A MANUAL

OF

EXPERIMENTAL PHYSIOLOGY

FOR

STUDENTS OF MEDICINE.

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TO

NATHAN SMITH DAVIS, M.D., LL.D.,

RECENTLY DECEASED DEAN EMERITUS OF THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL,

IN HUMBLE RECOGNITION OF THE STIMULUS WHICH HE GAVE TO EXPERIMENTAL MEDICINE IN AMERICA AND IN GRATEFUL REMEMBRANCE OF THE INSPIRATION RECEIVED FROM HIM THIS VOLUME IS RESPECTFULLY DEDICATED BY HIS PUPIL,

THE AUTHOR.

PREFACE.

This volume represents the accumulated experience of a decade in the presentation of experimental physiology to medical students.

The scope of the book has naturally been determined by the needs of medical students who are preparing for the practice of clinical medicine and surgery.

The preliminary lessons in *Cytology* are presented as a feature of the volume. This introductory course has proven to be a most valuable accompaniment to the beginning work in histology, as well as a most substantial foundation to general physiology.

The arrangement of the chapters has been determined by two considerations: (1) the degree of difficulty of the technique, and (2) the correlation of other work of the medical course. Cytology —the first chapter—involves the simplest microscopic technique, and the principles of cell life make the foundation of modern medicine and surgery. Electro-physiology involves a technique not too difficult for the earlier months of medical study, and, at the same time, it forms a most valuable basis for the experimental work that follows.

The order of the chapters on Circulation, Respiration, Hæmatology, and Digestion may easily be changed to suit the curriculum of the institution where the course is given.

The exercises have for years been furnished my students in the form of type-written syllibi, undergoing almost annual revision. They represent, therefore, a gradual evolution.

At no time during this development of a practical course in experimental physiology has the author lost sight of the fact that his pupils were preparing for clinical practice. The experiments

are carefully chosen and arranged to involve a considerable amount of *surgical work* and to present to the student those fundamental facts and principles of physiology which form the basis of *Internal Medicine.*

The author takes this opportunity to acknowledge the valuable assistance of his associate, Dr. C. J. Kurtz, who prepared the chapter on Normal Hæmatology, and of Professor Charles H. Miller, of the Department of Pharmacology, for his assistance in the preparation of the lessons on the physiological action of drugs.

W. S. H.

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EXPERIMENTAL PHYSIOLOGY.

INTRODUCTION.

The general method of presenting the subject of physiology is the same as that followed in all of the other experimental sciences, viz., the laboratory method, according to which the student is led to discover for himself certain facts and to formulate from his collected data conclusions which represent fundamental principles of the science.

This method of presenting the experimental sciences—chemistry, physics, and the biological sciences, including physiology, psychology, pharmacology, and pathology—is an expensive one, both as to the time and the money involved in it; but from the standpoint of pedagogy it is more economical than the text-book method, because it leads directly and surely to definite results.

In answer to the question as to just what these results are which follow the modern laboratory method of instruction, it may be stated first of all that it cultivates in the student the capacity of close and accurate observation; it affords an opportunity for valuable practice in the systematic recording of the observations; it develops the power of logical thought in drawing tenable conclusions from the observed data; and, in the formulation of conclusions, it stimulates the ability to express the thoughts in concise and unambiguous terms. In the second place, practice of this kind makes the student independent and furnishes him with just the mental equipment needed for later life in whatever line his activities may be directed. If such an education is more important for one profession than another, the medical profession is certainly the one in which its importance is greatest. The physics, chemistry, and biology studied in preparation for medicine, and the physiology, pharmacology, and pathology of the medical course should give the student a most admirable equipment for dealing with the complex problems of clinical practice.

From what has preceded, it will have been noted that the facts of an experimental science occupy a subordinate position. Facts are only stepping stones leading to principles. Principles are important. Quite as important as the principles to which the facts lead

is the method, the technique, through which the facts are observed. The technique guides one's hands and senses in the study of new phenomena, while the principles already mastered guide one's mind in dealing with the facts of the new phenomena. The hand and the mind working with technique and principles make the equipment of the scientific man of to-day.

As the application of this general method of the presentation of physiology, it may be briefly stated that, so far as the time permits, the student discovers for himself the facts and draws his own conclusions, defending them against the criticism of others. This is supplemented by demonstrations to the class, in which each student can observe phenomena which later become the subject of general discussion. Limitations in the time that may be devoted to work in the laboratory make it necessary to occasionally discuss phenomena and principles which the student has not observed and formulated; but these discussions have the purpose of permitting a more systematic presentation of the subject than would otherwise be possible, and the student is held responsible finally for what he has observed only.

Regarding Illustrations. The profuse illustration of a text-book is in perfect accord with the principles of pedagogy; that the profuse illustration of a laboratory manual is the reverse is evident from the following considerations:

The laboratory student receives from the demonstrator the material with which he is to work. If he receives a piece of apparatus which is new to him, a few questions or hints in his laboratory manual will lead him to discover, from an examination of the apparatus itself, the physical and mechanical principles involved and utilized in it. Most students will spontaneously make drawings showing the essential parts of all instruments; all students will willingly do so if required. This is a most valuable exercise for the pupil, which is likely to be omitted if the manual contains cuts of the apparatus.

