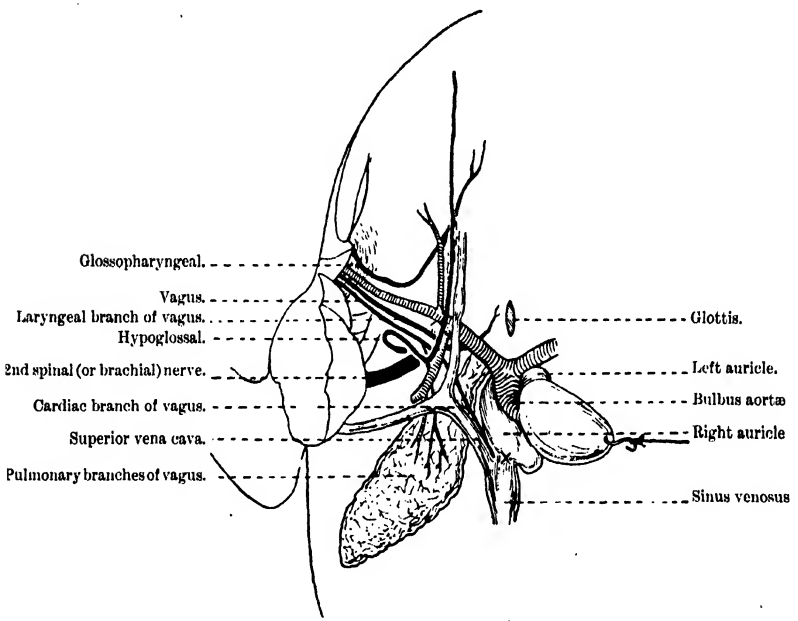

FIG. 67.—Relations of vagus nerve to other structures in the neck and thorax. The ventricle has been drawn over to the left side by a hook and the sinus venosus is thus exposed. ×, line of junction between sinus and auricles.

beat to return to normal before stimulating again. Note whether the heart you are using is pumping blood or is empty. Examine the records obtained by your colleagues and determine if the responses to vagus stimulation are affected in any way by the presence or absence of blood circulating through the heart.

Cut both vagus nerves a short distance from the skull and repeat stimulation of the medulla oblongata, recording the result at another level of the drum.

2. **Stimulation of vago-sympathetic trunk.**—Alter the commutator to stimulate the vagus nerve, recording the result.

With weak stimulation of the vagus in the frog the heart may beat faster and more strongly owing to excitation of the sympathetic fibres which have joined the vagus near the skull and are running with the cardiac branch to the heart; with stronger stimulation the heart will beat more slowly and less vigorously, or may stop altogether.

If you were unsuccessful in obtaining graded vagus effects from medullary stimulation, attempt to get graded effects from stimulation of the nerve.

3. **Action of nicotine.**—Take a record of the normal beat, and, with the drum still running, place one drop of a 1·0 per cent. solution of nicotine upon the sinus, recording the effect in a continuous tracing. The effect of this is at first to slow the heart, because the nerve-cells to which the vagus fibres are distributed are stimulated by the drug; subsequently they are paralysed, and the heart resumes its normal rate. After a short interval stimulate the vagus. No effect should be obtained, since nicotine blocks the junction of the preganglionic nerve-fibres with the distributing nerve-cells within the heart.

4. **Stimulation of sino-auricular junction.**—Stimulate the heart at the white line of the sino-auricular junction. (The electrodes must not be held in the hand, but must be fixed in position by plasticine.) The heart slows or comes to a standstill in diastole. Record this effect in a continuous tracing. Confirm, by stimulation of the vagus trunk, that the " paralysing " action of nicotine is still in evidence.

The effects of stimulation of the region of the sino-auricular junction are due to the postganglionic fibres of the vagus which are close to the surface at this place. The fact that effects are obtained after nicotinisation shows that though nicotine abolishes conduction between the preganglionic fibre and the ganglion cell, it does not prevent direct stimulation of this cell or its fibre. (Sometimes the heart rate increases when the sino-auricular junction is stimulated. If this happens, how do you account for it ?)

5. **Action of acetylcholine and atropine.**—Place a single drop of dilute solution of acetylcholine upon the sinus, recording the effect produced upon the rate and force of the heart. Now apply two or three drops of a solution of atropine sulphate (1 in 10,000) to the heart. After a few minutes once more apply acetylcholine to the heart: it will have no effect. Notice further that no inhibition can now be produced on stimulating either the vagus or the sino-auricular junction, *i.e.*, atropine prevents both vagal stimulation and vago-mimetic drugs—such as acetylcholine—from affecting the heart. There may, however, be *acceleration* on stimulating the vagus after atropinisation since the sympathetic fibres, the effects of whose activity are unaffected by atropine, pass to the heart in the cardiac branch of the vagus nerve.

Notice that in each case of acceleration or retardation of the

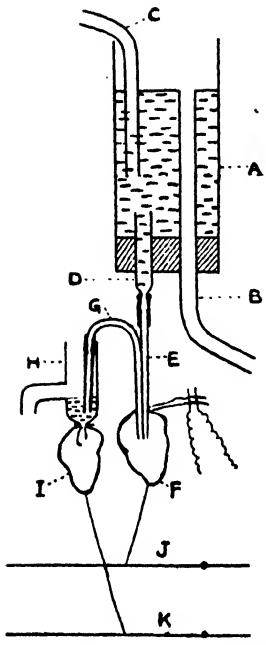

FIG. 68.—The perfusion apparatus A is provided with an overflow tube B, the height of which can be varied, thus altering the perfusion pressure. The apparatus is supplied with fluid through the tube C, which is connected with a reservoir of Clark's solution (not shown in diagram). The fluid from A passes through the tube D to the inflow limb E of the double cannula which supplies the donor heart F. After irrigating the inside of the donor heart, the fluid passes by the outflow limb G to the glass cannulated tube H, to which is attached the recipient heart I. This cannulated tube is provided with a lateral overflow, so that the hydrostatic pressure of the fluid supplied to the recipient heart remains constant. J and K are the levers to which the hearts F and I are respectively attached.

heart rate from stimulation there is an after-effect which at first continues the primary effect, but that this is often followed by a second after-effect of a nature contrary to the primary effect. How may these phenomena be accounted for ?

In analysing the results of the above experiments note the effects of the various procedures upon the conduction time between auricles and ventricles, in addition to the more obvious effects upon the rate and force of the cardiac contractions. The effect of vagus stimulation upon the excitability of cardiac muscle may also be investigated (see p. 69).

Mode of action of cardiac vagus (Demonstration). — That the cardiac vagus produces its effects upon the heart by the liberation of acetylcholine at the nerve endings, and the subsequent action of this substance upon the heart, is demonstrated by the following modification of Loewi's classical experiment. The liberation of the "transmitter" of the vagus effect is manifested by its humoral transference to another heart.

The arrangements of the experiment will be evident from Fig. 68. The receiver heart, preferably a small one, is first attached to its cannula and perfused from a reservoir as in the experiments described in the last chapter. The donor heart may be a simple preparation consisting of the isolated heart with its vago-sympathetic trunk on each side intact. A more satisfactory preparation consists of the heart, the medulla oblongata *in situ*, and the tissues surrounding the cardiac vagus nerves between their cranial exit and the heart. In this last preparation the

vagus is stimulated at its centre in the medulla, as in experiment on p. 96.

The superior venæ cavæ having been tied off—care being taken to avoid injuring the cardiac nerves, or including them in the ligatures—a fine double Kronecker cannula is introduced into the sinus venosus and secured when the point of the cannula has been pushed forward into the auricle. Or an incision may be made in the wall of the left auricle and the cannula inserted into this, a small incision having been made meantime in the interauricular septum. When the two hearts have been attached to their respective levers the flow from the donor heart is adjusted so that even when the donor heart is quiescent, as a result of vagus stimulation, fluid flows to the receiver heart in sufficient amount.

Observations.—1. When both hearts are recording regularly, stimulate the vagus centre of the donor for about a minute and observe the effects, if any, upon the receiver heart.

2. Repeat the observation a few minutes after the addition of eserine (1 in 200,000) to the perfusion fluid.

3. Repeat (2) after applying a few drops of 0·1 per cent. atropine sulphate solution to the fluid in the receiver cannula.

Success in this experiment depends on a high degree of sensitivity to acetylcholine in the receiver heart and to a diminished rate of hydrolysis of acetylcholine by both receiver and donor. If the receiver heart is perfused for an hour or so before making the observations its sensitivity will be increased, and the rate of hydrolysis of acetylcholine is diminished by using a perfusing fluid of pH 6 to 6·5. Choline esterase is specifically inhibited by eserine (physostigmine). Hence the potentiated effects in observation (2).

The liberation of acetylcholine by cardiac vagus stimulation may also be demonstrated by its action on the eserinised dorsal muscle of the leech (see p. 80).

CHAPTER XIX

OBSERVATION OF MAMMALIAN HEART *IN SITU*: PERFUSION OF MAMMALIAN HEART

Dissection.—The human heart or that of a sheep or dog should have been previously dissected to show its cavities and the blood-vessels connected with them, as well as the arrangement and action of the auriculo-ventricular and semilunar valves. The auriculo-ventricular bundle should also be observed. A dissection may be made in a rabbit or cat of the nerves accompanying the carotid artery (vagus, sympathetic, depressor, nerves of carotid sinus).

Observation of the heart of a mammal *in situ*.—An anæsthetised animal (cat) is decapitated by Sherrington's method, the carotids having been first ligatured and the vertebrals occluded by a wire passed immediately in front of the axis vertebra, drawn tightly and securely fastened behind. Another ligature includes all the remaining structures of the neck except the trachea. The head is cut off by an incision in front of these ligatures, passing between the occiput and atlas. Oozing of blood is stopped by application of dilute adrenaline and plasticine; the skin is fastened over the cut end of the neck. Before tying the carotids, a tube has been inserted into the trachea, and artificial respiration is kept up by pumping air into the lungs and allowing it to escape by a side tube. This air is warmed. The body is further kept warm after decapitation by placing it on a warmed plate and covering it with cotton-wool. In the decerebrated animal (see p. 155) the circulation is well maintained, but in the decapitated preparation the pressure is somewhat low, although the heart continues to beat and the tissues to live for several hours.

The heart is exposed by severing four or five ribs or rib-cartilages on each side with bone-forceps (a ligature having first been tied round the upper end of the sternum so as to occlude the internal mammary arteries), and with the same instrument the sternum is cut through near its lower end, and the detached part is forcibly raised, along with the cut ends of the ribs. It is not usually necessary to tie the severed intercostal arteries. The window thus opened discloses the heart within the pericardium; the latter may be cut open and the heart fully exposed. The systole, followed by diastole, of auricles and ventricles can be watched, and the hardening of the ventricles during their systole felt by applying the finger to their surface. By attaching one of the

ventricles and one of the auricles by fine hooks and threads to light levers (Fig. 69), the contraction of these parts can be recorded separately on a drum.

The effect of stimulating the vagus in the neck and the action of atropine in abolishing this effect can be demonstrated; also the effect of stimulating the accelerator fibres which pass from the stellate ganglion of the sympathetic to the cardiac plexuses. The same result is obtained by stimulating the ganglion itself; this may be found by following the cervical sympathetic downwards. In the cat the vagus

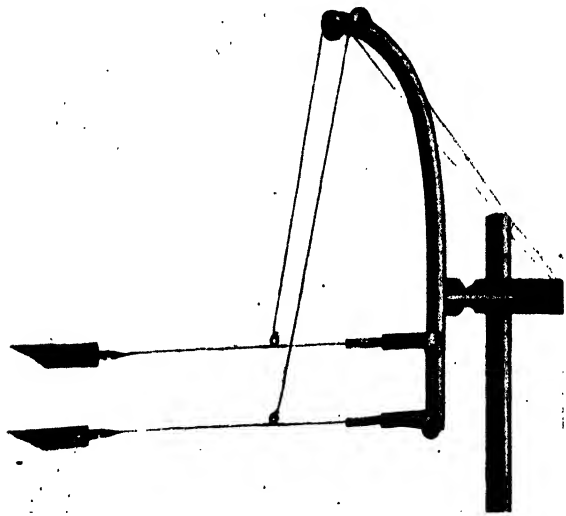

FIG. 69.—Cardiomyograph for obtaining simultaneous tracings from the auricle and ventricle of the exposed mammalian heart. The levers are formed of fine clock springs.

and sympathetic run in the same sheath in the neck, but they separate below and above. In the dog they are united in a common perineurium: in the rabbit they run separately from one another, and a third nerve —the depressor—accompanies them in their passage down the neck. (For a more detailed study of the cardiac nerves see p. 106.)

The effect of adrenaline on the heart can be shown in this preparation by injecting 1 milliliter of a solution of 1 in 100,000 into a vein; or even by merely dropping some of the solution on the base of the exposed heart. Similarly the action of acetylcholine can be shown. (See Chapter XXVI. for a more detailed study of the actions of drugs, etc.)

The effects upon the heart of increasing (by pressing gently on the abdomen) and decreasing (by partially occluding the inferior vena cava) the venous return can also readily be observed. (See also p. 104.)

Perfusion of the mammalian heart.—The heart is excised from a recently killed cat or rabbit, washed free from blood, and the aorta is at once tied on to a cannula through which Locke's solution (see Appendix), saturated with oxygen, is slowly dropping. The solution is warmed before reaching the heart to about 38° C., and oxygen is bubbled through it in a rapid stream. The heart should itself be enclosed in a warmed chamber or have warmed Locke solution dropping over it from a side-tube on the main supply. The cannula is directed towards the aortic valves, which are closed by the pressure (about 80 millimeters Hg.) of the perfusing fluid; this runs through the coronary vessels and escapes through the right auricle. The amount of fluid perfused can be measured by a tilter (see Fig. 83). The contractions are recorded by light levers, attached to the right auricle and right ventricle respectively, by threads which are passed round pulleys (Fig. 69).

1. *Effects of ion changes on mammalian heart.*—The effects of changes in the ratios of the different ions in the perfusing fluid can be investigated by having a second supply bottle, to contain the experimental solution, connected to the perfusing apparatus in such a way that it can be switched over to supply the heart at will. The effects of increasing and of decreasing the Ca, K, and H ion concentration of the perfusing fluid are to be investigated.

2. *Action of drugs and autacoids on mammalian heart.*—The action of drugs and autacoids is investigated by adding them to mammalian Ringer's solution (Locke's fluid). The best way to introduce a drug is to inject its solution with a hypodermic syringe into the rubber tubing which conducts the perfusion fluid to the heart. In this way the effects of adrenaline, acetylcholine, histamine, chloroform and ether upon the rate, amplitude, and character of the beat and upon the amount of fluid perfused can be investigated.

3. *Effect of temperature on pacemaker of mammalian heart.*—Carry a glass tube into the right auricle through the superior vena cava and allow it to project through the inferior vena cava. Arrange for water at any desired temperature to flow through this tube. By this means the temperature of the sino-auricular node will be affected. Investigate the effect of (*a*) warming, and (*b*) cooling the node.

4. *Effect of asphyxia on mammalian heart.*—Determine the effect of cutting off the oxygen supply to the perfusing fluid upon the rate, amplitude, and character of the beats.

The isolated auricle preparation.—The isolated auricles of a young rabbit, or the right auricle alone, suspended in a bath of Locke

solution at a suitable temperature, may be used instead of the perfused heart for some experiments. A bath similar to that used in the study of mammalian plain muscle is employed, but the lever should be a very light one—preferably of the isometric type. The fluid must be well oxygenated.

The effects of changes in ion ratios and in temperature, and the actions of drugs, can be shown in such a preparation.

CHAPTER XX

HEART-LUNG PREPARATION: CARDIAC NERVES OF MAMMAL: INTRACARDIAC PRESSURE CHANGES: FIBRILLATION

Output of the heart. Starling's heart-lung preparation.—A dog is anæsthetised, the chest opened and the heart exposed, artificial respiration being maintained. The left subclavian and brachiocephalic arteries are ligatured, also the azygos and right superior intercostal veins; and the phrenic nerves are cut. Cannulæ are tied into the brachiocephalic artery and the superior vena cava, and the descending aorta and inferior vena cava are tied off. A cannula for registration of venous pressure may be inserted in the inferior cava. The arterial and venous cannulæ (A.C. and V.C.) are then connected with the apparatus, as shown diagrammatically in Fig. 70. The blood with which the preparation is perfused is from another animal; to prevent clotting, heparin has been added to it. The course of the circulation is indicated by arrows. The blood is pumped by the left ventricle through a rubber tube to which is attached an air-chamber: the air in this represents by its resilience the elastic reaction of the arterial system. The arterial resistance is represented by R., which consists of a wide glass tube containing a rubber sleeve through which the blood flows. The resistance is increased by increasing the pressure round the sleeve by pumping air into the pressure bottle P.B. The venous filling of the heart is regulated by a screw clip on the tube leading from the venous reservoir V.R. to the cannula V.C. in the vena cava superior. The output of the heart is measured either by receiving the outflow into a graduated cylinder, or by an electrically recording tilter (not shown in the diagram).

Experiments

1. *Effect of venous inflow on output and rate of heart.*—Starting with a small venous inflow, determine the output per minute (minute-volume). Since output equals input this is the same as the venous inflow during the period. Increase the venous inflow up to, say, 2,000 milliliters per minute. The output is increased to the same extent without the rate of the heart being affected.

2. *Effect of arterial resistance on output and heart-rate.*—Have a venous inflow of moderate amount. Determine the output (minute-volume) when the arterial pressure is maintained at (*a*) 80 millimeters

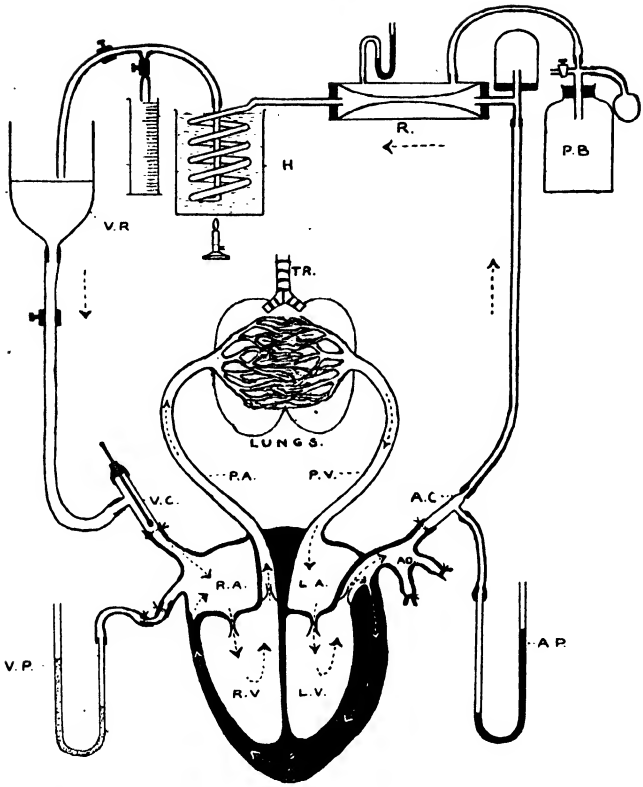

FIG. 70.—Diagram of heart-lung preparation. V.C., cannula in superior vena cava: it is provided with a thermometer and is connected to the venous reservoir V.R. by a tube bearing a screw clip, by adjustment of which the venous inflow to the heart may be varied. V.P., water manometer for recording of venous pressure, connected with cannula in inferior vena cava. R.A., right auricle. R.V., right ventricle. P.A., pulmonary artery. TR., trachea, into which is tied a cannula (not shown in diagram) connected with artificial respiration apparatus. P.V., pulmonary vein. L.A., left auricle. L.V., left ventricle. Ao., aorta. A.C., cannula in brachiocephalic artery, connected with mercury manometer A.P., for recording of arterial pressure. R., arterial resistance which can be altered by altering the air pressure in the space surrounding the sleeve through which the blood flows: the air pressure is increased by means of the pump attached to the pressure bottle P.B., decreased by allowing air to escape by the tap on side limb of pressure bottle outlet. Between R. and P.B. is the air cushion, which simulates the elastic reaction of the systemic arteries. H., heater. V.R., venous reservoir. Between the heater and the reservoir is a measuring cylinder. The output of the heart may be measured by opening the clip on the tube leading to the cylinder and closing that on the tube leading to the venous reservoir. The direction of the blood-flow in the system is indicated by arrows.

Hg.; (*b*) 100 millimeters Hg.; and (*c*) 120 millimeters Hg. The minute-volume remains the same under these different conditions, and the heart-rate, too, is unaffected.