Nearly every exercise requires the preparation of some simple appliance—*e. g.*, a frog board or a recording lever—whose adjustment will be much facilitated if the student is guided by a figure in his manual, but a model which the demonstrator has set up will be a better guide.

I have often seen students read their text descriptive of some organ—*e. g.*, a frog heart—and verify its statements from the accompanying figures, leaving almost unnoticed the object itself, which lay before them. A few brief questions or hints would have led them to discover from the object all of its essential features. Diagrammatic anatomical figures are sometimes useful in a laboratory manual, but true anatomical pictures are worse than useless—they bar the student's independent progress. If his laboratory manual contains illustrations of all apparatus and tissues, and of such experiments as admit of graphic records, the student makes similar draw-

ings in his notes, either unwillingly or dependently—frequently both. The laboratory work is thus robbed of much of the benefit it is intended to give the student. Independence and originality are completely defeated or aborted, except in the case of the rare student.

If the laboratory manual contains graphic records of experiments, much of the time of the demonstrator will be consumed in explaining to the students why the same physiological functions observed with slightly different apparatus and under slightly different circumstances may yield tracings which differ in minor detail from those in the book. The energies of both demonstrator and students will thus be partially diverted from their legitimate channel.

If there are no tracings in the text, students will naturally, by comparison of their tracings, discover the essential and the non-essential features and will seek the cause of the essential features of their tracings. After the student has made these independent discoveries he is in a position to gain the maximum profit from the comparison of his own tracings with those which others have taken, and from any explanations which the demonstrator may choose to add.

It is evident, then, that, from a pedagogical standpoint, the laboratory guide should be sparsely illustrated. On the other hand, the student's notes should be profusely illustrated.

Regarding Explanations. It may be well to introduce this topic by a statement of what the function of the demonstrator is not. It certainly is not to rob the student of the pleasure, exhilaration, and benefit of the independent investigation of a problem by introducing each laboratory period with an enumeration of the facts and principles which the work of the day is expected to establish. Such an introduction is worse than useless. The desirability of even asking the attention of the entire class to introductory remarks on the general bearing of the problem in hand is to be questioned. If the problem is well chosen and the work in the physiological laboratory properly co-ordinated with that in the recitation room and lecture room and that in the other departments, its significance will be at once evident to the intelligent pupil. If the introductory talk is omitted the prompt student may begin at once, upon entering the laboratory, the problem of the day, and will have a clear gain of ten or twenty minutes. Any supplementary introduction or hint may most profitably and economically be written upon the blackboard.

Most of the experiments given in this book cannot conveniently be performed by one individual working alone. After some experimentation it has been found most advantageous to divide the class into sections not exceeding thirty students, and to subdivide these sections into divisions of three students each. Each division is assigned a table. The assistant demonstrator places the material

needed for one day's work upon the table or where it is readily accessible.

Nothing should be done for the student which he can profitably do for himself. A small class with less limited time may easily construct much apparatus in the workshop. No class is so large as to debar the members from the privilege of setting up, adjusting, and readjusting all apparatus.

Nothing should be told a student which he can readily find out for himself. The function of the demonstrator is to guide the student by questions and by hints to discover facts and to formulate principles. Extended explanations on the part of the demonstrator may instruct the student, but they do not educate him.

Hints to the Students. It is a general principle that a student gets out of a course what he puts into it, and with interest. If he invests (1) intellectual capacity, (2) the spirit of inquiry and investigation, (3) the power of logical reasoning, and (4) the power to formulate conclusions, he will promptly receive interest upon the investment. Further, the greater the investment the greater the rate of interest. This may seem inequitable, but it is inevitable.

The value of taking full notes of laboratory experiments is unquestionable. The following hints regarding note-taking may be advantageous:

1. Make a careful description of each new instrument with which you work.
2. Formulate each problem definitely.
3. Describe the means used in the solution of the problem.
4. Enumerate the facts observed through the help of the means employed.
5. Seek for and note causes and interrelations of the facts as far as possible.
6. Differentiate the essential from the incidental.
7. Formulate conclusions from the collected data.
8. Make generalizations as far as they are justifiable.
9. A good note-book should possess the following qualities:

(*a*) It should be complete, containing an account of every problem studied.

(*b*) It should be full, containing a sufficient amount to guide another in performing the same experiments and in verifying the facts and conclusions noted.

(*c*) It should be logically arranged.

(*d*) It should be as neat and artistic as the student can make it in the time which he can devote to it.

PART I.

EXPERIMENTAL GENERAL PHYSIOLOGY.

This field of physiology is devoted to the laboratory observation of the general activities of the cells and tissues of living plants and animals. The study of cells is taken up under the head of Cytology.

CHAPTER I.

CYTOLOGY.

I. ALGÆ OR GREEN PLANTS OF LOW ORDER.

Cytology is devoted to the systematic treatise of the cell as a living organism, with reference to form as well as to function. In the unicellular organisms it is not profitable to make a sharp division between the discussion of the form and function; they should, rather, be discussed together.

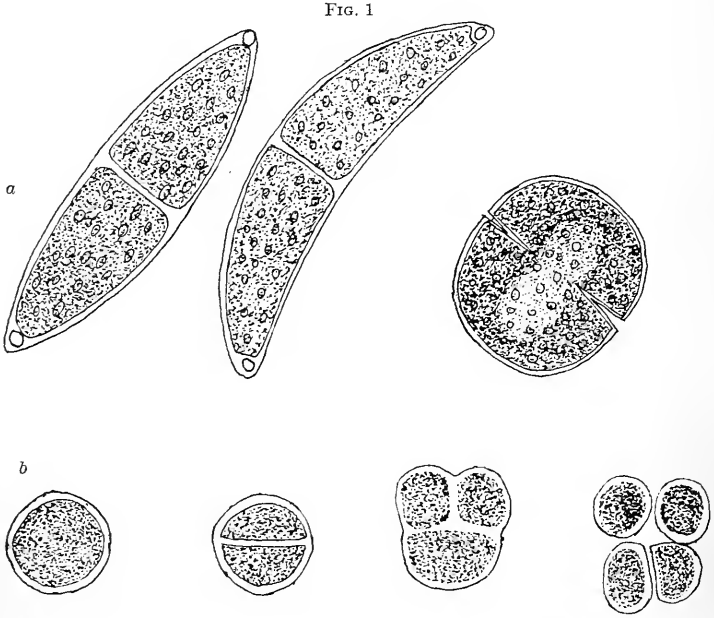
Interesting objects for the illustration of cell life are the Algæ, which are representatives of the lowest sub-kingdom of plants. Some of the Algæ are unicellular and some are multicellular. Some are motile and some are non-motile. All are provided with chlorophyll, which is a coloring matter, usually emerald-green, though sometimes a brownish-green and sometimes a bluish-green. Desmids, Protococcus, and Spirogyra are examples of non-motile Algæ possessed of green chlorophyll.

Desmids. These little plants are composed of a single cell, which may be circular, oblong, or crescentic. Each plant is divided into symmetrical halves, and the margins and the distribution of the chlorophyll are symmetrical and ornamental.

Protococcus. The green, dust-like coating of the tree-trunks and fence-posts is really composed of myriads of minute green plants, which are composed, like the desmid, of a single cell, though these cells are often loosely held together in colonies or families of three or four a short time after the young are formed. Reproduction in

these low organisms takes place by what is called fission. This process is a simple division of the cell body into two equal portions. The

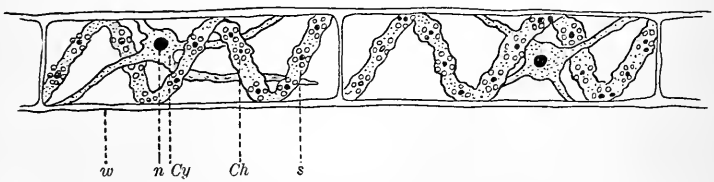
FIG. 1



a, *Desmids*. Common forms. Shaded portions of green chlorophyll, outer envelope of cellulose. *b*, *Protococcus*. Common forms, showing reproduction. A cellulose envelope enclosing chlorophyll.

first undivided cell is called the "mother cell" or the adult cell, while the two young cells resulting from the fission are called "daughter cells."

FIG. 2



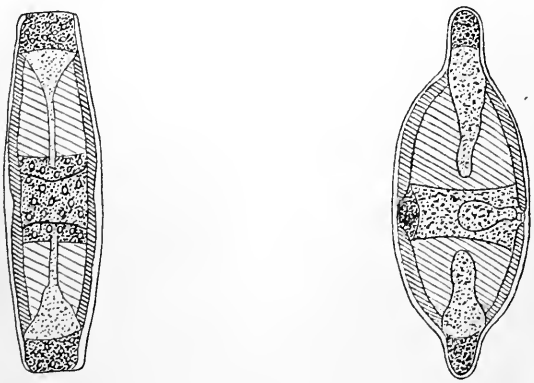
Spirogyra. Two cells of a long fibre are shown : *w*, cellulose cell wall; *n*, nucleus surrounded by cytoplasm (*Cy*), which sends out threads to the cell wall; *Ch*, chlorophyll band making two and one-half spiral turns around the inside of the cell wall; *s*, starch grain surrounded by several oil globules.

Spirogyra. This plant occurs in long, delicate threads which represent numerous cylindrical cells joined end to end. It receives its name from the spiral disposition of its chlorophyll. Each cell posesses two or three or four threads of chlorophyll, which are wound

spirally around the inside of the transparent cell wall, the threads appearing under the microscope to cross and recross in beautiful patterns. In reality, however, the threads do not touch each other.