3. Repeat the above observations, recording the volume of the beating heart with a cardiometer (see p. 107).

4. *Effect of temperature on output and heart-rate.*—Determine the minute-volume with moderate venous inflow and arterial resistance, at normal body temperature. Allow the temperature to fall several degrees. Note the effect on heart-rate and minute-volume. Note that although the heart-rate is decreased the minute-volume is unchanged —it has been maintained by an increased output per beat, resulting from the increased diastolic filling consequent upon the slowed rate of beat. Now raise the temperature to several degrees above normal. The heart-rate is increased but the minute-volume remains the same— the output per beat in this case being reduced owing to the lessened time available for diastolic filling consequent upon the increased rate.

Cardiac nerves of mammal.—A more complete study of the action of the heart nerves than is possible in the decapitate or decerebrate preparation can be made on an anæsthetised animal. The heart is exposed as before and attached to a cardiomyograph; for some observations it is an advantage to have the animal connected also with a string galvanometer. The arterial blood-pressure may be recorded. The vagi are exposed in the neck, and the right stellate ganglion with its cardiac fibres prepared for stimulation. The stellate ganglion is readily exposed from the front, and will be found, embedded in fat and in tough connective tissue, in the first intercostal space in line with the neck of the first rib.

1. **Accelerator nerves.**—*Effect on rate of beat and on conduction.* Stimulate the cardiac fibres passing from the stellate ganglion and note the effect on the heart-rate. There is usually a considerable latent period before the effect appears, and it may persist for a short time after cessation of the stimulation.

If the ECG is recorded, note the increased conduction rate resulting from sympathetic stimulation (reduced P-R interval).

2. **Vagus nerves.** (i) *Tonic action of cardiac vagus.*—Block both vagi by freezing—or by the passage of a constant current. The freezing may be effected by laying each nerve on a flat copper hook projecting from a thermos flask containing a freezing mixture. Note the effect on the heart-rate.

(ii) *Vagus standstill and vagus escape.*—Stimulate the peripheral end of the right vagus. Starting with a stimulus which brings the heart to a standstill, continue stimulation until " vagus escape " ensues.

(iii) *Effect of vagus on conduction and excitability.*—Use successively

weaker stimuli and observe the results. Look especially for (*a*) the lengthened A-V interval (P-R interval in the ECG) consequent upon the diminished conduction; (*b*) the appearance of varying degrees of heart-block (see p. 87) from the same cause; (*c*) the appearance of extra systoles as a result of the increased excitability during vagus stimulation.

(iv) *Reflex cardiac vagus effects.*—Remove the block from the left vagus. Stimulate the central end of the right vagus with different strengths of stimulus. In this case the effects are reflex through the opposite vagus.

(v) *Differences between action of right and left vagus.*—Now remove the block from the right vagus, block the left vagus and repeat observations (ii), (iii), and (iv) on this. Compare the results in the two sets of observations.

Cardiometry.—Place the heart in a cardiometer. This consists of a glass vessel of spherical shape, having a large aperture at one part and a side tube for connexion with a piston recorder. A rubber diaphragm is placed over the aperture and a hole made in this so that the heart may be passed into the interior of the sphere, the edge of the rubber making an airtight contact with the auriculo-ventricular groove; or the pericardium may be drawn over the open end and tied round. Record the volume changes of the heart. Now cut both vagi. Note the increased rate and the decreased diastolic volume and amplitude of the heart-beats. The minute-volume is maintained by the increased number of beats.

Stimulate the peripheral end of one vagus. Note the decreased rate, the increased diastolic volume, and increased amplitude of the beats. The heart being slowed, diastolic filling is increased, so that the minute-volume is maintained by the increased output per beat.

Intracardiac pressure changes.—A manometer of low inertia and high natural frequency such as the Wiggers optical manometer (Fig. 76) is used. A large dog is anæsthetised with chloralose, a glass or rubber catheter is passed down the right jugular vein into the right auricle and connected with the manometer by thick-walled rubber tubing, and the intra-auricular pressure changes during each cardiac cycle recorded.

The first wave is due to the auricular contraction, the second is caused by the closure of the mitral valve at the beginning of ventricular systole, while the third is due to the filling of the auricle. There may be a fourth wave in the opposite direction to the others, indicating the relaxed condition of the auricle when the auriculo-ventricular valves are open and blood is flowing into the ventricle.

To record the intra-aortic pressure changes the catheter is passed down the left carotid artery into the aorta, while to record intra-ventricular pressure changes it is passed through the wall of the ventricle, so that its open end lies in the ventricular cavity. Compare the pressure waves exhibited while the tube is in the aorta with those obtained when it is in the ventricle.

In the latter case note: (i) a small wave due to auricular systole; (ii) a sharp rise due to ventricular contraction, the aortic valves opening at the summit of this rise; (iii) a plateau where the pressure is maintained high; (iv) a rapid fall due to relaxation of the ventricle, the aortic valves having closed; (v) a stage of low pressure during ventricular diastole.

After examining the records, repeat the observations, listening to the heart sounds at the same time. If possible, obtain simultaneous records of the intracardiac pressure changes and the electrocardiogram.

Auricular and ventricular fibrillation.—Faradise one of the exposed auricles with a moderate strength of stimulus. Note the fibrillation which results and which ceases on, or soon after, removal of the stimulus. Now stimulate the ventricles. Fibrillation ensues and the *circulation is completely arrested.* Unlike the auricles, the ventricles do not usually recover spontaneously. If, however, some 10 per cent. KCl solution is injected into the ventricles they may come to a complete standstill in virtue of the action of the K ion. If the heart is now massaged, so as to drive out the potassium, the ventricles may resume their normal beat.

CHAPTER XXI

ACTION OF HEART IN MAN: HEART SOUNDS: ELECTROCARDIOGRAPH

OBSERVE the chest wall over the situation of the heart: notice and feel the impulse or apex beat, strongest at one spot; mark this with ink. Apply the ear directly or through a binaural stethoscope over this spot, and listen to the heart sounds. The first sound is most pronounced at this place (the mitral area).

Now listen over the second right costo-sternal articulation (aortic area). Note that the second sound is most pronounced here.

While listening to the sounds of the heart, feel the carotid pulse of the subject, and determine that the first sound is systolic —*i.e.*, is synchronous with the rise of pressure in the artery due to the contraction of the ventricle; the second sound which immediately follows being diastolic. The first sound is due to the vibrations set up by the sudden closure of the auriculo-ventricular valves, together with the murmur associated with the contraction of the ventricular muscle; the second is due to the vibrations of the aortic and pulmonary valves.

Other areas of cardiac auscultation are the pulmonary and tricuspid. Note the relations of the four areas to the cardiac valves, the positions of which are indicated in the chart (Fig. 71). Take every opportunity of listening to the sounds as obtained at the different points in different persons, both at rest and after exercise. Note the variations with the position of the subject.

Cardiographic tracing.—Apply the button of a cardiograph (Fig. 72) to the point where the impulse is most distinct, and take a tracing upon a moderately fast drum by the aid of a recording tambour.

An expiration should be made and the breath then held whilst the tracing is taken; this renders the apex beat more distinct and eliminates the movements caused by respiration. To obtain a good record a thin subject with a slow pulse should be selected, and he should be placed on a couch recumbent on his left side.

Rate of rhythm of the heart. Effect of position.—Count the rate of the heart-beat by placing the finger either over the apex beat or upon an artery (pulse). Do this with the subject (1) recumbent, (2) sitting up, (3) standing up. Note down any differences you may observe

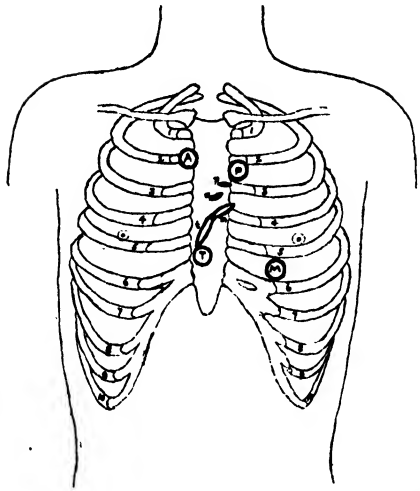

FIG. 71.—Diagram of human chest to show areas of auscultation and their relation to the cardiac valves. A, P, M, and T indicate the aortic, pulmonary, mitral, and tricuspid area of auscultation respectively, while *a*, *p*, *m*, and *t* indicate the anatomical position of the corresponding cardiac valves.

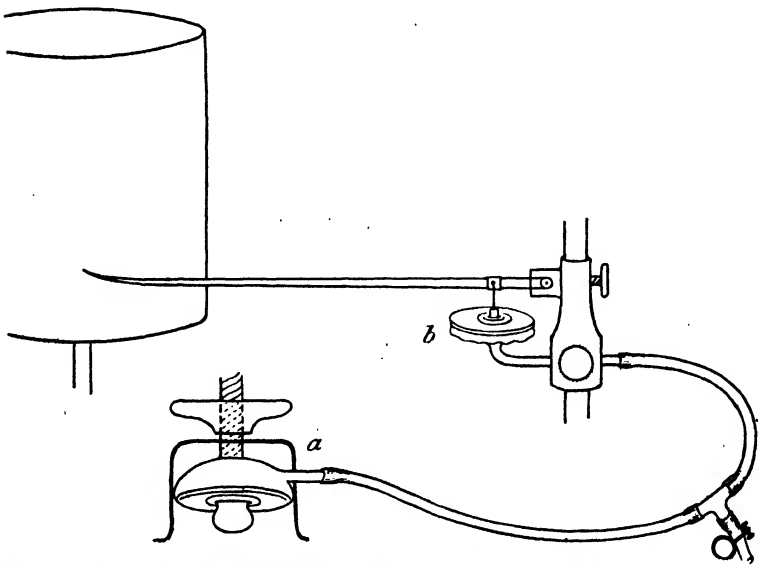

FIG. 72.—Diagram of Marey's cardiograph. *a*, receiving tambour for fixing over apex beat; *b*, recording tambour connected with *a* by rubber tubing, with a lateral opening closed by a clip.

in the rate and also in the character of the beat in these different postures.

Electrocardiogram of the human heart.—The principle of the string galvanometer has already been explained (p. 71).

In Fig. 73 is shown diagrammatically the usual type of circuit used in the electrocardiograph. The electrodes for connexion of the subject with the galvanometer consist of porous pots filled with NaCl solution.

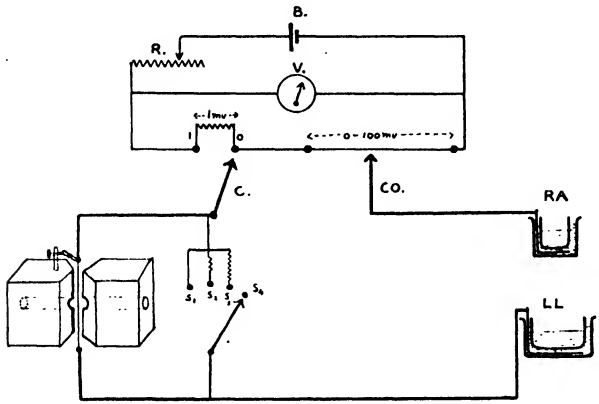

FIG. 73.—Diagram of electrocardiograph circuit. This consists of two parts, an external circuit containing a battery B, a voltmeter V, and a series of resistances; and the galvanometer circuit proper containing the electrodes RA, LL, the string galvanometer and shunts. The external circuit is for the purpose of calibrating the string and compensating for skin currents in the patient: it is connected with the galvanometer circuit by C and CO. The galvanometer circuit is completed by the patient, who places his right hand and left foot in the pot electrodes RA and LL respectively. The shunts S_1, S_2, and S_3 employed for the protection of the string during the preliminary adjustments allow $\frac{1}{1000}$, $\frac{1}{100}$, and $\frac{1}{10}$ of the current in the circuit to affect the string. The shunts are successively removed, drift of the string being compensated for by adjustment of the rider CO on the sliding resistance, and the string-tension so adjusted that, all the shunts being out, application of 1 millivolt by the movement of the switch C from the 0 to the 1 position causes the shadow of the string on the camera slit to move 1 centimeter.

These pots are immersed in larger pots containing $ZnSO_4$, and the wires to the galvanometer are connected with zinc plates immersed in the sulphate solution. An alternative form of electrode is afforded by a silver plate coated with silver chloride applied to the skin over a piece of lint soaked in normal saline.

The subject may be connected with the galvanometer in three different ways, and the resulting electrocardiogram is different in each

case. The two hands may be placed in the electrodes (lead I), or the right hand and the left foot (lead II), or the left hand and left foot (lead III).

The following is a brief résumé of the steps involved in obtaining an electrocardiogram: The subject having been connected with the instrument the light is turned on and casts a shadow of the string (magnified) on the camera scale. The compensating current is then switched on, also the current supplying the magnets. The subject is connected with the string through a series of shunts. These are removed one by one, and the shadow of the string brought to the centre of the camera slit by adjusting a sliding resistance, thus compensating for the patient's "skin currents" which are causing this drift. The tension of the string is now adjusted by means of a milled head on the string case until it gives a deflection of 1 centimeter when a millivolt is applied to it from the battery circuit. The time marker having been set in operation, the film or paper is set in motion, the slit opened, and the record obtained. This is developed and fixed in the usual way.

Make a drawing of the electrocardiogram provided. Note the lead employed, and mark the P, Q, R, S, and T waves. Compare the normal electrocardiograms from different subjects. Make a diagram showing the time relations between the electrocardiogram, the heart sounds, and the radial pulse.

CHAPTER XXII

METHODS OF INVESTIGATING PRESSURE CHANGES IN BLOOD-VESSELS OF ANIMALS

THE chief methods used can be practised upon a long india-rubber tube through which water is pumped from a reservoir by a rubber syringe actuated by an electro-motor. After passing through the system the fluid is again delivered through another rubber tube, controlled by a screw-clip, into the reservoir. A mercury kymograph (Fig. 74) and other manometers, such as Fick's or Hürthle's membrane-manometers (Fig. 75), or Wigger's optical membrane-manometer (Fig. 76) as well as Ludwig's stromuhr (Fig. 77), and other instruments for measuring or estimating velocity, may be connected by means of T-tubes with the main india-rubber tube, ordinary water manometers being attached to the latter at different points on both the "arterial" and "venous" sides. The capillary bed may be represented by rubber tubes interposed between the arterial and venous sides of the system and capable of being partially closed by screw-clips.

The use of each instrument is to be studied separately, the others being temporarily shut off by screw-clips.

Notice how the movement of any of the recording manometers may be damped by partially closing the tube connecting it with the main system. Observe the effect upon the pressure within the system (1) of increasing either the rate of the pump or the amount delivered at each stroke; (2) of diminishing or enlarging the outflow from the main tube by the screw-clip which controls it. This is equivalent to contraction or dilatation of the arterioles. Take a tracing with each form of manometer. Also record the amount of fluid passing through the stromuhr in one minute. Measure the diameter of the tubing in whose course the instrument is inserted, and calculate from these data the velocity of flow.

The velocity is found by dividing the amount flowing through the instrument in a second by the sectional area of the vessel $\left(V=\frac{v}{\pi r^2}\right)$.

In the recording of pressures such as those met with in veins or in the pulmonary artery, water manometers are employed, but for recording changes in mean systemic arterial pressure the mercury manometer, of Ludwig is used almost exclusively. The mercury

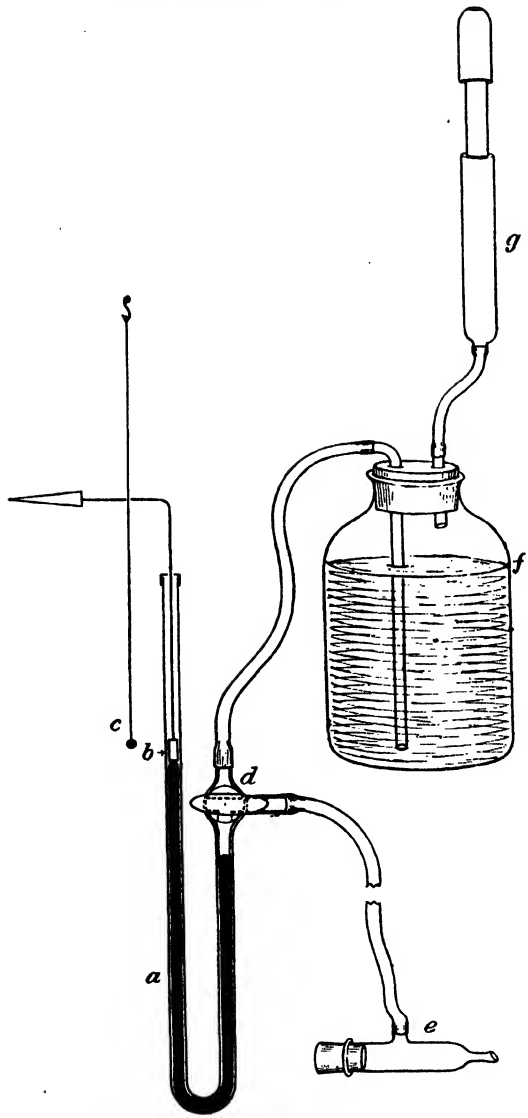

FIG. 74.—Diagram of mercury kymograph arranged for an experiment. *a*, bent glass tube containing mercury forming the manometer; *b*, aluminium float with rod, bent at right angles and ending in a writing point; *c*, small weight attached to silk thread suspended above writer and serving to keep it against the smoked paper; *d*, three-way stopcock; *e*, artery cannula; *f*, bottle of sodium sulphate solution; *g*, pump to raise the pressure: this may take the form of a rubber bag.

nanometer, while giving trustworthy indications of the average pressure in the arteries and of the slower fluctuations of pressure, is incapable by reason of its inertia of giving accurate records of rapid

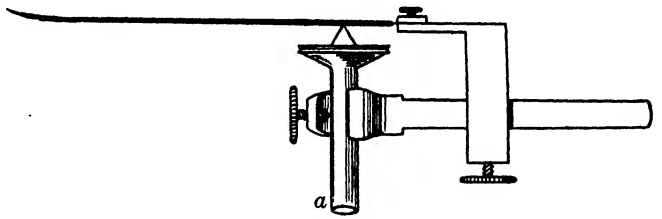

FIG. 75.—Diagram to show the principle of Fick's or Hürthle's membrane manometer. *a*, tube terminating above in a tambour-like enlargement covered by a stout rubber membrane upon which is a metal disc and a wedge actuating a very light lever. The tube is connected by rubber pressure tubing with a cannula like that shown in Fig. 71, and the whole is filled with sodium bicarbonate solution.

changes such as those occurring in the course of a cardiac cycle. For this purpose an instrument with low inertia and high natural frequency is required. For ordinary purposes the Fick or the Hürthle manometer may be used, but for more accurate work the optical

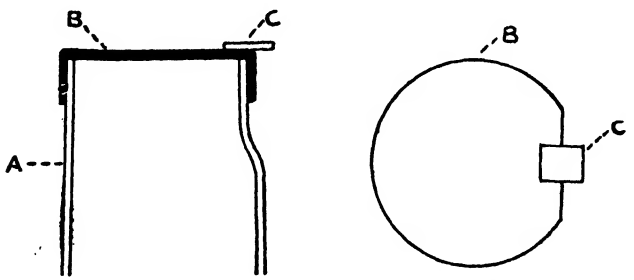

FIG. 76.—Diagram to show the principle of Wiggers' optical manometer. *a*, seen in vertical section. *b*, view from above. A metal tube A, flattened on one side, has covering its open end a tightly stretched rubber membrane B. In the middle of the flattened part of the metal tube is a light mirror C. The metal tube is connected with the arterial cannula by thick rubber tubing: the whole is filled with sodium bicarbonate solution.

manometer of Wiggers is employed. This, like the Fick or Hürthle, is a membrane manometer, but instead of the membrane operating a lever, which has mass and therefore inertia, a very small, light mirror is attached to the membrane, and a beam of light, reflected from this on to a suitable photographic recording apparatus, serves as a weightless

lever. The magnification obtainable with such an optical lever is almost unlimited, and therefore membranes of very high natural frequency can be used and accurate records of rapid pressure changes obtained. Such instruments must be calibrated by a mercury manometer.

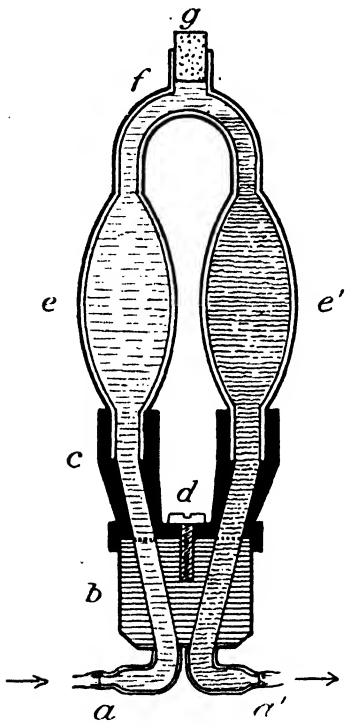

FIG. 77.—Ludwig's Stromuhr. *a*, *a'*, cannulas for tying into cut artery; *b*, block on which the part *c* rotates around the axis *d*; *e*, reservoir containing oil; *e'*, reservoir containing defibrinated blood; *f*, aperture for filling reservoirs, closed by cork *g*.

The systemic arterial pressure in animals.—In the anæsthetised or decerebrate animal, the femoral or carotid artery of one side is exposed. Two ligatures are placed around it, the most distal being tied. The artery is now clamped with a bull-dog-clip on the heart-side of the ligatures, a snip is made through its wall between the clamp and the distal ligature, a cannula (Fig. 74, *a*) is inserted into the artery through this snip, and tied securely in. If a mercury manometer is used the cannula is connected, by a lateral tube containing a saturated solution of sodium sulphate or sodium bicarbonate, with the proximal limb of the mercury manometer, as well as with a reservoir of the same solution, which is under pressure. By this pressure the mercury is forced up in the distal limb of the manometer, so that the recording style is about 50 millimeters above the abscissa or zero line. The communication with the reservoir is then closed, the clamp on the artery removed, and a record of the arterial pressure taken, a time-record (in minutes or fractions of a minute) being also inscribed. The cannula should at the time of insertion be filled with the sulphate or bicarbonate solution to prevent the blood clotting in its narrow part.

If a clot should form at any time during the experiment the artery is clipped and a quantity of the blood-pressure solution is allowed to flow through the cannula. If this fails to remove the clot, a fine feather, deprived of its barbs to near its extremity, is passed into the cannula and rotated; the washing-out process is then repeated. Sometimes

a clot may form inside the artery just below the nose of the cannula. This can usually be broken up by pressing on the artery between the forefinger and thumb. If the pressure in the cannula is then lowered to zero the fragments of clot will be forced into the cannula by the pressure of blood behind them, assisted if necessary by further manipulation of the artery. The cannula is then washed out as usual.

Trouble with clots will be greatly reduced if care is taken to avoid damage to the artery on inserting the cannula, and the cannula is well glazed at its point and is free from scratches or other roughnesses on its inside surface. The pressure bottle and the rubber tubing used for connexions should be scrupulously clean and the blood-pressure fluid (see Appendix) should be filtered before use.

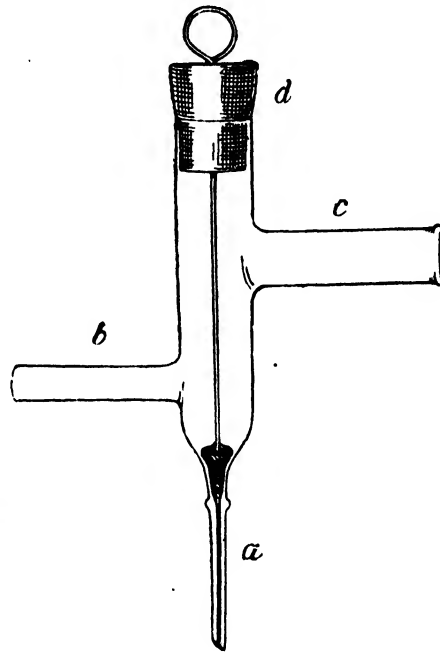

FIG. 78.—Cannula for insertion in the pulmonary artery through the wall of the right ventricle. *a*, open end of cannula with plug of Chatterton's cement attached to wire passing through cork *d*; *b*, lateral tube for connecting with recording apparatus; *c*, wash-out tube.

The pulmonary arterial pressure. — The blood-pressure in the pulmonary artery may be ascertained by using a specially constructed cannula (Fig. 78) which is passed into the commencement of the artery, through the wall of the right ventricle. The cannula, filled with half-saturated bicarbonate of soda solution, is tied in the wall of the ventricle by a purse-string suture and is furnished with a plug of Chatterton's cement to prevent escape of blood while the cannula is being inserted. Its construction will be understood from Fig. 78. It is connected with a vertical glass tube, with scale attached (Fig. 79), which serves as a manometer, giving the pulmonary pressure in centimeters and millimeters of the solution. The pressure is recorded upon the blackened paper of the kymograph by an Ellis piston-recorder connected with the open end of this manometer, and is written

down simultaneously with the pressure in the systemic artery recorded by the mercurial manometer.

As the thorax has to be opened to expose the heart and insert the

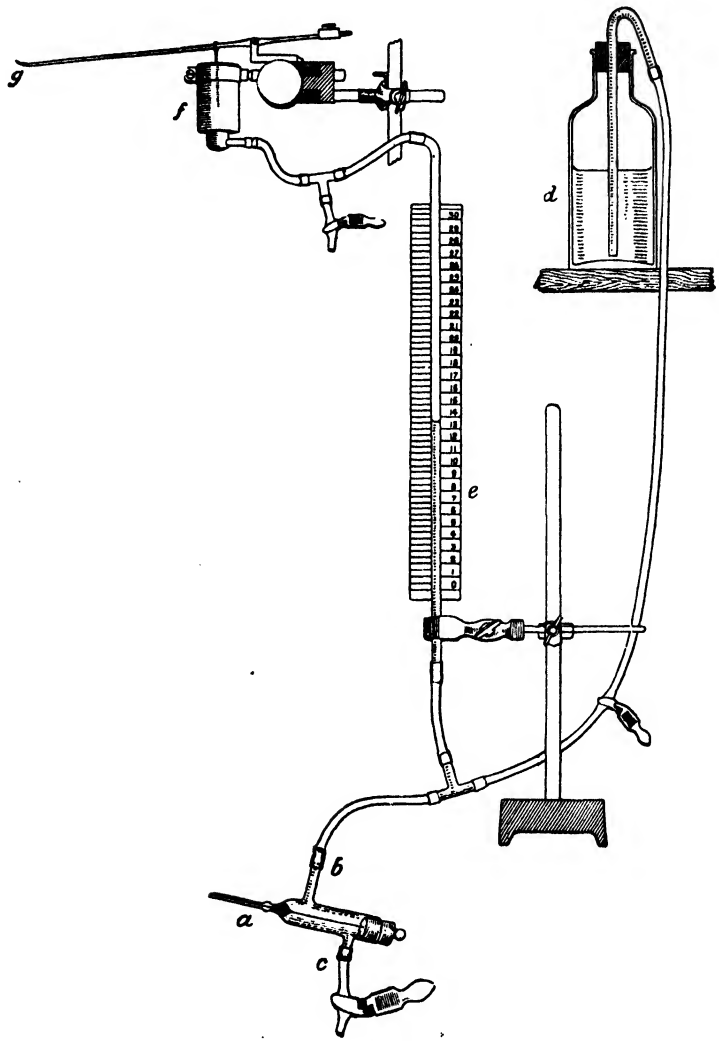

FIG. 79.—Diagram of method for recording the blood-pressure in the pulmonary system. *a*, *b*, *c*, as in Fig. 78; *d*, bottle of bicarbonate solution with loosely fitted cork; *e*, manometer tube with scale in centimeters and millimeters; *f*, piston-recorder; *g*, writing point.

pulmonary cannula, artificial respiration must be maintained throughout the operation (p. 100); but it is possible to sew up the thorax (with the lungs distended) and to continue the experiment with respiration under natural conditions.

The systemic venous pressure.—This is recorded by passing a special cannula with a long straight end (Fig. 80) into the vena cava via the

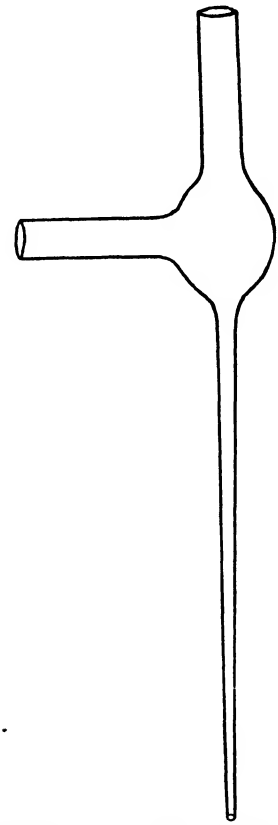

FIG. 80.—Diagram of cannula for recording of venous pressure $\times \frac{2}{3}$.

external jugular vein. The cannula is attached to a reservoir of half-saturated sodium sulphate or bicarbonate, and to a straight manometer, in the same way as the pulmonary arterial cannula (Fig. 79), but instead of the changes of pressure being transmitted to a piston recorder it is better to record directly from the manometer by means of a writing point attached to a capillary glass tube fixed to a glass or vulcanite float riding on the surface of the manometer fluid.

CHAPTER XXIII

PLETHYSMOGRAPHY AND PERFUSION OF BLOOD-VESSELS

Plethysmography. *Estimation of contraction or dilatation of vessels by measurement of volume changes in organ.*—The arm of a subject (who is to be seated comfortably) is placed in a Mosso plethysmograph

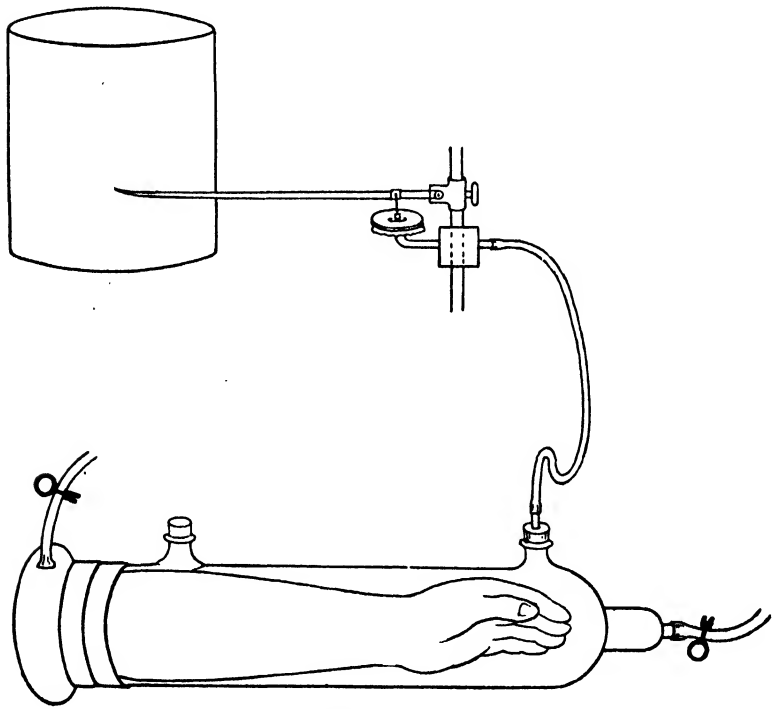

FIG. 81.—Mosso's arm-plethysmograph.

(Fig. 81), and this is allowed to rest on a table or in a sling. The junction with the arm is made by a broad rubber band or by a thin, hollow tube which is inflated. The interior of the plethysmograph is connected by rubber tubing with a recording tambour or piston-

recorder; the whole must be airtight. The lever of the recorder registers respiratory and cardiac movements upon the smoked surface, since these movements produce changes in general arterial pressure and thus in the amount of blood driven into the arm. Compress the brachial vein above the elbow; the swelling of the arm due to retention of blood is at once shown. The blood-flow through the limbs can be determined by means of the plethysmograph (see Lamb's "Human Physiology").

Plethysmographs for the kidney (Fig. 82), spleen, and other organs of animals are made of metal or vulcanite, on the same principle, but

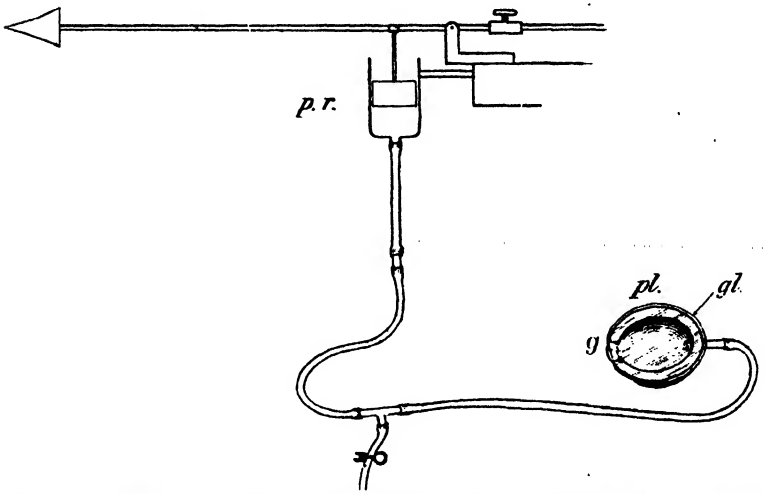

FIG. 82.—Diagram of kidney plethysmograph. *pl.*, plethysmograph; *gl.*, glass cover; *g*, gap for passage of blood-vessels and ureter; *p.r.*, piston recorder.

the form is adapted to each particular organ, and they are usually furnished with a glass cover. The cover as well as the gap (*g*) in the instrument through which the vessels and duct pass are made airtight by vaseline.

In Mosso's original instrument, and in Roy's oncometer, which works on a similar principle, the apparatus was filled with fluid (water, oil); but this is unnecessary, air-transmission to a piston-recorder or tambour gives as accurate results and is more convenient.

Perfusion of Vessels

Perfusion of frog blood-vessels. *Estimation of contraction or dilatation of vessels by measurement of perfusion rate.*—Tie a small glass

cannula into the aorta of a large frog killed by destruction of the *nervous system*; the cannula can either be passed directly into the cut aorta or more easily through an incision in the ventricle. In exposing the heart and aorta make as small an opening as possible. First remove a flap of skin, then cut through the upper part of the ensiform cartilage, and extend the incision on either side of the sternum: turn this up like a flap until the heart is sufficiently exposed.

The cannula must be filled with frog-Ringer, and connected through an india-rubber tube with a reservoir of the same fluid, which is allowed to drop slowly from it during the introduction; this is in order to exclude air bubbles.

Suspend the frog by a pin through the jaw, and fix the reservoir about 3 inches above the head so that the fluid flows into the vessels by gravitation. Make a cut into the sinus venosus to enable the fluid to flow freely out after it has traversed the blood-vessels of the body; the escaping fluid will drip from the toes, which should be tied together. A cut must be made through the skin of each leg to prevent accumulation of fluid in the lymph-spaces of the legs. Count the number of drops per minute, and repeat the counting twice; after the blood is completely washed out the flow should be fairly regular.

To test the effect of drugs or reagents upon the muscular tissue of the vessels the reagent is added in known quantity to the perfusing fluid or injected with a syringe and needle into the rubber tubing near the cannula. After addition of the drug, again count the number of drops per minute (three estimations), and thus determine whether the arterioles are becoming dilated or contracted as the effect of the reagent. This experiment may be tried with Ringer's solution containing acid (HCl, 1 in 5,000) and alkali (NaOH, 1 in 5,000), with a very dilute solution of adrenaline, with sodium nitrite, and with solutions of chloroform and ether in Ringer's solution. Normal Ringer's solution must invariably be substituted afterwards for that containing the drug, and a third determination made in the same way (average of three counts) to determine whether the effect obtained is reversible.

Perfusion of mammalian organs.—The same method is used for perfusion of the organs of mammals. The cannula is tied into the artery of the (excised) organ, which is placed in a jacketed funnel warmed to 38° C.: the perfused fluid, which must also be warmed before entering the organ, escapes by the vein and runs down the funnel into a measuring vessel. In this way perfusion can be conducted through the ear of the rabbit, the kidney of the dog or sheep, or through the vessels of a limb. In mammals it is usual to allow oxygen to bubble through the Ringer solution used for perfusion and to add gum arabic to the Ringer to prevent œdema (Bayliss). It is also

advantageous to render the pressure variable by intermittingly checking the inflow, thus imitating the arterial pulse.

The perfusion of mammalian organs is best effected by the Dale-Schuster pump. By its means both the stroke frequency and stroke output can be varied independently and accurately at will.

Methods of recording the outflow of fluid.—1. The sequence of drops of any fluid can be recorded by aid of an electric drop recorder connected with an electro-magnetic signal, which writes upon the smoked paper of a drum.

2. Another method of graphically registering the rate of flow, especially if the drops follow one another too fast to be recorded individually by a drop-recorder, is furnished by the "tilter" shown diagrammatically in Fig. 83. This is a small vulcanite or celluloid trough with open ends, with a septum across the middle; the trough is balanced on a vulcanite knife-edge. The drops are led over the middle, and, falling on the side of the septum which happens to be uppermost, they gradually fill that side of the trough. When full, it overbalances, and the trough tilts over to the other side, when the process is repeated. Each movement of the tilter is registered, either by an electrical or a pneumatic arrangement, upon the recording paper on which the time is also written. The capacity of the tilter being known, the amount of fluid flowing in a given time is ascertained. The record will continue automatically for long periods.

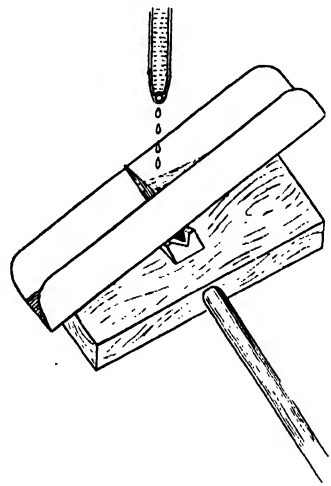

FIG. 83.—Diagram of tilter. The rocking movements are recorded either by allowing the accumulated fluid to actuate a tambour, or by an electro-magnetic signal.

3. A third method is to allow the fluid to flow into a vessel of known capacity which intermittingly and automatically empties itself by a siphon: each emptying being recorded graphically on the moving smoked paper.

The above methods may also be used to record the flow of secretions (Chapter XXVIII.).

CHAPTER XXIV

THE CAPILLARY CIRCULATION

The flow of blood in the smallest arteries and veins and in the capillaries is observed with the microscope in transparent parts of animals, such as the web, mesentery, and bladder of the frog, the tail of the tadpole, and the lung and tongue of the toad. Pithed frogs serve excellently for these experiments and should be pithed half to one hour before use.

1. **In the frog's web.**—The animal is laid on a flat cork with a slot at one end (Fig. 84, *a*): the margins of this are raised in the way shown in the figure, and the web of one foot is spread out over the slot with the aid of pins, but must not be stretched so tightly as to

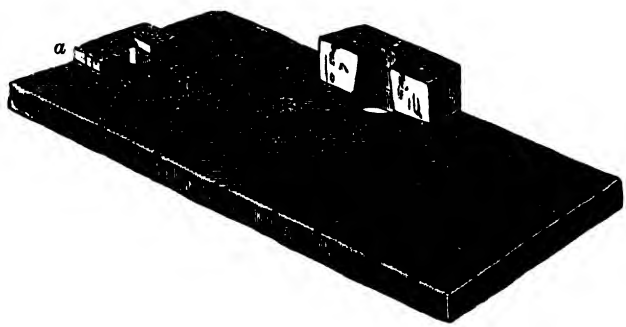

Fig. 84.—Frog-cork for displaying mesentery, lung, tongue, or web of foot.

obstruct the circulation. If a low power of the microscope is used the web need not be covered with a cover-glass.

2. **In the frog's mesentery.**—For this a hole is made at one side of the cork (Fig. 84, *b*) with its margins raised somewhat higher than for the web. The hole is covered by a circular disc of glass, not too thick, fixed to the cork with sealing-wax. A loop of intestine is drawn out through an aperture in the abdominal wall, and arranged round the glass disc in the manner shown in Fig. 85, with the mesentery resting on the disc. The mesentery must be kept wet with Ringer's solution.

3. **In the frog's bladder.**—The frog's bladder may be displayed in

the same way as the mesentery, care being taken to ensure that the circulation is not obstructed by pressure on the edge of the glass disc. It is usually necessary to slit the bladder open and pin the cut edges to the cork.

4. **In the tadpole's tail.**—To observe the flow of blood in this structure all that is necessary is to immobilise a tadpole with water shaken up with a little ether, and place it upon a glass slide in the same fluid. The slide should have a trough in its centre in which the body of the tadpole rests. The tail may then be made to lie flat on

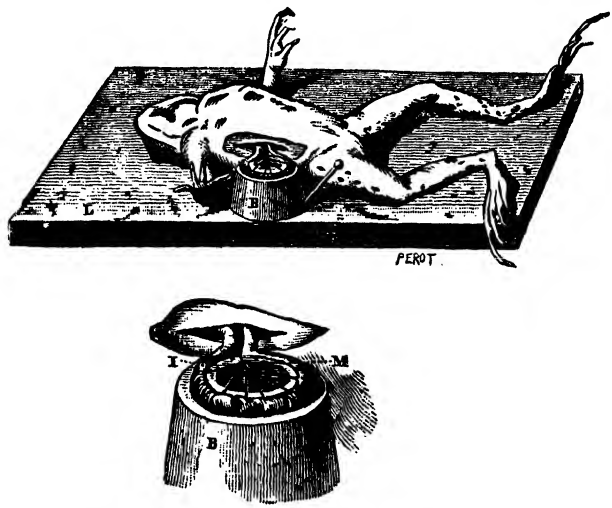

FIG. 85.—Method of displaying frog's mesentery for microscopic observation (Ranvier). L, glass or cork plate; B, cork, with cylindrical hole through it; I, intestine; M, mesentery.

the plane surface of the slide. The thin edges of the tail are observed with the microscope.