After the cool October weather comes, one is likely to find in the spirogyra a most interesting change in progress. During the spring and early summer the spirogyra grows by a reproduction of its cells by fission; these cells remain together and the reproduction thus produces the long, hair-like threads. As these delicate threads cannot live over winter, nature prepares for this season by causing a new method of reproduction. Two cells lying side by side put out projections which fuse or join together, making a communication from one cell into the other. Through this communication the contents of one cell flows into the other and the contents of both cells are thus mixed. This process is called "conjugation." After the conjugation

FIG. 3



Diatoms. Having silicious envelopes or shells outside the exochrome.

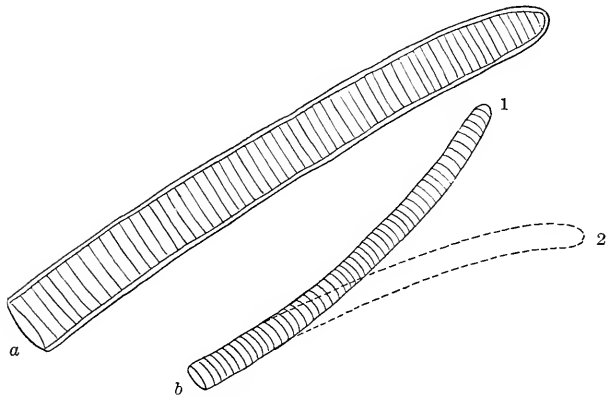
the new mass forms a thick cellulose covering and passes into a "resting stage" for the winter. With the warmth of returning spring the cellulose shell is burst and the new plant starts its summer growth.

Motion is so characteristic of the higher animals and a lack of voluntary motion so characteristic of higher plants, one naturally associates motion with animal life. But many of the lower orders of plants have the power of locomotion, while many of the lower animals—*e. g.*, corals and barnacles—are as fixed as a tree.

Among the algæ (motile) are diatoms and oscillaria.

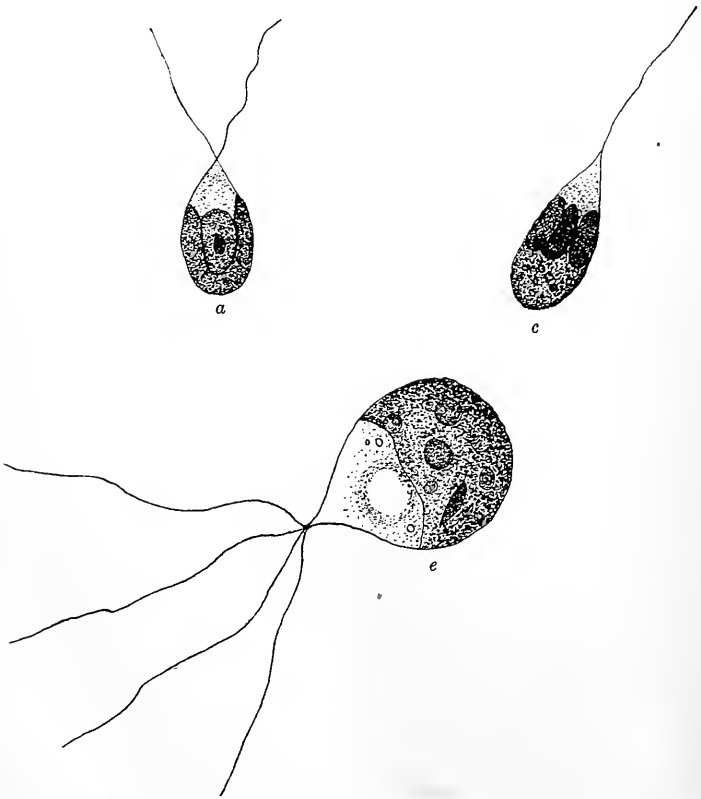
Diatoms. These are one-celled plants possessing a brownish-green chlorophyll and encased in two delicate silicious scales, which fit together like a box and its cover. They are boat-shaped and move slowly across the field of the microscope, pushed along by a

FIG. 4



Oscillaria. *a*, enlarged fibre; *b*, same, showing oscillation from position 1 to position 2, in sunlight.

FIG. 5



Swarm spores. *a*, of Acetabularia; *c*, of Botrydium; *e*, of Ulotrix.

trail of mucus which they give out in a continuous stream when in motion.

A high magnification usually shows fine transverse lines across the diatom shell. These lines are so fine in some species of diatom that these little plants serve as test objects to test the optical powers of a microscope.

Oscillaria. The oscillaria is a thread-like motile alga, provided with a bluish-green chlorophyll. The threads are not long and the motion consists in a jerky, oscillatory movement of the ends of the threads.

Swarm spores of the fresh-water algæ are unicellular and are the best examples of motile plants of the lower order. The first thought that comes to one while watching the active little plants is, Why do they move? The animal lives upon plants or other animals and must be provided with some means of catching its food. The green plant lives upon carbon-dioxide gas and water, with the salts dissolved in the water. But as the swarm spores live in water in which CO_2 and mineral salts are dissolved, why should they be endowed with locomotion?

Laboratory Exercises.

1. **Appliances.** Microscope with high and low power; slides, plain and celled; cover-glasses; pipette.