5. **In the toad's lung.**—This is prepared much in the same way as the frog's mesentery. The lung is allowed to protrude from an aperture in the side of the thorax and to rest on the mesentery disc. The toad is used in preference to the frog because its lung normally remains distended with air, and requires no special appliance for keeping it filled.

6. **In the frog's lung.**—If a frog's lung is cut open and laid out as directed for the mesentery or bladder, the pulmonary capillaries can be readily demonstrated. It is very instructive to compare the lung capillaries with those of the mesentery, web, or bladder.

7. **In the toad's tongue.**—This is on the whole the best object for

the study of the capillary circulation in cold-blooded animals: the frog's tongue is not quite as good.

The animal is immobilised as before, and is laid on the frog-cork on its back, with the end of the snout near the slot *a*. The lower jaw is then raised, and the tongue, which is naturally folded back in the mouth, is drawn forward, and is fastened, with as little injury as possible, by fine pins over the slot. There is a large lymph sac in the organ: this is to be distended by Ringer's solution with the aid of a hypodermic syringe. The mucous membrane which is now uppermost is of some thickness; it is slit up longitudinally with fine scissors, and pinned to either side, any escaped blood being got rid of by rinsing with Ringer. The delicate internal structures of the tongue are thereby exposed (muscle-fibres, connective tissue, and blood-vessels), and can be examined in detail with a high power of the microscope after covering with a light cover-glass.

8. **In the mesentery of small mammals such as rats and mice.**—The method is generally similar to that used for the frog-mesentery, but anæsthetisation must be produced by drugs such as urethrane or chloral hydrate administered either enterically or hypodermically, and special measures are necessary to keep the exposed mesentery warm and moist whilst under observation.

9. **In the human skin.**—By using a powerful illumination it is possible, with the aid of a good stereoscopic microscope, to observe the circulation in the capillary vessels of the skin of certain parts, *e.g.*, at the base of the finger nail.

Experiments on the minute vessels.—Observations on web, bladder, or mesentery.

1. **Identification of arterioles, capillaries, and venules.**—In a given field identify the different classes of vessels under the low power of the microscope by comparison of their relative sizes and the nature and direction of the flow of blood within them. Note the distortion of the red cells which may be associated with their passage along capillary vessels. Observe and note the characteristics of the leucocytes. Make a drawing of the vessels in a selected part of the field, viewed by a moderately high power.

2. **Effect of temperature changes on minute vessels.**—Cool the tissue you are examining by applying cold (5° C.) Ringer solution to it. Observe the effects and redraw the field you examined previously. Now warm the tissue with Ringer heated to 30° C. and again observe the effects and sketch the field.

3. **Effects of irritants.**—(*a*) Stroke the skin of the web gently with a sharp needle and note the reactions of the vessels.

(*b*) Choose a fresh field and, after observing it for some time, apply to it a small drop of croton oil. Note the reactions.

4. **Effects of hypo- and hypertonic solutions.**—For these observations the mesentery should be used. After observing the normal state of the vessels when the tissue is kept moist with Ringer solution, carefully remove excess Ringer with filter paper and place on the tissue a drop of distilled water. Note the reactions. Take a fresh field, examine it, and then add a drop of hypertonic salt solution.

5. **Effects of drugs.**—Examine part of the mesentery under low power: sketch the field and then add to it a drop of Ringer solution containing adrenaline 1 : 10,000. Observe, sketch, and comment on the changes in the vessels.

Take a fresh field and after examining it add a drop of Ringer containing histamine hydrochloride 1 : 10,000. Compare and contrast the effects with those of adrenaline.

6. **Effect of elasticity of arteries.**—While the web or mesentery is being examined, suddenly clamp the bulbus aortæ with a small bulldog clip. Note that though the cardiac output is thus suddenly checked, the flow of blood through the capillaries continues for some time. This is chiefly due to the elasticity of the arteries.

CHAPTER XXV

THE NERVOUS CONTROL OF THE CIRCULATION

A. **Vasomotor nerves.** (*a*) *Vasoconstrictor nerves and vasoconstrictor tone.*—A white rabbit is anæsthetised with urethane (see Appendix). The cervical sympathetic is isolated on one side and a thread placed loosely around it. The animal is placed so that a light may be shone through the ears showing up the blood-vessels. The ligature round the cervical sympathetic is now tied, thus severing the continuity of the nerve-fibres. The blood-vessels of the ear become dilated, giving the ear a bright red appearance, and making it much warmer to the touch than the other ear. Now stimulate the peripheral end of the ligatured nerve. Owing to the pronounced vasoconstriction the ear blanches and becomes colder to the touch than the normal ear: numerous vessels previously clearly visible disappear entirely. After removal of the stimulus the vessels revert to the condition of dilatation.

These observations demonstrate that the calibre of blood-vessels is under the control of nerves, and that under normal conditions the blood-vessels of the ear are maintained in a state of partial contraction or tone by impulses passing along the cervical sympathetic nerve. In this case the nerve is a *vasoconstrictor* and vasodilatation is brought about by a reduction or, as in this experiment, an abolition of vasoconstrictor tone.

> Before leaving this experiment compare the size of the pupils on the two sides. Note the effect of (1) section, and (2) stimulation of the cervical sympathetic upon the pupil.

(*b*) *Vasodilator nerves.*—A dog is anæsthetised with chloralose (see Appendix) and the lingual nerve on one side exposed. A cannula is inserted in the lingual vein of that side, the branch anastomosing with the vein of the opposite side having been tied off. The animal is given an injection of heparin to prevent blood-clotting. Any bleeding points are cauterised. Open the cannula in the lingual vein and allow the drops of blood issuing to operate a drop recorder or tilter (see p. 123). When the normal rate of flow has been determined, stimulate the peripheral end of the lingual nerve. A marked increase in the flow of blood will result, showing that the vessels have dilated. This demonstrates the action of a typical vasodilator nerve.

> The existence of vasoconstrictor fibres carried to the tongue vessels by the cervical sympathetic may also be demonstrated.

Stimulate the peripheral end of the vagosympathetic trunk. The flow will be reduced, indicating vasoconstriction.

Another method of performing the above experiment is to perfuse the organs *in situ* with warm oxygenated Locke solution to which gum arabic has been added. The tissues, however, do not maintain their excitability for long under these conditions. It is instructive to record the perfusion pressure by a manometer between the output of the Dale-Schuster pump and the lingual artery and to note the fall in pressure resulting from lingual stimulation and the rise with sympathetic stimulation, indicating decreased and increased resistance to the flow of fluid when the vessels are dilated and constricted respectively.

(*c*) *Mode of action of vasomotor nerves* (demonstration).—If in the perfused tongue the fluid issuing from the venous cannula is free from blood and samples are taken before and during stimulation of the lingual nerve and tested on the leech preparation (see p. 80), it is possible to demonstrate, even in an uneserinised animal, that the vasodilator nerves to the tongue vessels are cholinergic. In this instance the cholinergic fibres are atropine resistant, and while atropine will prevent the action of acetylcholine added to the perfusing fluid, it will not prevent vasodilatation from lingual nerve stimulation ("atropine paradox").

Under good conditions stimulation of the vagosympathetic trunk can be shown to confer on the perfusion fluid properties which render it excitatory to a frog's heart (arrangement of Fig. 66) or inhibitory to a piece of rabbit's intestine. The vasoconstrictor nerves are thus adrenergic: the substance they liberate is almost certainly adrenaline.

B. **Vasomotor and cardiac reflexes.**—The nervous regulation of the circulation is effected by two factors: (1) changes in the blood-vessels (vasomotor effects) and (2) changes in the heart (cardiac effects). The last have already been studied in detail (pp. 104, 106). The vasomotor reflexes are illustrated by the following experiments:—

A cat is anæsthetised with urethane, or a dog with chloralose, or, for some of the experiments, a Sherrington preparation may be used. The arterial pressure is recorded by a cannula in the femoral artery (see p. 116). A kidney, the spleen, a loop of intestine, or a limb is placed in a plethysmograph (see p. 121) and the volume changes recorded. The respiratory movements may also be recorded, by tambours or otherwise.

1. **Stimulation of cardio-accelerator nerves.**—Expose the stellate ganglion (see p. 106) and stimulate the cardiac branches. Note the effect on heart-rate and blood-pressure. The cardio-accelerator fibres are

affected reflexly in the intact animal in a manner which is described later.

2. ***Reflex effect of stimulation of a sensory nerve*** (pressor reflex).—Expose a limb-nerve, *e.g.*, the sciatic; free it for half an inch and tie a ligature tightly round it. This severs its fibres and may also serve to hold the nerve. Stimulate the end central to the ligature, thus exciting the afferent (sensory) fibres. Observe the reflex effects on arterial pressure, heart-rate, intestine (or other organ) volume, and respiration.

3. **Effects of splanchnic nerve stimulation.**—Expose the splanchnic nerve on one side.

(*a*) Stimulate its peripheral end and note the effects. Compare carefully with the previous record, noting especially the differences in the arterial pressure record and on spleen or intestine volume.

(*b*) Now ligature or clip the suprarenal vein on the same side so as to prevent adrenaline being passed into the circulation. Repeat the stimulation. Compare the records of (*a*) and (*b*) with one another.

4. **Effects of vagus stimulation** (cardio-aortic depressor reflex).—The vagi are exposed in the neck, and a ligature is tied tightly round the left vagus.

(*a*) Stimulate its peripheral end and note the effect upon the blood-pressure, etc. The effect is due to cardiac inhibition.

(*b*) Stimulate its central end and note the results. The effects in this case are reflex, and are due to (i) reflex cardiac inhibition, and (ii) reflex diminution of tone of the vasomotor centre, causing peripheral vasodilatation.

(*c*) That the results in (*b*) are due to two factors may be shown as follows. The left vagus being severed by the ligature, the right vagus is blocked by freezing (see p. 106), or by a galvanic current. Both vagi are now out of action. Note the effects on heart-rate and blood-pressure. (There is also a slowing of respiration, but this effect will be tested later.) Now repeat the stimulation of the central end of the left vagus. Compare the result with (*b*) above. There will be a fall of blood-pressure without cardiac inhibition, *i.e.*, reflex vasodilatation alone.

Atropine also dissociates the cardiac and vasomotor effects. It prevents the vagi from affecting the heart-beat but does not interfere with the vasomotor responses. To demonstrate its action remove the block from the right vagus. Repeat (*a*) and (*b*). Then inject into the saphenous or femoral vein one milliliter of a 0·1 per cent. solution of atropine sulphate in normal saline. Repeat (*a*) and (*b*). It will not be possible after administration of atropine to show any other results in which the cardiac vagus is involved.

The reflex studied above is one which operates in the intact animal in response to any sudden rise of blood-pressure. The afferent endings are situated mainly in the arch of the aorta.

The vagus also carries fibres which are stimulated by a fall of pressure in the aorta. Hence under some circumstances, especially in the dog, a rise of blood-pressure may be obtained by stimulating the central end of a cut vagus as in (*b*) above.

5. **Stimulation of carotid sinus** (carotid sinus pressor-depressor reflexes). (*a*) *Depressor reflex.*—A reflex similar to that studied above is also set in operation by a rise of pressure in the carotid arteries. The sensory receptors are situated in the carotid sinus.

Exert caudal traction on the right common carotid, or on both common carotids, or, alternatively, expose the carotid sinus on the right side and stimulate it electrically. Observe the effects on blood-pressure and heart-rate. Block the right vagus, the left being already tied, and repeat.

(*b*) *Pressor reflex.*—The opposite effect to (*a*) is produced when the pressure in the carotid arteries falls. Occlude both common carotids for 30-40 seconds by means of bulldog clips. Note the effects on heart-rate, blood-pressure, etc., and compare with the effects produced in experiments 2 and 3 above.

Perfusion of carotid sinus.—A large dog is anæsthetised with chloralose. One of the common carotids is exposed and cleared to beyond its bifurcation. The external and internal branches are ligatured as far from the bifurcation as possible. All the branches central to these ligatures are tied off, care being taken to avoid damaging the sinus nerve. The ligature on the lingual artery must be as distal as possible. To test whether all branches have been secured, raise the common carotid with one hand, grasp the artery just below the bifurcation between the thumb and forefinger of the other hand, and "milk" the blood backwards, thus emptying the artery. If blood flows into the emptied artery from the head end, the small branches responsible must be secured before going further. If the artery remains empty, ligature the common carotid, cut it open distal to the ligature, and tie a cannula in the free end. Tie another cannula in the lingual artery, proximal to its ligature. Attach the carotid cannula to the output of a Dale-Schuster pump and perfuse the isolated carotid sinus with Locke solution or, preferably, defibrinated blood. The perfusing fluid leaves the sinus by way of the lingual artery.

Arrange for the perfusion-pressure and the animal's femoral pressure to be recorded, also the volume of the spleen or of a loop of intestine or of a limb. It is advantageous to clip off the carotid artery which is not being used.

1. Starting with the perfusion-pressure and stroke-frequency of the

pump about the same as the animal's arterial pressure and heart-rate *respectively, first inscribe a normal record.*

2. Raise the perfusion-pressure and observe the results.

3. Bring the perfusion-pressure to normal and, when everything is functioning as in (1), lower the perfusion-pressure and again observe the results.

4. Dissociate the cardiac and vasomotor parts of the depressor response (as in 4 (*c*) on p. 130).

5. Finally, destroy the continuity of the fibres of the sinus-nerve by placing a ligature round the internal carotid artery, drawing it tight and releasing it. Note that the reflexes can no longer be elicited.

Afferent endings of nerves affecting the respiratory centre are also situated in the carotid sinus region. The effects of the above procedures upon the respiratory movements may be noted (see also p. 145).

CHAPTER XXVI

EFFECTS OF DRUGS AND AUTACOIDS AND OF HÆMORRHAGE UPON THE CIRCULATORY SYSTEM

Effects of autacoids and drugs upon the circulation.—A cat, anæsthetised with urethane or chloralose, is used for the following demonstration experiment. The systemic arterial pressure, the venous pressure, the volume of a limb, kidney, the spleen, or a loop of intestine, and the respiration are recorded. A small cannula which fits the nozzle of a hypodermic syringe is tied into one of the femoral veins, towards the thorax: this is for the purpose of giving intravenous injections. Administer the following substances, allowing conditions to return to normal between each successive injection.

(*a*) Adrenaline (10 γ in 1 milliliter saline, washed in with further 1 milliliter saline). Note the rapid rise of pressure, the diminution in organ-volume, and the cardiac inhibition (from the operation of the aortic and carotid sinus depressor reflexes, produced by the rise in blood-pressure).

(*b*) "Pituitrin" (0·1 milliliter—1 unit—in 1 milliliter saline, washed in with a further 1 milliliter saline). Compare the effects with those obtained by adrenaline.

(*c*) Acetylcholine (10 γ in 1 milliliter saline).

(*d*) Amyl nitrite (3 minims by inhalation through trachea tube).

(*e*) Histamine (10 γ in normal saline). Compare with acetylcholine. Note any differences in the effects produced on the heart and on the venous pressure. Acetylcholine dilates the arterioles, histamine constricts the arterioles but dilates the capillaries.

(*f*) Give a small dose of atropine (1·0 milligram) and repeat (*a*), (*b*), and (*c*), noting the differences.

Further observations on depressor drugs.—An anæsthetised or spinal cat is used for this experiment in which the effects of physostigmine and atropine on the actions of acetylcholine are contrasted with their effects on the action of histamine. The arrangements of the experiment are the same as in that just described. Inject successively, allowing conditions to return to normal between each injection, (*a*) acetylcholine, 5 γ; (*b*) histamine, 10 γ; (*c*) physostigmine (eserine), 0·25 milligram; (*d*) acetylcholine, 5 γ; (*e*) histamine, 10 γ; (*f*) atropine, 5·0 milligrams; (*g*) acetylcholine, 5 γ; (*h*) histamine, 10 γ; (*i*) acetylcholine, 1·0 milligram.

Physostigmine inhibits the choline esterase responsible for the rapid destruction of acetylcholine in the body. Thus after eserinisation, acetylcholine, whether produced by the activity of cholinergic nerves or introduced to the blood-stream from without, produces a greater effect by reason of its slower destruction. Atropine prevents the "muscarine" actions of acetylcholine upon tissues, and the actions of most postganglionic cholinergic nerve fibres: the "nicotine" actions of acetylcholine are, however, unaffected. Histamine acts independently of autonomic innervation and its actions are unaffected by choline esterase, and hence also by eserine. Similarly, actions of histamine are unaffected by atropine. Amyl nitrite, studied in the previous experiment, also acts independently of autonomic innervation and relaxes all plain muscle. Histamine contracts most of the plain muscle of the body, and its depressor effect upon blood-pressure is chiefly due to its dilator action on capillaries.

Further observations on pressor drugs.—A spinal cat is suitable for this experiment, in which the actions of nicotine, adrenaline, "pituitrin," and ergotoxine are studied. Part of a 2 per cent. solution of nicotine tartrate is diluted to give a 0·5 per cent. solution. Inject successively 0·1, 0·2, and 0·4 milliliters of the 0·5 per cent. solution, making each injection up to 1 milliliter with saline and washing in with a further 1 milliliter of saline. Then inject 0·5 milliliter, 1 milliliter and, if necessary, a further 1 milliliter of the 2 per cent. solution, *i.e.*, continue the injections until the dose has no effect on the blood-pressure. Note the effects of the early injections on blood-pressure, heart-rate, etc. Compare with observation (*i*) in previous experiment. How does nicotine cause a rise of blood-pressure and how do the effects become less with successive injections?

Now inject 10 γ adrenaline and, when the effect has passed off, inject 0·5 milligram ergotoxine ethanesulphonate (or ergotamine tartrate) and, later, a further milligram. When the blood-pressure has returned to a steady base line, again inject 10 γ adrenaline. Finally, administer 1 unit of "pituitrin."

Note that nicotine, which, as far as the autonomic nervous system is concerned, exerts both its "stimulating" and its "paralysing" action on autonomic ganglia (see p. 97), does not affect the actions of adrenaline. Adrenaline acts peripherally, on the tissues supplied by adrenergic fibres, and not on the ganglia. Nicotine, however, causes an outpouring of adrenaline from the suprarenal glands, in virtue of the fact that the medulla of the gland itself represents the ganglion cells and post-ganglionic fibres of the ordinary autonomic pathway. The secretory nerve supply to the suprarenals is thus unique in that it consists of preganglionic autonomic fibres. Since all preganglionic fibres appear to be cholinergic, the liberation of adrenaline in response to splanchnic

nerve activity is brought about by the liberation of acetylcholine at the nerve terminations within the gland. This is a "nicotine" action of acetylcholine and is therefore unaffected by atropine.

We have already seen (Chapter XV.) that ergotoxine and ergotamine first stimulate directly all tissues with a motor adrenergic innervation, and then, in sufficient dose, prevent the motor actions of adrenergic nerves and of adrenaline. Ergotoxine neither stimulates nor subsequently prevents the inhibitor actions of adrenergic nerves or of adrenaline. Hence such inhibitor actions are unmasked and alone become evident after ergotoxinisation. "Pituitrin" acts on plain muscle independently of its autonomic innervation, and hence its actions are unaffected by ergotoxine.

Effect of hæmorrhage, etc., upon the circulation.—Allow the animal to bleed from an artery until about 25 milliliters of blood is shed. Note the effects on blood-pressure, heart-rate, etc. Draw further quantities of blood, always providing that the total amount removed does not exceed about 20 milliliters per kilogram of the animal's body weight. Note the effects of the hæmorrhage.

Now inject at intervals into a vein 20 to 30 milliliters of normal saline. Note the effect upon blood-pressure and heart-rate.

Finally, give injections as above but of 6 per cent. gum arabic in normal saline (gum-saline solution) and compare the effects of this with the effects of the saline alone. How may the differences be accounted for?

CHAPTER XXVII

ARTERIAL AND VENOUS PULSE, AND ARTERIAL BLOOD-PRESSURE IN MAN

The pulse in the arteries.—Feel the pulse in the radial artery and determine and note (1) its rate and regularity, (2) its quality, whether hard or soft, bounding, readily compressible, etc. Examine a Dudgeon sphygmograph (Fig. 86); apply it over the radial artery. The position of the artery should first be marked, and the instrument placed on the wrist so that the button lies directly over this mark. Using the cam

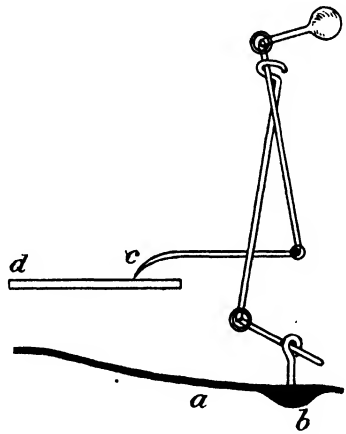

FIG. 86.—Diagram to show the lever-mechanism of the Dudgeon sphygmograph. *a*, spring; *b*, button to be applied to the radial artery; *c*, writing point of jointed lever attached to spring; *d*, glass plate or paper on which the tracing is recorded.

of the sphygmograph, exert such pressure upon the spring as will allow the variations in pressure within the artery to be most manifest. The tracings are taken on slips of glazed paper smoked over a candle. Note the effect upon the records of varying the tension of the spring. Write on each slip the name of the subject of the experiment and the pressure which was employed; varnish and preserve.

Make an enlarged drawing of the record, marking the different parts of the wave and appending notes regarding their significance.

The first part of the wave is called the percussion wave or anacrotic limb, and is due to the rise of pressure in the artery during the ejection phase of ventricular systole. The descending or katacrotic limb is interrupted by one or more waves. The first and principal of these is a depression, the dicrotic notch, due to the sudden closure of the aortic (semilunar) valves. Study the records obtained from different subjects and take the opportunity of palpating the pulse in as many different persons as possible.

Effect of muscular exertion.—Determine the effect upon the pulse-rate of different positions of the body, viz.: lying down, sitting, standing; and also the effect of muscular exertion, such as lifting heavy weights or ascending stairs. Find out how soon after such exercise the pulse-rate returns to normal.

The venous pulse.—In a recumbent subject hold a small receiving tambour without rubber membrane (a small thistle funnel will do) over the place in the lower part of the neck where the venous pulse in the jugular is most distinct. This receiving tambour, the edges of which can be made airtight by vaseline, is connected by rubber tubing to a recording tambour, and the curve is written on paper moved slowly by clockwork. A tracing of the carotid pulse can be obtained on the same paper, another tambour provided with a rubber cover and a button being fixed over the artery.

A convenient apparatus for taking such tracings is the *polygraph* of Mackenzie. In this instrument there is a continuous roll of white paper on which the tracings are recorded with ink.

Make enlarged drawings of the jugular and carotid pulse-tracings, and indicate in your notes the significance of the various waves of increased or diminished pressure shown upon them.

The jugular pulse-tracing has three—sometimes four—positive waves with three negative waves dividing them. The former are known as *a*, *c*, and *v*, while the latter are termed *x*, *x'*, and *y*. The *a* wave is synchronous with auricular systole; *c* is due to the rise of pressure transmitted from the carotid during ventricular systole; while *v* is due to accumulation of blood in the veins during contraction of the ventricle. The *a-c* interval represents the interval between contraction of auricles and ventricles respectively, and as a measure of auriculo-ventricular conduction-time is of importance clinically. The carotid pulse-tracing is simpler, consisting, like the radial pulse-tracing, of a rapid rise and a fall interrupted by the dicrotic notch. The interval between a notch on the rise (anacrotic) and the appearance of the dicrotic notch on the fall

represents the time between the opening and closing of the aortic (semilunar) valves, and thus represents the "ejection time" of ventricular systole.

Having now studied the heart sounds, the electrocardiogram, the arterial and venous pulses, and the intraventricular pressure changes, complete your drawings by marking upon them the time relations between the various events in the cardiac cycle. Especially mark the points where the auriculo-ventricular and aortic valves open and close.

Arterial pressure in man.—The pressure of the blood within the human arteries is determined by the *sphygmometer*, of which many forms are in use. All have a ring-shaped rubber bag (Fig. 87, *a*) which is enclosed by canvas or leather and is placed round the upper arm. The bag is distended with air by a pump, the amount of pressure used being recorded either by a mercury manometer (Fig. 87) or an aneroid. As the distension progresses the manometer shows not only the gradual increase of pressure but also oscillations due to the pulse. These oscillations increase in magnitude up to a certain point. The mean reading when the oscillations are greatest is the measure of *diastolic pressure*, as the artery will expand maximally when the pressure outside is just equal to the arterial pressure during diastole. On further raising the pressure the oscillations again become smaller, as the brachial artery is now becoming occluded. When it is completely occluded the pulse ceases to be felt at the wrist; this point is the measure of *systolic* or *maximal pressure*. It should be confirmed by very gradually diminishing the pressure and noting the point at which the radial pulse reappears.

It is not always easy to determine the exact point at which the pulse ceases to be felt, *i.e.*, the point at which the artery is occluded. The difficulty is lessened by listening with a stethoscope over the brachial artery at the elbow. As the pressure is raised over the upper arm the pulse beats become heard as distinct taps in the artery at the elbow, but when the pressure is sufficient to occlude the brachial the taps cease. On letting out air and thus diminishing pressure in the armlet the taps reappear. The point at which they again become distinct indicates the systolic pressure in the artery, and is always a little higher than that obtained by palpation of the radial. As the pressure is further lowered the sounds become louder and assume a blowing character; suddenly the loud blowing sounds become much fainter. This point gives the measure of diastolic pressure.

To recapitulate: *Diastolic pressure* may be determined by (1) inspection of oscillation in manometer (mean point where

oscillations are maximum); (2) auscultation of brachial artery (where "blowing" sounds becomé faint on deflation). *Systolic pressure* may be determined by (1) palpation of radial artery

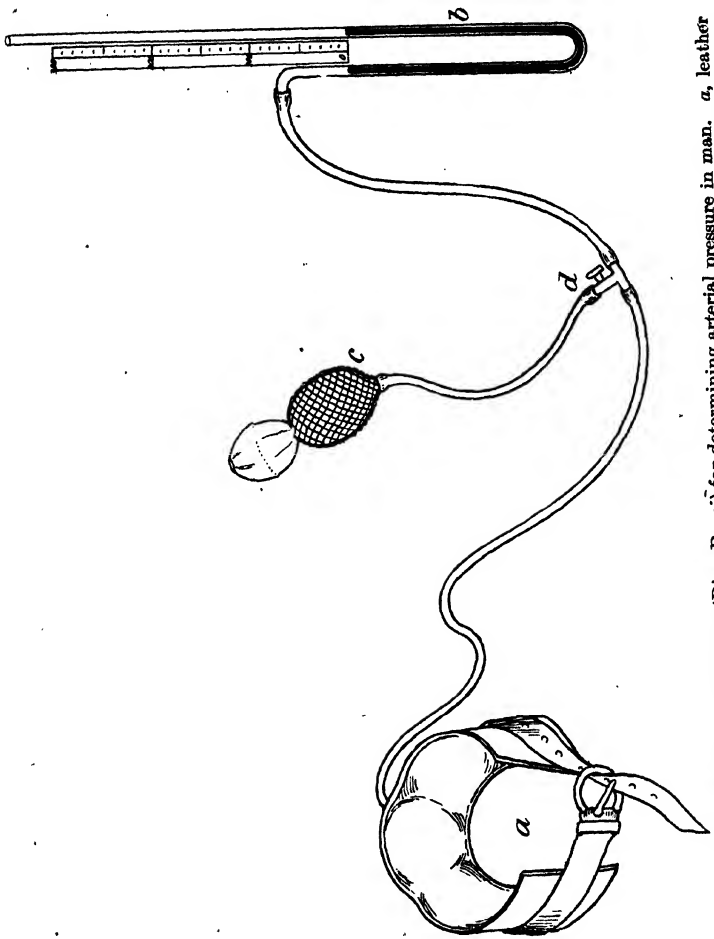

FIG. 87.—Diagram of apparatus (Riva-Rocci) for determining arterial pressure in man. *a*, leather arm-band with rubber bag as lining; *c*, pump for forcing air into the bag; *d*, valve for letting air out of the system; *b*, mercury manometer.

(mean of the readings (*a*) where the radial pulse disappears on inflation and (*b*) where it reappears on deflation); (2) auscultation of brachial artery (point where sound appears on deflation).

Determine the diastolic and systolic pressures by the above methods, noting the results.

Though determination of diastolic pressure by inspection of the manometric oscillations and of systolic pressure by palpation of the radial artery gives a clear idea of the principles involved in the measurement of blood-pressure in man, it is the auscultatory methods which are used almost exclusively in practice; these should therefore be mastered by the student.

CHAPTER XXVIII

SECRETION

THE process of secretion is studied in the salivary glands, the pancreas, the liver, and the kidneys. The influence of nerves upon secretion is illustrated by the salivary glands, that of drugs and autacoids by the pancreas and liver. The secretion of the kidneys is also dependent partly upon chemical agents, but largely upon the blood-pressure and blood-flow through the organ. The following experiments are suitable for demonstration.

The submaxillary gland.—A dog, having been anæsthetised with chloralosane, is fixed on its back and a vein-cannula inserted into the femoral vein. An incision is then made on one side through the skin and fascia below the mouth, extending from the chin backwards for 3 or 4 inches nearly parallel with the line of the lower jaw. At the posterior part of the wound the submaxillary gland may be observed. The anterior belly of the digastric, which comes into view and conceals the hilum of the gland, is drawn over laterally by a weighted hook: or it may be cut away. Any oozing from small vessels is controlled by rinsing the wound with dilute solution of adrenaline; larger vessels are tied. A muscle—the geniohyoid—is now exposed: it is composed of transverse fibres. When it is cautiously cut through, the duct of the submaxillary gland (Wharton's duct) is seen passing obliquely forwards towards the floor of the mouth. It is accompanied by a smaller duct, that of the sublingual. Crossing these ducts is a conspicuous nerve—the lingual branch of the 5th. If this is drawn towards the middle line with a blunt hook, it is seen that just before it crosses the ducts it gives off a small nerve—the chorda tympani—which curves sharply backwards and enters the hilum of the submaxillary gland, whence Wharton's duct is emerging. Tie a thread tightly round the lingual above the place where the chorda leaves it, and clear a short length of the chorda so that a small pair of flat electrodes can be placed underneath it. Do not injure the nerve. Stimulate by induction shocks: the duct will fill with saliva.

Place a wet thread round the duct and slip a pointed piece of paraffined paper under it. Make a snip into it with fine scissors; pass a "finder" into the aperture. Substitute for the "finder" a very fine metal or glass cannula, and tie this in: a piece of fine rubber tubing (drainage tubing) can be used to conduct the secretion beyond

the edge of the jaw, where it may be allowed to drop into a measuring vessel, or operate an electrical drop recorder.

On the same side make a longitudinal incision through the skin and fascia of the neck, and separate the muscles so as to expose the carotid artery and the common trunk of the vagus and sympathetic nerves. Ligature and cut this combined nerve low down, and place the upper cut end on a second pair of electrodes. The two pairs of electrodes—for chorda and sympathetic respectively—are connected to a commutator without cross wires, and this with the secondary coil, so that stimulation can be led into either pair at will.

1. Stimulate the sympathetic. A few drops of thick viscid saliva are secreted. (In the cat, stimulation of the sympathetic yields an abundant secretion.)

2. Stimulate the chorda. There is a rapid flow of watery saliva, lasting as long as the excitation is continued.

3. Inject 0·005 milligram (5 γ) acetylcholine intravenously. If no effect is obtained double the dose.

4. Inject slowly 0·5 milligram eserine sulphate.

5. After a few minutes repeat 2. The effects will be potentiated.

6. Repeat 3. Again the effects will be greater than before the administration of eserine.

7. Inject a small amount of pilocarpine nitrate (0·5 milligram). This produces an intense secretion.

8. Inject a small amount of atropine sulphate (1 milligram) into the vein. The flow produced by pilocarpine immediately stops.

9. Stimulate the chorda. The strongest stimulation produces no effect. The atropine has prevented the effect of chorda stimulation.

10. Again inject acetylcholine. No effect will be obtained.

11. Stimulate the sympathetic. Saliva will be secreted as before. The dose of atropine has been insufficient to abolish the action produced through the sympathetic.

The pancreas.—A dog is anæsthetised with chloralosane and a cannula tied into the femoral vein. The abdomen is opened by an incision in the linea alba. The duodenum is found and brought to the surface: the pancreas is seen in the mesentery within its curve. The main duct of the gland—canal of Wirsung—may be found without difficulty near the lower end of the part of the gland which is in contact with the duodenum.[1] Isolate a short length of the duct with forceps; pass a wet ligature round it and slip a pointed piece of paraffined paper under it. Make a snip into the duct with fine scissors, introduce a finder, substitute for the finder a fine metal or glass cannula, and tie this in. Attach a piece of fine rubber tubing to the cannula, bring

[1] In the cat the main duct enters the duodenum along with the common bile duct, from which it can easily be distinguished.

the end of this outside the wound, and let the drops of secretion fall into a measuring glass or on to an electrical drop recorder.

1. Inject into the femoral vein 5 milliliters of an extract of duodenal mucous membrane of any animal. The extract is made by boiling the chopped mucous membrane with 0·5 per cent. hydrochloric acid, cooling the decoction, neutralising with dilute alkali, and filtering. It contains a hormone (*secretin*), which has the effect of producing a rapid flow of pancreatic juice when injected into the circulation.

2. Inject pilocarpine nitrate and compare the effect with that of secretin.

> In the above experiment it is instructive to record pancreatic and bile secretion simultaneously, the latter by a cannula in the common bile duct, the cystic duct having been tied off. Intravenous injection of crude secretin will cause increased bile secretion because of the bile salts in the extract. Intravenous injection of bile salts alone (*e.g.*, sodium glycocholate) will increase bile secretion but will not affect pancreatic secretion.

The kidney.—In an anæsthetised rabbit or cat tie a cannula into the jugular vein and connect an artery with a manometer for registering blood-pressure. Make an incision through the skin and muscles on the left side of the abdomen near the back over the situation of the kidney, which is easily felt. After exposing the kidney, bring it towards the surface, partly clear it of fat, and allow it to lie in a suitable plethysmograph (Fig. 82), the margins of which have been vaselined, and place over it a glass cover also well vaselined: the cover is clipped down on to the plethysmograph. The blood-vessels and ureter pass out at the chink (*g*) left on one side of the plethysmograph: the chink is made airtight with vaseline. A tube leads from the plethysmograph to a piston recorder (*p.r.*) writing on smoked paper.

Make another incision in the lower part of the abdomen in the middle line; find the urinary bladder; hold it up with two pairs of clamp forceps; loop a ligature round it just beyond these; make an incision into it, and introduce the middle limb of a glass T-piece, which is then tied into the bladder. The horizontal limb of the T-piece is cannulated at one end; the other end has a short piece of rubber tube attached to it which is tied up or closed by a clip. To the cannulated end a longish piece of rubber drainage tubing is attached, the end of which must be below the level of the bladder so that this organ is constantly drained. Complete drainage of the bladder in this experiment is facilitated by having a piece of cotton wick threaded through the T-piece, so that part of the wick lies in the bladder, the other end

coming to the cannulated part of the T-piece. The drops can be registered by a drop-recorder (p. 123).

If the normal rate of flow of urine is low, slowly inject 20 milliliters of Locke solution intravenously, and when the flow is fairly constant proceed with the following observations :—

1. Lower the blood-pressure by stimulating the vagus (either central or peripheral end of cut nerve), or by injection of acetylcholine.

2. Raise the blood-pressure by stimulating an afferent nerve, or by occluding both carotids.

3. Inject 5 milliliters of Locke's solution slowly into the vein.

4. Repeat this experiment, using the same amount of Locke's solution, but with the addition of half a gram of urea.

5. Inject once more 5 milliliters of Locke's solution, but with the addition of half a gram of sodium sulphate.

6. Inject 2 units of pituitary (posterior lobe) extract into the vein.[1]

7. Inject 5 milliliters of Locke's solution containing 5 milligrams of caffeine citrate.

In each case note the effect on blood-pressure, kidney volume, and amount of urine secreted.

8. Inject atropine sulphate (1 milligram) similarly. This has no effect on the secretion of the kidney (compare its effect on salivary secretion).

[1] The effects on urinary secretion seen in this experiment illustrate the response of the *anæsthetised* animal to pituitary (posterior lobe) extract. In the unanæsthetised animal and in man, urine formation is *diminished* by pituitary extract.

CHAPTER XXIX

RESPIRATORY MOVEMENTS IN ANIMALS

In an anæsthetised animal (rabbit, cat, or dog) note the respiratory movements, paying particular attention to the abdominal and costal components respectively. Record the respiratory movements by means of a Marey tambour lightly strapped to the chest wall and communicating with another tambour the movements of which may be inscribed on the recording drum. If a rabbit is used the movements of a diaphragm slip may be recorded in addition (Head's method).

The control of the respiratory movements.—Expose the trachea by a mid-line incision in the neck. Perform tracheotomy and tie a Y-shaped tube into the trachea. Expose and clear both vagi for about 3 centimeters. Prepare the carotid sinus of one side so that it may be perfused at a later stage of the experiment. The following experiments are to be made, the respiratory movements being recorded :—

1. Allow the animal to breathe air containing excess CO_2.
2. Occlude both limbs of the trachea tube at the height of an inspiration ; or alternatively, inflate the lungs.
3. Occlude both limbs of the trachea tube at the end of an expiration ; or deflate the lungs.
4. Block (by freezing, see p. 106) or cut one vagus. This may have no effect on the respiratory movements.
5. Block or cut the second vagus.
6. Now repeat observations 1, 2, and 3 with the vagi cut.
7. Stimulate the central end of one vagus with (*a*) weak, and (*b*) strong stimuli. If the stimuli are suitably graded opposite results will be obtained.
8. If the vagi have been blocked and not cut, remove the blocks. The respirations will resume their original character.
9. Perfuse the carotid sinus of one side with well oxygenated defibrinated blood.
10. When the respirations are regular allow blood which has an increased CO_2 content to perfuse through the sinus.
11. Resume perfusion with the " normal " blood.
12. Denervate the carotid sinus (see p. 132) and repeat 10.
13. Kill the animal by occluding both limbs of the trachea tube. Record the respirations and blood-pressure in the different stages of asphyxia.

The above experiments illustrate the dual control of the respiratory movements—the nervous control by the vagi from afferent endings in the lungs themselves, stimulated mechanically, and the humoral control by the CO_2 in the blood chemically stimulating special sensory receptors in the region of the carotid sinuses. As the blood is also circulating in the respiratory centre in the medulla oblongata, this will also be influenced directly by chemical changes in the blood, as can be shown by perfusion of the head or, more simply, by making observation 1 (above) before and after denervation of both carotid sinuses.

CHAPTER XXX

RESPIRATION IN MAN

The respiratory movements in man.—Examine the bared chest during quiet respiration, and notice the parts in which movement is most evident; the same with deep respiration. Observe the alteration in obliquity and other changes in position of the ribs, rib-cartilages, sternum, and epigastrium. Apply the ear directly or through a stethoscope to the chest wall and listen to the vesicular murmur; listen also over the larger bronchi and trachea. Count the rate of respiration and compare it with that of the pulse of the same individual.

Measurements of the chest and abdomen in deepest inspiration and in deepest expiration.—Determine these upon yourself (*a*) at the level of the armpits, (*b*) at the level of the lower end of the sternum, (*c*) at the level of the umbilicus, using a tape measure. Note down the results.

Record of respiratory movements.—For the following experiments the slowest rate of drum is to be used, and the subject must not be allowed to see the tracing which is being taken.

1. **Normal respiration.**—Apply a stethograph (Marey's or Sanderson's) (Fig. 88) to the chest, and register the movements of respiration by means of a recording tambour. The subject's attention should be diverted from the experiment by his being engaged in reading a book.

2. **Effect of swallowing on respiration.**—Allow the subject to swallow (*a*) water, (*b*) solid food. Note the effects.

3. **Effect of voluntary forced breathing** (leading to washing out of CO_2 from the alveoli). Remove the lever of the recording tambour from contact with the drum. Cause the subject to take a number of deep respirations at a rate of about 20 per minute, rapid and vigorous movement being avoided. Then let him cease these voluntary efforts. Take a record of the breathing which succeeds them. There will probably be a pause (apnœa), followed by respirations which are at first shallow but gradually become of the ordinary character. Note the appearance of the subject and ascertain his subjective symptoms following the forced breathing. Compare the effects of forced breathing in different subjects, some of whom may exhibit Cheyne-Stokes respiration. If forced breathing has been continued for two or three minutes the succeeding apnœa may produce signs of oxygen lack (anoxæmia).

4. **Effect of voluntary abstention from breathing** (leading to CO_2 increase and O_2 lack).—Next, let the subject abstain from breathing

(after an ordinary expiration) for the space of half a minute, and record the respiratory movements which succeed the abstention (dyspnœa).

5. **Effect of increased CO_2 in inspired air.**—Lastly, allow the subject to respire a mixture of air and CO_2 (10 per cent.) contained in an oil-silk bag or a gasometer, and again record the movements of the chest wall.