2. **Preparation.** Several well-stocked aquaria, stocked with pond scum, slime, ooze, and pond water gotten from stagnant ponds which lie in not too close proximity to a manufacturing district or railway. By stocking aquaria from different ponds one is likely to find all the above-named forms (except protococcus) and perhaps many other forms. Keep the aquaria near the windows, where they will get the warm sunshine during the day. In addition to the above, one will do well to prepare an infusion of hay and keep the same in a 500 c.c. beaker. The amœba, paramœcium, and dileptus are likely to appear in the liquid after a few days, while it will swarm with myriads of bacteria.

3. **Observations.** (1) Take a drop of water from near the top or bottom of one of the aquaria, place it upon a slide, put a clean cover-glass gently over it, and focus under the low power of the microscope. Look for any of the organisms above described. As the aquaria will probably differ somewhat the one from the other in the organisms which they contain, the student will do well to examine the contents of all the aquaria. Study all forms of life. Determine, if possible, in the first place whether an organism is plant or animal. If it seems to be plant, determine whether or not it represents one of the common algæ above described. If so, make a careful study of it.

(2) Draw a careful figure of all forms studied, making the figure large enough to show the details of structure clearly. Indicate in the figure the location of the chlorophyll. If a nucleus or other prominent mass is seen within the cell, locate it carefully in the drawing.

(3) Note carefully whether or not the organism moves. If it does, determine the cause of the movement and describe it minutely.

II. THE YEAST PLANT.

The yeast plant (saccharomyces) belongs to a fungi. The fungi and the algæ belong to the lowest sub-kingdom of plants—the Thallophytes—which are characterized by the absence of root, stem, and leaf. The student will remember that all the algæ which he has studied have possessed chlorophyll or green coloring matter. This is true of the algæ in general. The fungi, however, have no green coloring matter. The toadstool is a fungus, and it is wholly without the green color which most plants possess. The yeast plant is a fungus and it has no chlorophyll.

What is the work which the chlorophyll does for the green plants?

How does the yeast plant get its living?

Laboratory Exercises.

1. Put a bit of fresh yeast about as large as the head of a pin upon a glass slide, mix it with normal saline solution (0.6 per cent. NaCl in aq. dest.) and study under high power. Draw and describe the cells. The yeast reproduces by budding or gemmation. It reproduces also by the formation of internal spores (ascosporæ) (Fig. 6). Let your figure show various forms and combinations of cells. Is the protoplasm of the cells homogeneous or is it granular? Is a reticulum visible under your microscope?

2. Put a piece of fresh yeast about the size of a hazelnut into 30 c.c. of Pasteur's fluid, and stand this for a period of one hour in an incubator kept at a temperature of 35° to 40° C.

Pasteur's Fluid:

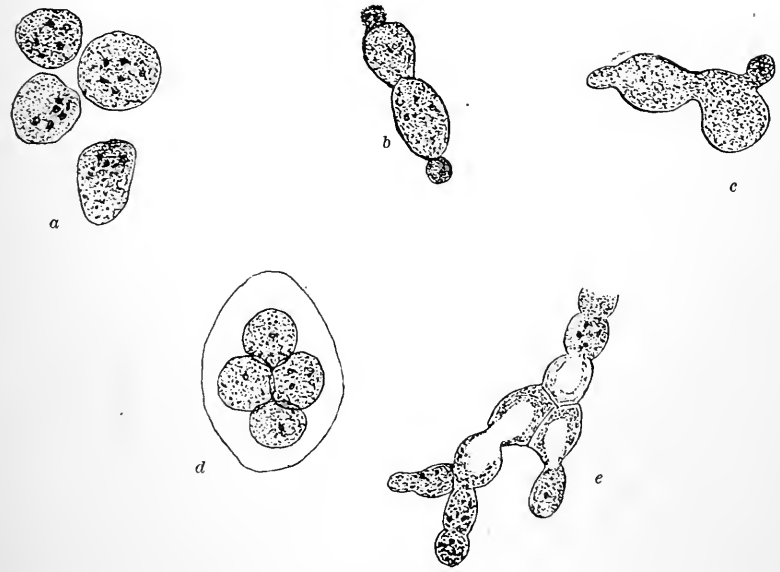
Potassium phosphate	2.0 grams.
Calcium phosphate	0.2 "
Magnesium sulphate	0.2 "
Ammonium tartrate	10.0 "
Saccharose (cane-sugar)	150.0 "
Aqua q. s. ad	1000.0 "

After a half or three-quarters of an hour remove the yeast from the incubator and examine the mixture carefully. Note the rapid escape of bubbles from the liquid. If the liquid be placed in a

small flask and a tube led from the closed flask into a solution of $Ca(OH)_2$ the character of the gas will be revealed—it is carbon dioxide, CO_2.

The cane-sugar gives the Pasteur solution a sweet taste. Taste an yeast mixture that has been in the incubator twenty-four hours. It has lost its sweetness and gained the taste of alcohol and CO_2. The yeast plant consumes sugar and breaks it up to CO_2 and H_2O. The alcohol and CO_2 are waste products which the yeast throws out because they are useless to it.