Measurement of tidal air passing into and out of lungs.—Using either an airtight mask or a mouth-tube provided with valves (in this case the nostrils must be closed by a clip) allow the subject to breathe during one minute into a carefully balanced spirometer (see

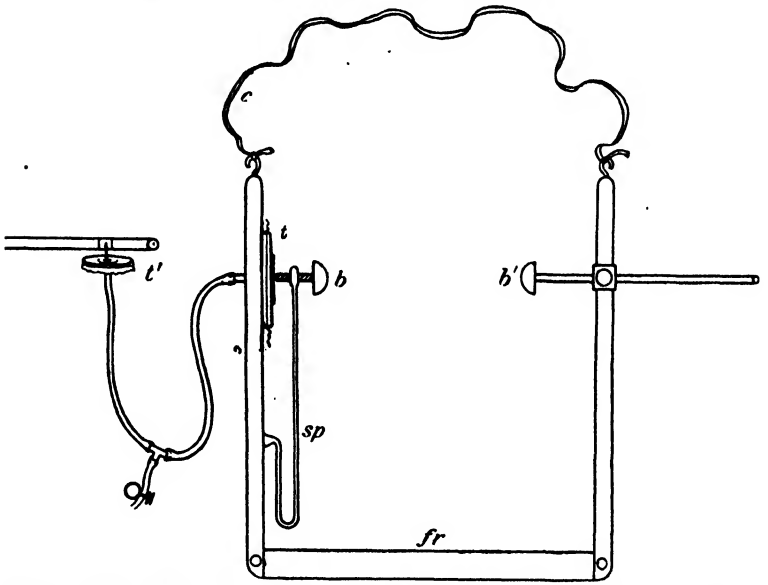

FIG. 88.—Sanderson's stethograph. *fr*, frame suspended over the shoulder by cord *c*; *b*, *b'*, buttons applied to opposite sides of the chest wall; *sp*, steel spring; *t*, receiving tambour; *t'*, recording tambour.

diagram, Fig. 89). Count the number of respirations in a given time, and note the amount of air which has been breathed in that time. From these results calculate the tidal air passing through the lungs with each respiration. The observation should not be begun until the subject is breathing regularly and unconsciously, and he must not be permitted to see the movements of the spirometer.

Reserve air, supplemental air, vital capacity.—Determine in your own person with the spirometer the amounts of each of these, and note down the results.

Reserve or complemental air is the volume which can be inspired after an ordinary inspiration; supplemental, that which

can be expired after an ordinary expiration; while the vital capacity is the total volume which can be expired after a maximum inspiration, and is thus the sum of reserve, supplemental and tidal air.

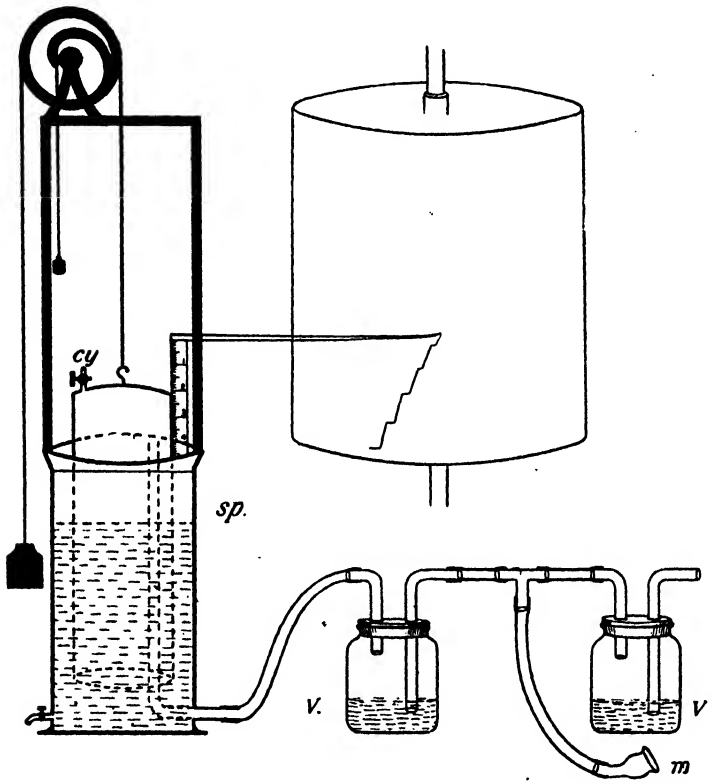

FIG. 89.—Spirometer arranged to register, upon a slowly revolving drum, the amount of air respired. *sp*, body of spirometer; *cy*, measuring cylinder with scale and writer attached; *v*, *v'*, water valves; *m*, mouthpiece.

Artificial respiration in man.—Place the subject flat on the ground in the prone position with the head on one side. Kneel or squat by the side of or across the lower part of the body, facing the head, and place your hands flat on the loins with the thumbs nearly touching at the spine (Fig. 90). Throw the weight of your body forward on the hands, keeping your arms straight (*A*), and count slowly *one*, *two*, *three*, *four*, *five*. Whilst counting *four*, *five*, swing backwards (*B*) so as to take the weight off your hands. Then swing forwards again,

counting, as before, *one, two, three*; and backwards, counting *four, five*; and so on twelve to fifteen times a minute. The effect of the pressure is to force the abdomen and lower part of the chest against the ground so that the viscera are pressed against the diaphragm. In this way air is driven out of the lungs. On relaxing the pressure the parts resume their former position; the diaphragm descends and air is drawn into the lungs.

The amount of air thus pumped through the lungs in a minute may

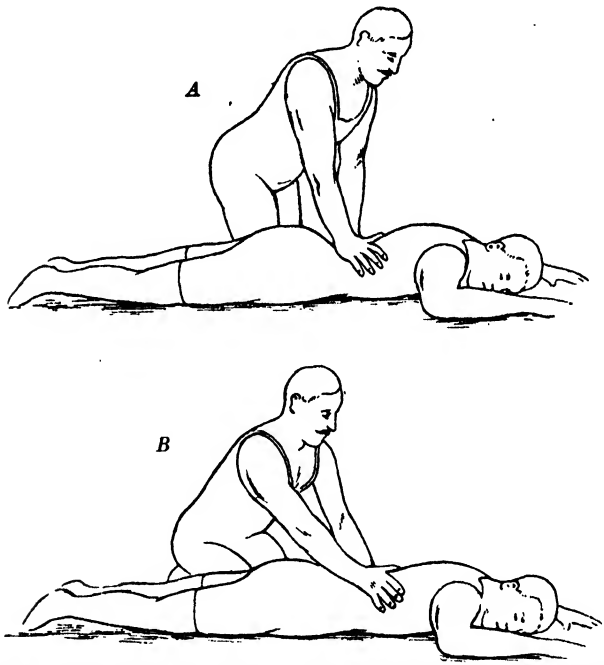

FIG. 90.—Prone-pressure method of artificial respiration. *A*, pressure being applied; *B*, pressure removed.

be measured by the spirometer in the same way as the tidal air measured in natural respiration.

Pressure within thorax.—Introduce through an intercostal space into the pleural cavity in the human cadaver or in any dead animal a sharp-pointed cannula or a trochar connected by rubber tubing to a water manometer. Notice that as soon as the trochar communicates with the pleural space the water in the distal part of the manometer sinks and registers a certain amount of pressure within the thorax (Donders' experiment). This pressure is sub-atmospheric and is often incorrectly referred to as "negative pressure."

CHAPTER XXXI

REFLEX ACTION IN THE FROG: LAW OF SPINAL ROOTS

A FROG, the brain of which has been removed,[1] is used for the following experiments. Note the position of the animal when placed on the table, and the absence of spontaneous movements. Suspend the preparation by the lower jaw (Fig. 92). Have ready a large jar or beaker of water (*a*),

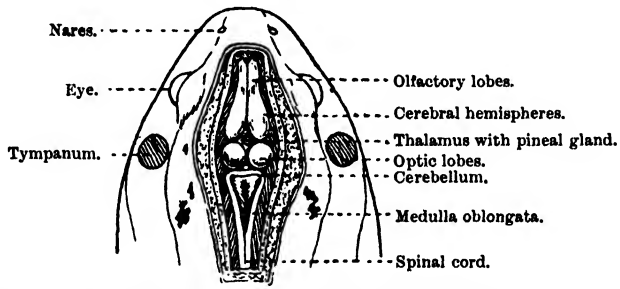

FIG. 91.—Brain of frog *in situ*, exposed by removing the roof of the cranium.

a small beaker of 2 per 1,000 sulphuric acid (*b*), and some small pieces—about 4 millimeters square—of filter paper, moistened with 5 per cent. acetic acid. A watch, with seconds hand, or a metronome is also required.

[1] For some of the experiments on reflex action the whole contents of the skull are destroyed. This can be done without hæmorrhage by inserting a sharp-pointed plug of wood through the occipital foramen. An animal with the whole brain destroyed and only the spinal cord left is known as a "spinal" animal. For other experiments only the cerebrum or brain proper is destroyed, the optic lobes and medulla oblongata being left. This is effected either by crushing the anterior part of the skull with Spencer Wells forceps; or by opening the skull and removing the hemispheres in an anæsthetised animal; or by cutting away all the part of the skull in front of the tympanic membranes with a razor or strong scissors. Such an animal is termed "decerebrate."

The reactions of the spinal and decerebrate frog may be compared. It is best to perform the operation of destroying the brain or removing the cerebral hemispheres some hours before the experiments on reflex action are to be tried in order to eliminate the effects of the immediate shock of the operation.

1. **Effect of strength of stimulus.**—Gently pinch the toe of one foot with the fingers or with forceps; the leg is drawn up. When again quiescent, pinch the toe more firmly; not only the one pinched, but both legs are drawn up, and there may also be a movement of the upper limbs (*irradiation of reflexes*).

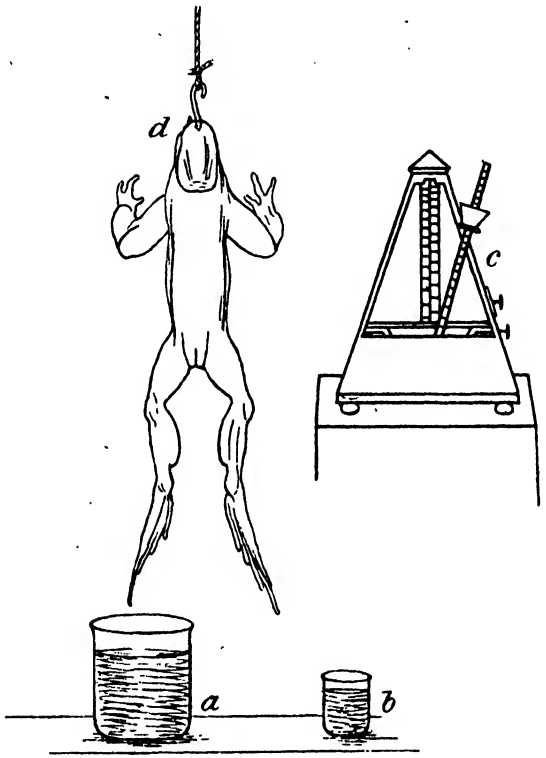

FIG. 92.—Türck's method of determining time of reflex to acid-stimulus applied to toes of decapitated frog. *a*, beaker of water; *b*, beaker of dilute acid; *c*, metronome; *d*, hook suspending frog by lower jaw.

2. **Effect of summation of stimuli.**—Stimulate the toe (*a*) with single and (*b*) with a succession of interrupted induced shocks. Weak stimuli only need be used. Determine and note down at what distance of the secondary coil from the primary the reflex response is elicited in each case. Or, stimulate with simple shocks at different frequencies and compare the number of stimuli required at each frequency (*summation of subminimal stimuli*).

3. **Relation of duration of stimulus to duration of reflex response.**—

Note that the reflex elicited in response to a series of stimuli persists for some time after cessation of the stimulation (*after-discharge*).

4. **"Purposeful" nature of reflex action.**—Place on one flank a 4-millimeter square piece of paper moistened with 5 per cent. acetic acid; the foot of the same side is raised to rub off the irritant; if that foot is held down, the other foot may be used.

After the observation do not leave the acid in contact with the flank, but wash it off by bringing a large beaker of water up over the legs and lower part of the trunk. The experiment may be repeated upon other places; *e.g.*, the inside of one thigh, the back, and the abdomen, *always washing the acid away after each observation.*

5. **Time of reflex response; Türck's method.**—Having allowed the frog to become quiescent, allow the extremity of the toes to dip into a small beaker of dilute sulphuric acid (2 per 1,000). Count the time in seconds which elapses between the application of the acid and the withdrawal of the toe. Wash the acid off immediately after the withdrawal. Repeat this observation three times at intervals of a few minutes; calculate and record the average time of response.

6. **Inhibition of reflex.**—Place a crystal of salt upon the optic lobes (or on the upper cut end of the cord if the whole brain has been removed), and again determine the time of response after application of dilute sulphuric acid to the toes (**Setschenow's experiment**).

A similar result (inhibition) is obtained by fixing a bulldog clip on the skin of the abdomen.

7. **Effect of drugs on reflex action.**—(1) Determine the normal reaction-time to acid-stimulation of the skin of the foot in a spinal frog (Türck's method). Then inject a very small dose of strychnine nitrate (1 drop of a 1 per 1,000 solution) under the skin and wait for a few minutes until it is absorbed into and distributed by the circulation. Determine the reaction-time once more. It will be found that the reaction-time is not only diminished and the amount of reaction increased, but that there is eventually produced not simple purposeful reflex actions, but convulsive contractions of all the muscles in the body.

(2) In another spinal frog, determine by Türck's method the normal reflex time. Inject a few drops of a 2 per cent. solution of potassium bromide under the skin and, a few minutes later, again determine the reflex time. It will probably be much prolonged, or no reflex reaction at all may be obtained.

8. **Reflex inhibition of heart.**—Fix a decerebrate frog securely on its back upon the frog-cork; expose the heart sufficiently for its beats to be observed. Tap the abdomen smartly with some small heavy instrument, such as the handle of a knife. The effect is to produce a slowing or complete stoppage of the heart, which will,

however, soon recommence beating. The same result is obtained if the abdomen is opened and a loop of intestine strongly stimulated by faradisation (**Goltz's experiment**).

For this experiment the medulla oblongata must be left.

9. **Reciprocal innervation and contraction of antagonistic muscles.**—In a spinal frog attach the tendo Achillis and the tendon of tibialis anticus each to a separate lever. Stimulate the abdomen of the frog and observe the reflex reciprocal contractions of the two muscles—when the gastrocnemius contracts the tibialis muscle relaxes, and *vice versa*. Repeat after section of the nerve supply to one or other of the muscles.

Bell-Magendie Law of spinal roots.—Decapitate a large frog and fix it securely in a prone position on the frog-cork. Cut away the skin along the whole length of the spine. With a pair of strong but fine scissors sever the neural arches on each side, working from above down, and removing them so as to expose the spinal cord and the nerve-roots. The dorsal roots are distinguished both by their position and by the ganglia through which they pass; they are especially large and long in the lumbo-sacral region. Cut them in this region on one side of the body.

1. Tetanise the skin of the corresponding foot. No reflex movement is produced: although, if the skin of the opposite foot is stimulated, strong movements are produced in both limbs.

2. Stimulate the distal end of one or more of the cut roots. If care is taken that the current does not spread to a ventral root, no movement results.

3. Stimulate the proximal end. Strong reflex movements are caused. Now cut the ventral roots on the same side in the same region. Notice that on cutting them the leg muscles contract.

4. Stimulate the peripheral end of one or more of the cut ventral roots. There is strong contraction of muscles of the corresponding limb.

5. Stimulate the central end of the same. No effect is observed.

The excitation used for the roots may be mechanical, such as a pinch or snip of the scissors near the cut end. In this case errors which with electrical stimulus may arise from spread of current are obviated. But if the Helmholtz arrangement is used, and only weak induction shocks employed, the risk of spread is much reduced.

CHAPTER XXXII

REFLEX ACTION IN MAMMALS

THE reflex actions of mammals can be studied in decerebrated or decapitated preparations, reflexes being elicited in various ways, as by touching or pricking an ear or a paw or the skin of the flank or the side of the thorax, or by imparting passive movements (changes of posture) to the head. If records are taken both from the muscles concerned in producing the reflex movement and from the antagonistic muscles, it will be seen that any tonic contraction which the latter may be exhibiting is lessened or inhibited. Tendon-reflexes, such as the knee-jerk and the ankle-clonus, obtained by forcibly bending the foot at the ankle, can also be well observed in such a preparation.

The following experiment (devised by Sherrington) on the isolated quadriceps extensor femoris illustrates some of the phenomena associated with reflex activity in the mammal :—

A cat is decerebrated by the guillotine method.[1] The severance is effected from a point 30 millimeters behind the fronto-parietal suture, the knife emerging into the mouth through the coronoid processes of the lower jaw.[1] The guillotine knife passes between cerebrum and cerebellum, usually removing from the latter the foremost part of its median lobe : the hemispheres and basal ganglia of the cerebrum and both colliculi are removed with the head.

A characteristic rigidity (*decerebrate rigidity*) is produced, those muscles which are normally employed in the maintenance of the erect posture—the anti-gravity muscles—being in a state of exaggerated reflex tonus.

In such a decerebrate preparation the left quadriceps extensor muscle is isolated as follows : The left femoral nerve is exposed and followed up into the psoas muscle to where it branches. The lateral and medial branches are severed, the main (middle) branch which supplies the quadriceps being left intact. The ilio-psoas is cut across about the level of Poupart's ligament. This operation is repeated on the right femoral region, but the nerve to the quadriceps is severed on that side.

The left sciatic is now exposed and its hamstring division severed. The rest of the nerve is dissected downwards and the peroneal and tibial

[1] Details of this method of decerebration and accounts of other experiments on reflex action are given in Sherrington's *Mammalian Physiology*.

divisions ligatured as a single trunk; the nerve is cut distal to the ligature, about 5 centimeters being cleared centrally. The operation is repeated on the opposite side.

All the nerves of the limb have now been severed except the supply to the quadriceps itself; the bony and fascial attachments of the muscle are undisturbed. To complete the preparation proceed as follows: Pass a strong ligature through the skin and superficial part of the calf muscles of the left leg about halfway between knee and ankle, and secure the whole circumference of the limb in the ligature. Amputate the leg 2 centimeters distal to the ligature. Transfix the pretibial skin and the muscles near the end of the stump with a strong needle. Insert a drill pin through the condyle of the left femur from the medial side; leave this pin in the bone and secure it to a vertical stand. Fix a thread to the pretibial skin needle and carry it to an isometric recording lever. Two induction coils, each with a signal in parallel with the primary circuit and stimulating electrodes connected with the secondary, complete the equipment.

The following observations are to be made :—

1. **Reflex posture (proprioceptive postural reflexes).** (*a*) *The "shortening reaction": plastic tonus.*—Note posture of left knee joint. Steady the thigh with one hand and gently raise the limb stump below the knee. On releasing the stump the extended posture thus passively given is more or less maintained. This is called the "shortening reaction," because in it the tonic (postural) length of the muscle is decreased. It is a proprioceptive reflex.

(*b*) *The stretch reflex.*—Steady the thigh and press gently but firmly on the leg stump below the knee so as to flex the knee. Note the resistance offered by the extensor muscle thus stretched. This resistance is active and is reflex—the "stretch reflex."

(*c*) *The "lengthening reaction."*—Continue the application of pressure to the leg stump as in (*b*) above. At a certain degree of pressure the knee will be found to yield, and when released will retain approximately the degree of flexion imposed upon it. Since this reaction is the reaction of an isolated muscle, it is obviously a proprioceptive reflex of that muscle. It is called the "lengthening reaction" because in it the postural (tonic) length of the muscle is increased.

2. **Reflex inhibition of posture and post-inhibitory rebound.**—Making use of (*a*) above, give the knee an extended posture. Stimulate the central stump of the left (ipsilateral) sciatic nerve. The leg stump drops, due to reflex inhibition of the postural tone of left quadriceps. Cessation of the stimulus may be followed by a return to or beyond the previous condition of tone (post-inhibitory rebound), or it may not.

3. **Reflex contraction (crossed extension reflex).**—Attach electrodes to right (contralateral) sciatic and excite. Extension of the left knee

ensues, *i.e.*, reflex contraction from stimulation of the contralateral afferent nerve. Note the latent period, the "recruitment," if any, and the after-discharge. Compare with simple tetanisation of a muscle through its motor nerve.

4. **Reflex inhibition of contraction.**—Stimulate the right sciatic so as to produce reflex contraction of the quadriceps as above. Three or four seconds later stimulate the left sciatic for a few seconds while the right is being stimulated. Discontinue stimulation of the left nerve and, four seconds later, discontinue stimulation of the right. Note that stimulation of the ipsilateral afferent causes not only inhibition of the contralateral reflex but inhibition of postural tone as well, despite the fact that stimulation of the contralateral afferent is maintained unaltered all the time.