FIG. 6



Saccharomyces, or yeast plant: *a*, isolated yeast cells; *b*, *c*, gemmation; *d*, endogonidia or ascosporæ; *e*, budding of the endogonidia.

3. Place 5 c.c. of the yeast mixture in a test-tube and insert the tube in boiling water for a minute, or hold the tube over a Bunsen burner until the mixture comes to a boil. What is the influence of the heat upon the life of the yeast? Is there any evidence that the yeast has been killed; if so, what?

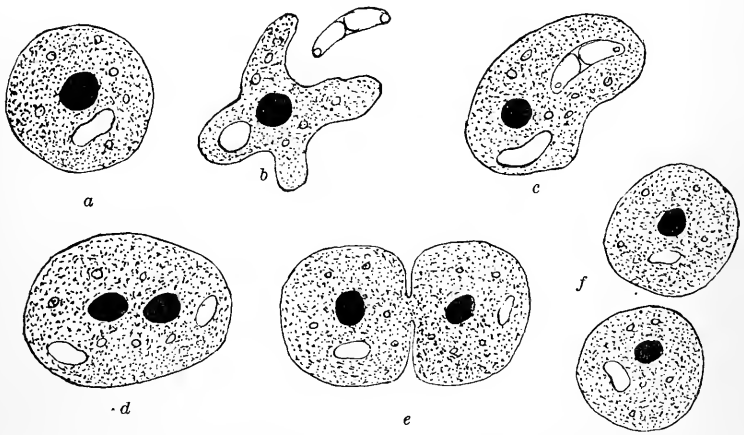
4. Place 5 c.c. of an active mixture in a test-tube and add alcohol (95 per cent.) drop by drop until a change is noticed in the activity of the yeast. Has the yeast been stimulated? If not, what has the effect been? Account for the results.

5. To another 5 c.c. of active yeast mixture add crystals of common salt, stirring gently to cause solution, and note results.

III. PROTOZOA OR ONE-CELLED ANIMALS.

We come now to an entirely different order of beings—the protozoa, or one-celled animals. They live in the water and feed upon unicellular plants. They sometimes contain granules of chlorophyll, sufficient in quantity to give them the appearance of motile plants, but the chlorophyll has been taken up with the plants which they have eaten. Chlorophyll thus absorbed is retained only a short time and is then excreted. The protozoa are classified as follows:

FIG. 7



Amœba. *a*, at rest; *b*, extending *pseudopodia* in search of food; *c*, food enclosed; *d*, reproduction by fission, beginning with division of nucleus and vacuole; *e*, cytoplasm dividing; *f*, reproduction complete.

Protozoa. One-celled animals.

1. Rhizopoda, protozoa possessing bodies of changeable shape.
   (*a*) Helizoa, naked rhizopoda. Ex. amœba (Fig. 7).
   (*b*) Foraminifera, marine rhizopoda with porous shells.
   (*c*) Radiolaria, marine rhizopoda with concentric spherical shells.
2. Infusoria, protozoa possessing bodies of fixed shape.
   (*a*) Flagellata, infusoria that swim with a whip-like flagellum. Ex. Euglena (Fig. 8).
   (*b*) Ciliata, ciliated infusoria with mouth and anus. Ex. Paramœcium (Fig. 9). Vorticella (Fig. 10).

Laboratory Exercises.

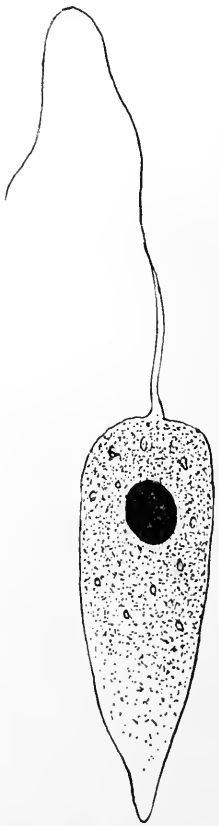
1. **Appliances.** Microscope with $\frac{1}{4}$-inch to $\frac{1}{6}$-inch objective; cell slides; covers; aquaria well stocked with protozoa; drop-tubes; filter

paper and absorbent cotton; 50 per cent. alcohol; ether; saturated salt solution; aqueous 10 per cent. solutions of iodine, of tannic acid, of picric acid, and of nitrate of silver.

2. **Observations.** Place a small drop of water containing euglenæ, amœbæ, paramœcia, vorticellæ, or other protozoa on the center of a perfectly clean cover-glass. If there are any drops of fibrous algæ, as conferva or spirogyra, the cover may be inverted upon a clean slide and the animals studied. If, however, there are no plants present, the cover-glass may be supported upon a hair laid across the slide, otherwise the cover will settle down upon the animals and prevent their free movements.

After the cover is properly supported and the organisms focused, make a careful study of the forms present, describing their structure and such activities as are observed.

FIG. 8



Euglena viridis. A flagellate infusorian.

To Determine the Influence of Carbon Dioxide upon the Activity of Animal Cells.

1. **Appliances.** Microscope with high power; ventilated, deep celled slide; ventilating apparatus, consisting of reservoir, siphon, and pressure bottle with connections as shown in Fig. 11 (p. 34); aquarium stocked with protozoa; normal saline solution (NaCl 0.6 per cent.); CO_2 gas generator.

2. **Exercises.** (1) (*a*) Set up the ventilating apparatus as shown in Fig. 11. The slide should be clamped upon the stage of the microscope with the help of the stage clips. Disjoin the CO_2 flask at *S* and *d;* fill and clamp the siphon; fill the flask with CO_2 from the generator and replace it in the apparatus; close both clamps.

Mount a hanging drop of protozoa from the aquarium; focus under high power. While watching the movements of the protozoa loosen the siphon clamp; after the siphon starts, loosen the gas clamp slightly to admit a little of the CO_2. If after a half-minute or more no appreciable change takes place in the rate of movement of the cilia, repeat the dose of gas. What is the effect of CO_2 upon the activity of protozoa?

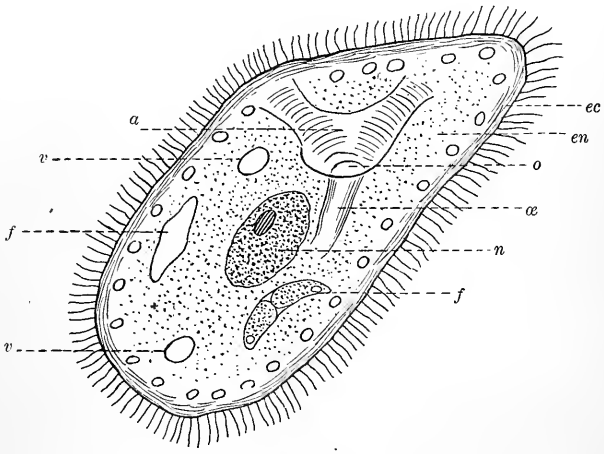
(*b*) After the effect of the gas has become apparent, clamp the

tube at *S;* disjoin the gas tube at *d,* and gently draw air through the cell, thus ventilating it and restoring practically the normal condition. Do the cells resume their normal movement?

(*c*) How many times may the cells be brought under the influence of the CO_2 and then, by ventilation, be brought to the normal condition again?

Do you see evidence that any of the animals eat green plants? Do you note any of the reproductive changes in any organism? What is the method of locomotion of the forms studied? Do the organisms respond to such mechanical stimuli as a gentle rapping upon the slide with pencil or scalpel?

FIG. 9



Paramœcium bursarium. *ec*, ectoplasm; *en*, endoplasm; *n*, nucleus with nucleolus; *a*, vestibule; *o*, oral aperture; *œ*, œsophagus; *v*, vacuoles; *f*, ingested food.

(2) With a drop-tube place a drop of saturated salt solution at one edge of the cover. By placing a piece of dry filter paper at the other edge of the cover, the capillary attraction of the paper will draw the salt solution under the cover and thus mix it with the aquarium water. Study the effect of this upon the animal organism present and describe minutely everything observed.

(3) Place a drop of 50 per cent. alcohol beside the cover-glass and draw it under as described above. Note results as above.

(4) In a similar way study the effect of iodine, tannic acid, picric acid, and nitrate of silver.

(5) Take a deep-celled slide; upon the top of the cell invert a clean cover-glass to which is clinging a "hanging drop" taken from an aquarium well stocked with protozoa. The "hanging drop" should be a small one for two reasons: (1) objects within a small hanging

drop are more readily focused with a high-power objective; (2) if the drop is small, vapors penetrate more readily to the organisms. Focus upon the drop through the cover-glass. If you find active protozoa, study their movements and note the degree of activity of these movements.

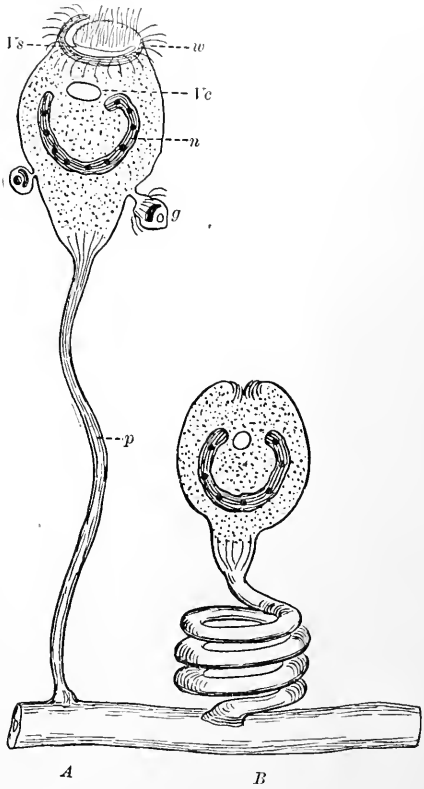
Prepare a little roll of absorbent cotton about as large as a pea, saturate it with 50 per cent. alcohol and place it in the bottom of the cell, at one side in order not to interrupt the light.

Replace the cover-glass and focus again upon the organisms which you studied a few moments before. Note carefully whether or not there is any change of activity; if so, describe minutely what you have observed.