Excitation of the cortex cerebri.—A monkey, anæsthetised with ether, is best used for this demonstration. A considerable portion of the skull cap is removed on one side by trephining the skull and enlarging the aperture by bone-forceps. The dura mater is then cut through below and reflected towards the middle line, thus exposing the cerebral surface. A pair of blunt-pointed platinum electrodes, with their points 1 millimeter apart, is connected with a key in the secondary circuit of an induction coil (use the Helmholtz modification) and is applied to various spots in the excitable region of the frontal lobe, to the first temporal gyrus and to the occipital lobe, and the results are noted.

> The monopolar method of stimulation may also be employed for these observations (Sherrington). In this case one electrode is a flat pad of wash-leather wetted with strong salt solution and laid on any part of the body (the skin should first be shaved) or in the mouth; the other [stimulating] electrode is a fine spiral wire with blunt platinum point, which is applied to the surface of the cortex.

CHAPTER XXXIII

REFLEX ACTION AND REACTION TIMES IN MAN

Tendon-reflex; knee-jerk.—In a subject seated in a chair with one leg crossed over the other, or seated on a table with the legs dangling, strike the patellar tendon with the handle of a knife or the back of a thin book. Notice the sudden jerk forward of the leg owing to the contraction of the vastus internus. The contraction can be recorded by a transmission myograph (see p. 41).

Reinforcement of tendon-reflex.—Just before striking the patellar tendon cause the subject to clench his fist. The movement of the leg will be stronger, or will be elicited with a slighter tap on the tendon.

Other reflexes to be studied in man are :—

1. **The Achilles jerk (ankle-jerk).**—Kneel on chair with one foot at right angles to leg. Experimenter hits tendo Achilles a sharp blow. A reflex contraction of the gastrocnemius results.

2. **The ankle reflex (ankle-clonus).**—Pressure on sole with flexion of foot causes rhythmic contractions (clonus) of calf muscles.

3. **The plantar reflex.**—Stroke sole of foot; flexion of toes results. If extension of the big toe occurs the result is abnormal. This abnormality is known as *Babinski's sign*, and is usually associated with a lesion of the cerebral cortex or of the tracts connecting it with the spinal centres.

4. **The abdominal reflex.**—Stroke abdomen with pencil just below ribs—contraction of abdominal muscles.

5. **The cremaster reflex.**—Stroke inner side of thigh with pencil—strong contraction of cremaster muscle.

6. **The pharyngeal reflex.**—Touch back of pharynx with pencil—violent contraction of pharynx and movement of tongue. Prolonged stimulation causes irradiation to diaphragm, and vomiting.

7. **The sneezing reflex.**—Elicited by tickling interior of nostril.

8. **The corneal reflex.**—Touching surface of cornea leads to reflex closure of eyelids and to activity of the lacrimal glands.

9. **The pupil reflexes.**—Reaction (*a*) to light, and (*b*) associated with accommodation to near vision (see p. 163).

Reaction-time in man.—The reaction-time in man may be determined by an arrangement of electro-magnetic signals, but more simply by Waller's apparatus (Fig. 93). This consists of two wooden levers lying across a piece of large india-rubber tube. One end of this is closed; the other is connected with a tambour which writes upon a

drum, the speed of which should be moderate. A screen hides the movements of the experimenter from the person experimented on, who sits at the table with one finger resting lightly on the extremity of one of the levers. He is to respond by pressing the lever the instant he (1) *feels* a movement of that lever, his eyes being shut; (2) *hears* a tap on the second lever; (3) *sees* a movement which is imparted to the second lever by the experimenter, who presses it down on the other side of the screen. In each case two marks are recorded upon the abscissa: one being that which is made by the experimenter in

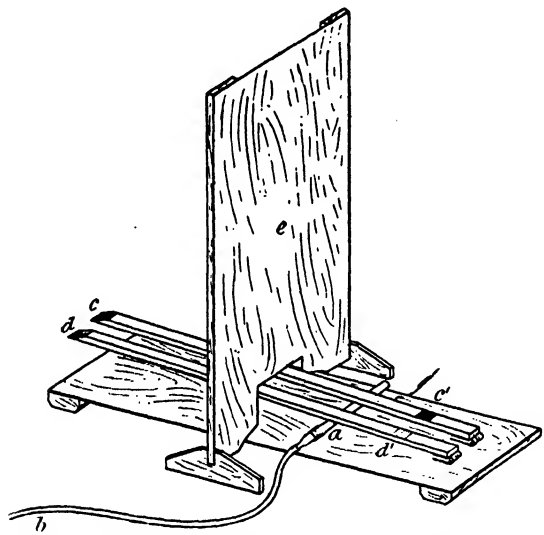

FIG. 93.—Waller's apparatus for reaction-time. *a*, rubber tube closed at one end and at the other connected by *b* with a tambour (not shown); *c*, *c'*, *d*, *d'*, levers (with coloured patches) hinged near *c'*, *d'*, and resting on the rubber tube *a*; *e*, wooden screen. (The tube *a* may be placed on the other side of the screen.)

imparting the stimulus, and the other that made by the observed person in responding. The interval between the two marks, which can be measured by the aid of a time tracing, indicates the time between stimulus and response—*i.e.*, the reaction-time—in the case of each of the three senses. To determine this with any accuracy several observations must be made with each method of stimulation and the shortest time taken.

Discrimination time.—For the measurement of this the observed person places one finger over each lever. It is agreed beforehand that he is only to react to a stimulus received on the one side, not on the

other. Or it may be agreed that it is only the hand on the side which receives the stimulus which is to be used for the response. The experimenter may stimulate either. It will be found that the reaction-time is lengthened by a certain interval, and this increase of reaction-time is termed the *discrimination-time.*

Variations of the above experiments can be made by the employment of different sounds and exhibition of different colours, but the methods for recording the reaction-times are essentially the same. For rapid and accurate work it is usual to employ a specially constructed clock or other apparatus which can register the time of a reaction to a small fraction of a second.

CHAPTER XXXIV

CUTANEOUS SENSATIONS

THE sensations of pain, warmth, cold, and touch are experienced only on adequate stimulation of certain points on the skin, which points are different for each sensation. The following observations should be carried out on an area of skin 1 centimeter square, and the results plotted on an enlarged chart of the area. The subject should be blindfolded.

Pain spots.—Explore with a sharp pin or needle a portion of the skin of the palmar aspect of the forearm of another person, pressing the point firmly here and there, but without penetrating the surface. Notice that whereas at some places the prick is painful, at others no pain is caused, the feeling being either one of touch or of pressure. Indicate on a chart the distribution of the pain spots.

For gauging the amount of pressure of the needle which is needed to elicit a definite sensation of pain, an instrument termed the algesimeter is employed, the pressure being produced by compression of a spiral spring, and the amount in grams indicated on a dial.

Warmth spots.—Substitute for the pin a thick copper rod with a smooth, blunt point at one end; the rod may be provided with a cork handle. Warm the rod by immersing it in water heated to 45° C. Explore the skin by passing the point of the rod slowly over it. It will be found that the sensation is one of warmth only at certain points where it is very distinct; at others it is merely a sensation of touch.

Cold spots.—Repeat with the same or a similar rod cooled by immersion in ice-cold water. In this way spots sensitive to cold alone flash out on passing the point slowly over the surface of the skin: they are not the same as those which are sensitive to warmth.

The various spots may be mapped out upon a patch of the skin with coloured inks or pencils, and may be tested again later. According to most observers they are constant in position.

Touch spots: Determination of the relative delicacy of different parts to touch.—The adequate stimulus for touch is light mechanical pressure. Take a fine bristle or coarse hair 2 inches long, and fix it with sealing-wax to a match to serve as a holder (Fig. 94). Explore in another person (who is not to see the part which is touched) any part of the skin, determining the spots which are most sensitive to the pressure of the hair. The point of this is to be brought vertically on

the skin without lateral movement and pressed down only just enough to bend it slightly. The subject is asked to indicate when he feels the pressure of the hair. By using a number of bristles of different thickness a certain rough scale of delicacy of touch on different parts of the body can be made out. Notice that the slightest side-movement greatly increases the sensitiveness of any part to the touch, especially if hairs are deflected. This can also be shown with a scrap of cotton-wool, the touch of which may be imperceptible until it is removed.

A series of bristles just described form collectively v. Frey's æsthesiometer.

A more elaborate æsthesiometer on v. Frey's principle may be employed in which a bristle is made to protrude from the end of a fine tube to a variable extent, which can be exactly measured. The longer the part protruding the more easily is the bristle bent on pressure. Bristles of different diameter can be used. The delicacy of touch is gauged according to the length of the protruding part, which is just felt when pressed upon the surface of the skin until it bends over.

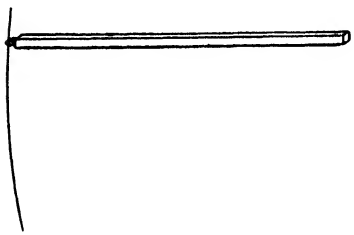

FIG. 94.
v. Frey's hair æsthesiometer.

Graham Brown's æsthesiometer consists of a convex piece of steel with a polished surface, a part of which can be made to project beyond the rest by turning a truly cut micrometer screw. The relative delicacy of touch is gauged by the power of feeling different degrees of projection. This is especially applicable to the lips or to the palmar surfaces of the finger-tips.

Touch discrimination of two points.—For this purpose a pair of compasses with blunted ivory points is used: their distance apart is measured upon a scale after each observation. Or the points may be permanently connected with the scale, one being fixed at its zero and the other sliding along it (Sievekings' æsthesiometer). Test in this manner in another person various parts of the integument (back and front of arm, fingers, lips, tip of tongue, etc.) and record the distances at which the two points are discriminated as separate, causing them always to be applied simultaneously, and without lateral movement.

Accuracy of localisation of touch sensation.—This is investigated by lightly touching any part of the skin and causing the subject immediately to place a finger upon the part touched.

In all the above experiments the subject should be blindfolded.

CHAPTER XXXV

EXPERIMENTS ON THE DIOPTRIC MECHANISM

Dissection.—An eye (ox, sheep, or pig) is to be dissected.

1. After cleaning away from the globe all remains of muscles, fat, etc., cut a window out from the back, removing the sclera and choroid and exposing the retina. Notice that when the cornea is turned to the window, or preferably toward a T-shaped source of light, a reversed image of this is formed upon the retina. (The eye of an albino rabbit is best for demonstrating the inversion of the retinal image, which can be seen through the non-pigmented sclera.)

2. Cut away a small portion of the sclera at the edge of the cornea. A greyish ring of plain muscular tissue is exposed, the fibres passing from the corneo-sclerotic junction backwards over and into the choroid. This is the ciliary muscle.

3. Cut the eye in two at its equator. Notice, in the posterior half, from which the jelly-like vitreous humor flows away, the retina—usually somewhat opaque and crumpled after death—spreading out from the entrance of the optic nerve; in the anterior half the lens within its capsule, the suspensory ligament around the margin of the lens, the radiating ciliary processes.

4. Snip through the suspensory ligament all round the lens, which can be removed within its capsule; the iris is now seen projecting into the anterior chamber.

Pupillary reflexes.—*Light reflex:* The subject faces a window, his eyes covered by the hands of the observer. One eye is then uncovered: the pupil contracts. Similarly if the rays from an electric torch are suddenly directed on the eye the pupil contracts: it dilates again when the light is extinguished.

Consensual reflex: If, in performing the previous experiment, one eye is kept in the shade while the other is illuminated, note that the pupil of the shaded eye usually also contracts.

Accommodation reflex: The subject is directed to accommodate by looking at a near object: the pupil constricts. On directing the gaze to a distant object the pupil dilates.

Accommodation; Change in shape of the lens.—That the lens bulges forward in accommodation is shown in various ways.

1. Stand at the side of another person and let him fix his vision on a distant object, looking beyond a near object such as a needle or

pencil held a few inches from the eye. Notice his iris, which can be seen through the edge of the cornea lying against the front of the lens. Now let the subject look at the near object. His iris is seen to advance, being pushed forwards by the bulging lens; the pupil at the same time contracts.

2. *Sanson's images.*—In a dark room hold a candle at one side of the eye of a subject, and, standing on the other side, observe the reflected images—a bright one from the front of the cornea, a less bright one from the front of the lens, and a duller, small, and inverted image difficult to see—from the back of the lens. The subject as before is to have his vision fixed at first on a distant object, and is then to transfer his gaze to a near object in the same line. The image reflected from the front of the lens becomes smaller and moves nearer to that reflected from the front of the cornea; the other images remain unaltered. This change of the second image is due to bulging of the anterior surface of the lens.

3. *Phakoscope.*—The same experiment may be performed with a phakoscope (Fig. 95) with less trouble, since all the points are fixed. The instrument—a triangular box with truncated angles *a*, *b*, *c*—is used in a darkened room. A lamp is placed in such a position that the light from the two square window prisms at *c* falls upon the observed eye at *a*. The observer looks through the opening at *b* and sees in the observed eye three pairs of images—two bright squares (reflected from the anterior surface of the cornea), two larger but less distinct squares, and two smaller and much dimmer squares. The two last pairs, being reflected from the anterior and posterior surfaces of the lens respectively, can, of course, only be seen within the pupil. The last pair is difficult to make out. If the subject is asked first to look *past* the needle *n* at a distant window, and then *at* the needle, the middle double image becomes smaller and slightly brighter during accommodation for the near object; the squares approaching each other and coming nearer to the corneal image; the other two double images remain unaffected.

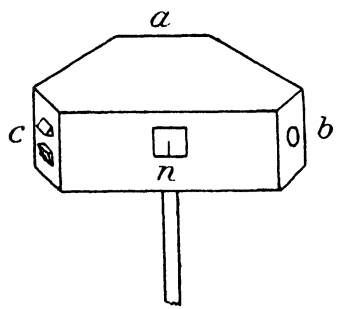

FIG. 95.—Phakoscope: *a*, situation of observed eye; *b*, ditto of observer's eye; *c*, lenses; *n*, aperture with needle.

Near and far points of distinct vision.—A wooden scale about 12 inches long is marked in half-inches or in centimeters. One end of the scale is placed close to the eye, and a needle is put in about 5 inches off. If

the eye is normal, the needle should be seen sharply at this distance and at any point beyond; but if it is brought nearer the eye its image becomes blurred. If the eye is myopic the needle may be brought nearer than 5 inches without its image being blurred; when blurring occurs the near point for that eye has been passed.

If the eye is hypermetropic the needle will already appear blurred at 5 inches, and it may be required to be moved considerably farther from the eye before the near point of distinct vision is passed.

Scheiner's experiment.—The observation is rendered easier and more striking by the device of observing the needle through two pin-holes made close to one another and side by side in a card fixed vertically at one end of the scale (Fig. 96). In this case, when the needle is nearer

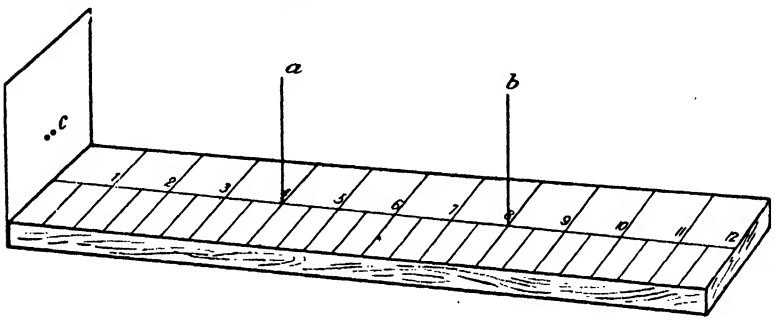

FIG. 96.—Board with perforated card for Scheiner's experiment. *a*, *b*, needles; *c*, perforations in card.

to or farther from the near or far points of distinct vision, its image appears not blurred but double.

That the eye cannot simultaneously obtain sharp images of a near and a distant object is shown by taking two needles and fixing one at about 5 inches along the scale and the other some inches farther. If now, in Scheiner's experiment, the eye is focused on the near needle, the far one looks double, and *vice versa.*

The Ophthalmoscope.—*Direct method.*—The instrument which is used for examining the interior of the eyeball consists essentially of a small concave mirror with a hole in the centre. Practise first on an artificial model of the eye and then on the living subject. [It can be practised upon a rabbit: a drop or two of a 1 per cent. solution of atropine should previously be instilled into the eye. Or a frog, with the body wrapped in a cloth, may be employed.] Only a limited part of the retina is seen at one time, but it is much magnified.

The subject is seated in a darkened room with a light, not too bright, near his ear. The observer sits in front of, and on a slightly higher level than, the subject, close to him. The observer holds the

mirror in front of and close to his own (corresponding) eye, and, throwing the beam of light into the subject's eye, asks him to look upwards and inwards. The observer then moves the mirror, with his eye close behind it, backwards and forwards, looking through the hole in the centre, and when the proper distance is found (2 to 3 inches), the retina comes into view with its vessels running in different directions on a red ground. The mirror is moved about until the optic disc (entrance of optic nerve) is seen as a whitish circular area with the central artery and vein of the retina emerging at its centre. The image of the fundus is virtual (erect), and is enlarged because the refracting surfaces of the subject's eye magnify the parts under observation.

Indirect method.—With the subject as before, the observer places himself about 18 inches in front of the patient and throws the light on the pupil as in the direct method. He then takes a small biconvex lens (2 to 3 inches focus) in his left hand, and, holding it vertically between the thumb and forefinger at a distance of 2 to 3 inches from the patient's eye, moves his own eye with the mirror in front of it backwards and forwards and from side to side until the optic disc and other parts of the retina are seen. The image is real (inverted), and is only slightly magnified.

Ophthalmoscopy is rendered easier if the pupil has been previously dilated by homatropine. If either the patient or observer has abnormal vision this is corrected by suitable lenses placed behind the aperture in the mirror of the ophthalmoscope.

Entoptic appearances of the lens and vitreous humor.—By a series of very simple experiments the student will be able to see certain details of the structure of the lens, vitreous humor, and retinal vessels of his own eye.

If a small source of light is looked at through a strong biconcave lens (—16 diopters) held about 50 centimeters from the eye, the light source will be seen as an almost circular spot of light, surrounded by a serrated margin. This serrated margin is produced by the margin of the iris of the observer's own eye, while the bright spot of light corresponds to the pupil. If the other eye is illuminated, the pupil under observation can be seen to contract. When the biconcave lens is brought still nearer the eye, irregular fibre-like markings can be seen in the spot of light which corresponds to the pupil; these markings are caused by the fibres of the lens. If the biconcave lens is approached to within a few centimeters of the eye, the markings disappear and become replaced by irregularly moving spots; these are due to floating cells in the anterior part of the vitreous.

Instead of a biconcave lens, an ordinary microscope, the field of which is evenly illuminated, may be used. The observer looks into the eyepiece from a distance of about 20 centimetres; under these circum-

stances he will see in the eyepiece the pupil as a circular spot of light, surrounded by the serrated margin of the iris. As the eye of the observer is brought nearer to the eyepiece the spot of light becomes larger until, at a point about 5 centimeters away, the fibre-like structure of the lens appears. If the eye is brought still closer, vitreous "floaters" can be seen moving across the illuminated field.

The part of the vitreous lying immediately in front of the retina can be inspected by looking at an evenly illuminated surface, such as a bright but cloudy sky. Numerous irregular spots and threads will appear moving across the field of vision; these are due to tissue elements in the vitreous humor. They are the well-known *muscæ volitantes*.

CHAPTER XXXVI

STIMULATION OF THE RETINA

Inversion of retinal image.—That the image which falls upon the retina is inverted can be confirmed directly in the excised eye of a mammal (see p. 163). The following experiment demonstrates this inversion in man.

Close one eye and with the other look at the sky through a small hole in a piece of black paper held about 3 inches away. Hold a pin, head upwards, close to the eye on the near side of the paper. The pin will appear to be inverted, because the light passing through the hole in the paper casts a direct shadow of the pinhead on the retina, and this shadow is the same way up as the pin itself.

Electrical changes.—That an electrical change results from the action of light upon the retina can be shown in the frog. The eye is enucleated and is placed on non-polarisable electrodes, one of which is in contact with the cut optic nerve, the other with the front of the eye. The electrodes are connected with a galvanometer or electrometer (see Chapter XIV.), and the preparation is placed in a dark box. On letting light into the eye through an aperture in the box an electric change is produced in the retina, and the galvanometer or electrometer is deflected. On removing the light there is another deflection in the same direction.

Blind spot. *Mariotte's experiment.*—Make a mark of any sort (such as a small cross) upon a piece of paper, and fix one eye—say the right—upon it, closing the other eye and placing the head about 6 inches from the paper. It will be found that about 3 or 3½ inches to the right of the cross, over a considerable area of irregular shape, the point of a pen or pencil will not be visible because its image falls upon the place where the optic nerve enters the retina. Even a large black dot made in this area upon the white paper is quite invisible as long as the eye is fixed upon the cross. The experiment shows that the optic nerve-fibres are insensitive to light. Map out the blind area, noting distance of eye from paper.

Macula lutea. *Maxwell's experiment.*—Close the eyes for a minute; then, opening one of them, hold a bottle with parallel sides containing a solution of chrome-alum—which has a greenish colour—between the eye and a uniform source of light such as a white cloud. The middle of the field of vision will be occupied by an oval rose-coloured area;

the alteration in colour of this part is due to absorption by the yellowish pigment of the macula of some of the rays transmitted through the chrome-alum, which is dichroic, transmitting greenish-blue and red rays: the former are absorbed.

Retinal blood-vessels. *Purkinje's experiment.*—This is performed in a darkened room. Stand about 8 feet from a sheet of white or grey paper fixed to the wall and get an assistant to illuminate the retina through the sclerotic by means of an electric torch held at one side of the head. Look steadily at the paper with one eye, accommodating for distance. In a minute or two a number of branching figures will be seen like the roots of trees. These are the shadows of the retinal blood-vessels. When the light is moved the shadows are also seen to move. This experiment shows that the receptive visual cells lie behind the blood-vessels of the retina. The presence of the vessels may also be appreciated by moving the field in relation to the retina. Look at the sky through a pinhole in a sheet of cardboard, give the card an up and down, side to side, and lastly a rotatory motion, and the branching shadows will be perceived with a central free space corresponding to the macula.

The retinal vessels can be seen still better by using a very small electric bulb mounted on a stem (electric ophthalmoscope torch). The bulb is pressed directly backwards on the lower eyelid of the closed eye, and moved about slightly; in a certain position, which has to be found by trial, the observer will see his own retinal vessels standing out as red streaks on a darker red ground. The vessels disappear into a dark area, the blind spot. In carrying out this experiment both eyes should be closed.

Retinal blood corpuscles.—The red corpuscles can be seen moving in the retinal vessels by looking at a bright sky, or a piece of white cardboard strongly illuminated by a mercury arc, through a piece of dark-blue glass. After a few seconds the field of vision exhibits minute silvery spots in rapid movement; these are caused by the shadows of the red corpuscles within the retinal capillaries falling on the light-sensitive rods and cones.

Mapping of visual field. *Perimetry.*—The perimeter is an instrument for testing and recording light perceptions in different parts of the retina. The eye is fixed upon a point in the centre of the concave hemisphere forming the perimeter, and a white disc is brought gradually from the edge of the hemisphere nearer and nearer to the centre until it is perceived. This is repeated along different meridians at 20 degree intervals, and the results are marked on a chart at the back of the instrument. By using coloured discs instead of white, and noting the points in the different meridians at which the colour is just recognisable, the area of the retina which is sensitive to each colour can be

ascertained. Map out your own fields of vision for white, red, yellow, and blue.

Testing for colour vision. Edridge-Green's lamp and spectroscope. Holmgren's wools. Edridge-Green's card test. Ishihara's card test.—The best practical method for testing colour vision is by the use of a lamp provided with glasses of different colour, the subject being expected to name the colour which is exhibited. A more accurate method of obtaining spectral colours for testing purposes is the employment of a spectroscope so arranged that only a definite part of the spectrum with a pure spectral colour is visible at one time.

A method which has been considerably used for testing colour vision is to take a box full of skeins of wool dyed with different colours, and, selecting one skein, to ask the person who is being tested to pick out any that match it. If he is colour-blind he is liable to make serious mistakes, matching grey with red, green with grey or red, and so on; but it occasionally happens that persons who fail completely with the lamp test are able, probably by judging from the intensity of the reflected light, to match the Holmgren wools fairly well. Other tests for colour vision which have more recently come into use are the card tests of Edridge-Green and of Ishihara. Examine and compare the two types of card.

Simultaneous contrast. *Meyer's experiment.*—Place a grey disc upon a yellow ground, and cover the whole with thin tissue paper: the grey disc at once appears blue; the contrast colour of the yellow. If a blue ground is used the disc will appear yellow. The same experiment may be repeated with red and green grounds. On the white ground the grey disc will appear darker; on a black ground lighter.

Successive contrast.—Fix the vision upon a white spot on a dark ground. After one minute look at a uniform white surface such as a white ceiling. A dark spot now occupies the centre of the field of vision.

This experiment is varied by employing colours—*e.g.*, a yellow spot on a blue ground or *vice versa*; and a red spot on a green ground or *vice versa*. In each case the vision is transferred to a uniform white surface and the contrast colours are observed. In all instances the after-image will be of the complementary colour.

For the grounds, coloured paper is used; for the spots, either paper discs or wafers.

Colour mixer.—This usually takes the form of a revolving circular plate on which sectors of different coloured cards can be arranged. Owing to the fact that retinal impressions have an appreciable duration, the colours appear blended during the revolution of the plate, and the mixing of the colours on the retina can thus be studied.

Stereoscope.—The fact that in stereoscopic vision the mind combines

the effects produced by slightly dissimilar pictures falling on the two retinæ is illustrated by the ordinary stereoscope.

Persistence of visual impressions.—The fact that a visual impression persists for a brief interval after removal of the stimulus is shown by the apparent luminous line described in the air by rapidly moving the glowing end of a burning stick. It is also familiarly illustrated by kinematograph pictures.

Measurement of visual acuity.—Examine the Snellen test letters. These are such that the angle subtended by the details (breadth of stroke, etc.) of the letters of each group is one minute at the distance marked. Stand at a distance of 6 meters and find the smallest type you can read with the right eye alone. (Cover the left eye with a piece of black cardboard; do not hold the eye, as this interferes with accommodation.) If the smallest legible type is marked x meters, then the acuity of the right eye is $6/x$. Find the visual acuity for the left eye, and for both eyes together. If the subject is hypermetropic his performance will be similar with and without a positive (convex) lens in front of the eye, whereas if he is myopic his performance will be markedly improved by a negative (concave) lens. How do you explain this ?

CHAPTER XXXVII

PHONATION AND AUDITION. THE SEMICIRCULAR CANALS

Use of the laryngoscope.—The laryngoscope consists of a small circular plane mirror fixed to a handle at a suitable angle. As a source of light a large concave mirror with a hole in the centre is strapped to the operator's forehead, and reflects the light of a lamp behind the subject. Alternatively, a small electric lamp is attached to the laryngoscope and furnishes direct illumination.

Practise first on an artificial model of the larynx and afterwards on the living subject. The latter is placed on a stool with a lamp over his right shoulder, a little above the level of his mouth. The observer sits opposite and close to the subject with the large mirror attached to his forehead. The subject is asked to open his mouth, incline his head slightly backwards, protrude his tongue, and hold it down with a handkerchief. The observer manœuvres his head until the back of the subject's throat is brightly illuminated; he then takes the small mirror in his right hand, warms it slightly in a flame to prevent moisture condensing on its surface (the back of the mirror should be just perceptibly warm to the cheek), and, holding the handle as one does a pen, pushes it horizontally backwards until it touches the uvula. First the dorsum of the tongue is seen in the mirror, then, as the handle is depressed, the epiglottis; then the glottis and vocal cords come into view. The image of the larynx thus obtained is an inverted one. In ordinary breathing the glottis is open; if the patient is asked to sound a high note the vocal cords may be seen to come together and to vibrate, and if he is asked to take a deep breath they separate; the interior of the trachea and even its bifurcation may then be seen through the widely open glottis.

Any tendency to retch when the mirror comes in contact with the soft palate may be diminished by the application of a solution of cocaine to the mucous membrane.

The movements of the laryngeal cartilages are studied in a model which represents them articulated together. The action of the muscles can be imitated by threads, and the vocal cords by thin flat rubber bands stretched between the thyroid and arytenoids.

The production of vowel sounds.—Notice that the production of the vowel sounds (*ah, eh, ee, o, oo*) is accompanied by changes in the

shape and size of the resonating chamber formed by the throat and buccal cavity.

The production of consonants.—Notice that most of the consonants are produced by an interruption, complete or incomplete, of the blast of air which is producing the vibration of the vocal cords, the interruption occurring either at the back of the palate (gutturals), at the front of the palate (linguals), or at the lips (labials). Notice also that the character of the interruption is a factor in determining the quality of the consonant: thus, with some, such as *k*, *b*, and *t*, it is sudden or explosive; with others, such as *m* and *n*, the nasal cavities are brought in as resonators; with others, such as *ch*, *f*, and *s*, the blast is continuous, but is made to traverse a narrowed part of the cavity: whilst with *qu* there is an actual vibration of the narrowed part.

The production of sounds by vibration of the vocal cords, and the dependence of the pitch of sounds on the tension of the cords.—Take a sheep's larynx and tie a glass tube into the trachea. Fix the larynx securely on a board, with the dorsal surface downwards, by wires or strong pins through the cricoid cartilage and epiglottis. Pass a string through the lower part of the thyroid cartilage. When this string is pulled vertically upwards, the vocal cords are stretched in proportion to the pull, and become approximated. If air is blown through the tube in the trachea, the edges of the cords are set in vibration when thus approximated and a sound is emitted, the pitch of which varies with the tension of the cords. The blast of air should be of the same strength throughout.

Analysis of sounds of musical instruments and of the voice.—The analysis can be made by applying different Helmholtz resonators to the ear of the observer. König's manometric flames, which are provided with these resonators and are examined with the aid of vertical rotating mirrors, are also employed for this purpose. But the foregoing instruments are now being replaced by the kathode-ray oscillograph (see p. 72).

Audition: Inspection of the tympanic membrane.—Using a mirror with a central aperture, throw the reflection of a lamp into the meatus of the subject, whose external ear must be drawn somewhat backwards and upwards.

Propagation of sound to the internal ear.—The mode of transmission may be studied with the aid of a model showing the bones of the middle ear and their attachments to the membrana tympani and the fenestra ovalis. The model shows that when the tympanic membrane —to which the handle of the malleus is attached—is pressed inwards, the base of the stapes, which fits into the fenestra ovalis, follows the movement; but when the tympanic membrane is forced outwards

beyond a certain point the stapes is not dragged after it, owing to the nature of the articulation between malleus and incus. The model also shows the effect of the tensor tympani in pulling inwards the handle of the malleus and with it the membrana tympani, and the effect of the stapedius in pulling the head of the stapes backwards and causing the base to be tilted within the fenestra ovalis, thus rendering tight the ligament which fixes it in that aperture.

Determination of range of pitch for audition.—The highest and lowest notes which can be appreciated are determined by the use of Galton's adjustable whistle. The highest audible pitch is very variable for different persons.

Conduction of sound by the bones of the skull. *Rinne's test.*—Stop the ears with wool. Set a small tuning-fork in vibration, and hold it with the base applied to the top of the skull, to the teeth, or to the mastoid process. The sound is propagated to the cochlea by the bones of the skull. After the sound has become inaudible by bone conduction it can usually still be perceived by holding the fork near the pinna (positive Rinne's test); when the membranes or ossicles of the middle ear are defective, however, the opposite is observed (negative Rinne's test). In incomplete inner ear (nerve) deafness Rinne's test is positive though, of course, the duration of audibility is reduced.

Auditory localisation. (i) *Position of source of sound.*—The subject closes his eyes and the observer makes faint clicks, by means of two coins, at different points near the subject's head. The subject states the supposed position of the source of sound.

(ii) *Weber's test of uni-aural deafness.*—The base of a vibrating tuning-fork is applied to the crown of the head. In obstructive or middle ear deafness the sound seems to come from the side of the deaf ear, in nerve deafness from that of the "good" ear. The former point may be tested by carrying out the experiment after plugging one meatus with cotton-wool.

Auditory intensity or acuity. (i) *Watch test.*—Plug one of the subject's ears with cotton-wool and move a watch slowly away from the other ear until he just fails to hear its ticking. Advance it again slowly until he just hears it. Repeat the observation several times and take the average distance. Test the other ear in the same way after reversing the position of the subject, so that the watch is moved in the same direction in the room as before. The results give a measure of the relative acuity of audition in the two ears.

(ii) *Tuning-fork test.* (*a*) *Measure of total hearing.*—Strike a tuning-fork a blow on the knee and hold it close to the pinna of the subject, who is to indicate the instant at which he ceases to hear it. The observer notes the time in seconds from the striking of the fork to this instant. Repeat the observation several times with each ear, and with

the ears made artificially deaf with light plugs of cotton-wool. In making these experiments note that the time during which the fork is heard under any given circumstances is independent of the initial blow given to it. (*b*) *Measure of cochlear hearing.*—In the above experiment the sound had to pass through the middle ear, and the results thus give a measure of total hearing. If the base of the fork is applied to the mastoid process the sound reaches the cochlea through the bones of the skull, and the presence or absence of cochlear hearing can be tested in this way (see *Rinne's test*, p. 174) and its extent measured. Middle ear losses are shown by the difference in readings for total hearing and for cochlear hearing.

Semicircular canals. (i) *In the bird.*—For demonstrating the effects of injury to semicircular canals, a bird (pigeon) is employed. An opening is made in the side of the skull of the anæsthetised animal with a very small trephine, and through the aperture a special instrument is introduced and passed underneath the dura mater until one of the bony semicircular canals (which in the bird project above the surface of the petrous bone) is met with; the canal can then be broken across.

(ii) *In mammals.*—The effects of passively changing the posture of the head upon the position of the limbs (Magnus & Klijn) can be studied in an animal (cat) decerebrated by Sherrington's method (see p. 155), and suspended by a broad bandage round the trunk. These effects are due to stimulation of the nerves of the labyrinth (utricle, saccule) by pressure of the otoliths.

(iii) *In man.*—The effects of stimulating the nerve-terminations in the ampullæ of the semicircular canals by movements of the endolymph are studied by the aid of a turn-table, upon which the subject (blindfolded) is seated. The several canals are affected according to the inclination of the head—the external when the head is erect, the superior with the head inclined forwards, the posterior with the head inclined laterally. On ceasing the rotation varying movements of the body and eyes are produced, involuntary in character and accompanied by giddiness.

Experiments involving the semicircular canals can be made without the use of any special apparatus: (i) Stand erect and spin round several times on one heel, driving yourself with the other foot. Do not spin so fast that you cannot maintain complete control of your balance. Stop suddenly and attempt to draw a vertical line, on a wall board, in the same position as one previously drawn. After a few minutes repeat the spinning, and stop facing your partner who is at once to examine your eyes for nystagmus. Again repeat the rotation. As soon as you stop lean your head well over towards the right or the

left shoulder. Have someone at hand to catch you. These observations should be repeated with the opposite direction of spin. (ii) Stoop so that your trunk is horizontal. Walk rapidly two or three times in a circle around the point below your eyes as centre. Rise erect and try to walk towards some definite goal, or along a straight line chalked on the floor.

CHAPTER XXXVIII

TASTE AND SMELL

Taste.—(*a*) To test the localisation of taste, direct the subject to close his eyes and put out his tongue. This organ is then dried, and different parts are touched with a small brush or glass rod moistened with the sapid substance in solution. After each observation the mouth must be rinsed with water. The following solutions may be used for testing the four qualities of taste, viz.: for *bitter*, 1 per cent. solution of quinine sulphate; for *sweet*, 5 per cent. solution of sugar; for *acid*, 2 per cent. solution of citric acid; and for *salt*, 5 per cent. solution of common salt. Notice the time which intervenes between the application of the sapid substance and its effect. Record your results as regards localisation upon an outline plan of the tongue.

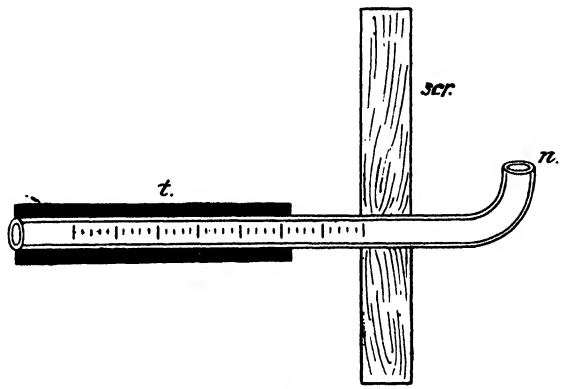

FIG. 97.—Zwaardemaker's olfactometer. *n.*, nose-piece; *scr.*, screen; *t.*, porous tube sliding over graduated glass tube.

(*b*) Test different parts of the tongue by applying closely set electrodes arranged to conduct a weak faradising current.

(*c*) Chew a leaf of *Gymnema sylvestris*, or paint the tongue with 2 per cent. cocaine solution, and determine whether the taste of any of the varieties of sapid substances is affected.

Smell.—The sense of smell is tested by Zwaardemaker's olfactometer, which consists of a glass tube with one end adapted to the nostril, while over the other end a tube, which can be impregnated with the odoriferous substance, slides, so that a greater or less amount of its inner surface is exposed to the air which is passing through the glass tube into the nostril (Fig. 97).

APPENDIX

USEFUL INFORMATION

Solutions for amphibian tissues.

Normal saline.—0·6 per cent. NaCl in distilled water.

Ringer's solution.—NaCl, 6 g.; KCl, 0·075 g.; $CaCl_2$, 0·1 g.; $NaHCO_3$, 0·1 g.; distilled water to 1,000 ml.

Clark's Ringer solution.—NaCl, 6·5 g.; KCl, 0·14 g; $CaCl_2$ (anhydr.), 0·12 g.; $NaHCO_3$, 0·1 g.; NaH_2PO_4, 0·01 g.; glucose, 2 g. (this may be omitted); distilled water to 1,000 ml.

Solutions for mammalian tissues.

Normal saline.—0·7 to 0·9 per cent. NaCl in distilled water.

Locke's Ringer solution.—NaCl, 9·2 g.; KCl, 0·40 g.; $CaCl_2$ (cryst.), 0·24 g.; $NaHCO_3$, 0·15 g.; distilled water to 1,000 ml. To the above, when employed for perfusion of the mammalian heart, 1 g. pure glucose is added just before use.

Dale's Ringer solution.—NaCl, 9 g.; KCl, 0·42 g.; $CaCl_2$ (cryst.), 0·24 g.; $NaHCO_3$, 0·5 g.; $MgCl_2$, 0·005 g.; distilled water to 1,000 ml. Suitable for mammalian plain muscle.

In preparing the above solutions dissolve the NaCl, KCl, and $NaHCO_3$ (and phosphate, if any) in the greater part of the water. Then, while stirring the fluid vigorously, add the $CaCl_2$ dissolved in a little water.

Volatile anæsthetics.

Of the volatile anæsthetics ethyl ether is the safest for all animals. If the nature of the experiment does not contraindicate it, a small dose of atropine may be given before anæsthetisation to prevent excessive secretion of mucus and saliva.

For dogs it is sometimes an advantage to combine morphine with ether. Half a grain of morphine-sulphate is given subcutaneously half an hour before beginning the administration of ether.

Induction of anæsthesia in dogs is, however, best accomplished by the administration of a mixture of chloroform and ether (chloroform 1 part, ether 2 parts). Premedication is then unnecessary.

Non-volatile anæsthetics.

For cats and rabbits a very good non-volatile anæsthetic is urethane. This is dissolved in saline and injected subcutaneously about an hour before beginning the experiment. The dose is 1·6 grams per kilogram of body weight.

For cats and dogs chloralose (Martindale) or "chloralosane" (Kuhlmann, Paris) may be used. This is administered intravenously in a dose of 0·08-0·1 gram per kilogram of body weight. The animal is placed under ether or chloroform-ether anæsthesia for the administration.

Blood-pressure fluid.

Probably the best blood-pressure fluid is an almost saturated solution of sodium sulphate. The solution should not be sufficiently concentrated for crystals to separate out in the pressure bottle on a cold day. A suitable concentration is 300 grams sodium sulphate (crystals) to 1 liter distilled water. Filter before use.

Stock solutions of adrenaline and acetylcholine.

Concentrated stock solutions of these drugs, which are indefinitely stable if kept in the cold and protected from light, are prepared as follows: *Adrenaline*—Add N/10 HCl to a quantity of the base to give a solution containing 10 milligrams base per milliliter. A small quantity of this solution diluted with distilled water to 0·1 per cent. forms a suitable stock from which further dilution can be made for any day's work. Dilutions of even 1 in 100,000 with *distilled water* are stable for some hours and can be used in class for addition to Ringer's fluid to give the desired final concentration for experiments. Such additions to Ringer's solution should be made immediately before use. *Acetylcholine*—0·1 gram acetylcholine chloride (Roche Products, Ltd.) from a sealed tube is washed with distilled water into a volumetric flask and made up to 100 milliliters with a 5 per cent. solution of NaH_2PO_4 and boiled for a few minutes. When cool it may be distributed into small sterile hard-glass tubes, each containing 1 milliliter, which are then corked and kept in the ice chest. Dilute solutions for class use are made by adding the contents of one of the tubes (1 milligram) to distilled water to give the concentration most suitable for subsequent addition to Ringer's fluid.

Varnish for smoked papers.

The following varnish gives an almost matt surface and does not cause the paper to become brittle when dry. It is made by dissolving 150 grams pure colophony resin (powdered) in 2 liters rectified spirit. With use the varnish will tend to thicken by evaporation of the spirit: addition of further spirit at intervals will prevent this.

INDEX