(6) If a change in activity is noticed, remove the cover-glass, expose it to the air for two or three minutes, then invert it over a clean, dry cell, and note whether there is a partial or complete return to the normal activity.

(7) Repeat this experiment, using fresh protozoa and ether (50 per cent.) instead of alcohol.

FIG. 10



Vorticella. *A*, expanded condition; *n*, nucleus; *Vc*, vacuole; *w*, peristome; *Vs*, vestibule; *g*, gemma; *p*, pedicle; *B*, contracted condition. The pedicle is thrown into a spiral coil, drawing the body to the point of attachment.

(8) Repeat, using dilute ammonia or oil of peppermint.

IV. NORMAL CILIARY MOTION.

1. **Appliances.** Microscope, cell slide, and cover-glass; physiological operating case (see Appendix, 3); normal saline solution; frog or clam; camel's-hair brush or absorbent cotton; frog board (see Appendix, 2) and cork board.

2. **Preparation.** **To Pith a Frog.** (1) Grasp it with the left hand, holding the legs extended, one on either side of the little finger, in

such a way as to bring the dorsum of the frog toward the palm of the hand.

(2) With the thumb and index finger grasp the frog's nose and press it ventrally.

(3) Place the point of a narrow-bladed scalpel in the median dorsal line over the space between the occiput and atlas—*i. e.*, over the occipito-atlantal membrane. This point is most readily located by using the eyes as a landmark.

The occipito-atlantal membrane lies at the apex of an equilateral triangle whose base has its extremities in the center of the corneæ and whose apex extends posteriorly. Having located the point of incision, press the knife through the skin, the intervening connective tissue and the occipito-atlantal membrane, and cut the spinal cord transversely. Withdraw the knife.

(4) Insert the apex of a slender probe or of a blunt needle into the incision, turning it sharply forward so as to enter the cranial cavity. By sweeping the distal end of the probe from side to side the contents of the cranial cavity may be functionally destroyed. When it is required simply to pith a frog it is understood that the operation is complete as described above. It may, however, frequently be necessary to destroy the spinal cord as well as the brain. To accomplish this insert the needle as described under (4); but turn the point of the probe so that it shall enter the neutral canal of the vertebræ. Pass it along this canal to a point nearly opposite the anterior end of the ilia. Withdraw the probe.

A pithed frog can suffer no pain, but will respond reflexly to certain stimuli. A pithed frog whose spinal cord is destroyed cannot with the skeletal muscles respond reflexly to any stimuli. Having pithed the frog destroy its spinal cord, pin it to a frog board, with dorsum down and legs extended.

To Remove the Œsophagus of a Frog. (1) Place the head of the frog nearer to the operator. With forceps lift the mandible and with stronger scissors sever the whole floor of the mouth transversely and as far posteriorly as possible. Divide the skin in the median line as far posteriorly as the pubes.

(2) Separate the two lateral halves of the sternum by dividing the median sternal cartilage and carry the incision through the xiphoid appendix and abdominal walls. Withdraw the pins which fix the anterior extremities; separate the lateral halves of the sternum by lateral traction upon the anterior limbs.

(3) With the forceps grasp a fold of the mucous membrane which surrounds the puckered anterior end of the œsophagus. While making gentle traction with the forceps make, with fine scissors, a circular incision through the mucous membrane surrounding the opening of the œsophagus.

(4) Grasp the pyloric end of the stomach, sever the duodenum,

lift the stomach up vertically above the sternum, and make moderate traction. The delicate and elastic submucosa about the end of the œsophagus will yield to the traction and the whole œsophagus will be readily separated from the surrounding tissues and wholly removed from the frog.

(5) Open the stomach and œsophagus by means of a longitudinal incision through their walls; stretch them on a cork board, fixing with pins, and gently wash off mucus with normal saline solution and camel's-hair brush. Remove the excess of liquid with the help of filter paper.

3. **Observations.** (1) Place a small piece of cork upon the anterior end of the œsophagus. Does the cork move? If so, in what direction and at what rate?

(2) Will the cork pass over the boundary line between œsophagus and stomach, and will it move over the surface of the stomach?

(3) To determine the cause for the movement of the cork, cut a minute portion of mucous membrane from the crest of one of the folds, place it in a hanging drop of saline solution mounted over a cell slide, and examine with a microscope. If the preparation has been properly made the margin of the tissue should, at certain points, show the cause for the phenomena above observed. Study the character of the ciliary movements. Describe.

(4) Study ciliary movement with higher power. It is probable that the first preparation is not suited to observation with a high power. If the cilia cannot be readily brought into focus, prepare a second one as follows: From the ciliated surface—clam-gill or frog-œsophagus—scrape a few epithelial cells with the point of a scalpel, place the minute bit of tissue upon a cover-glass, add a small drop of saline solution, gently tease the tissues with needles, and invert the cover upon a slide, allowing one edge to rest upon a hair, to avoid undue pressure upon the tissue.

Focus under high power (300 to 600 diam.). If the preparation is successful, groups of ciliated cells may be seen and the character of the ciliary movement studied.

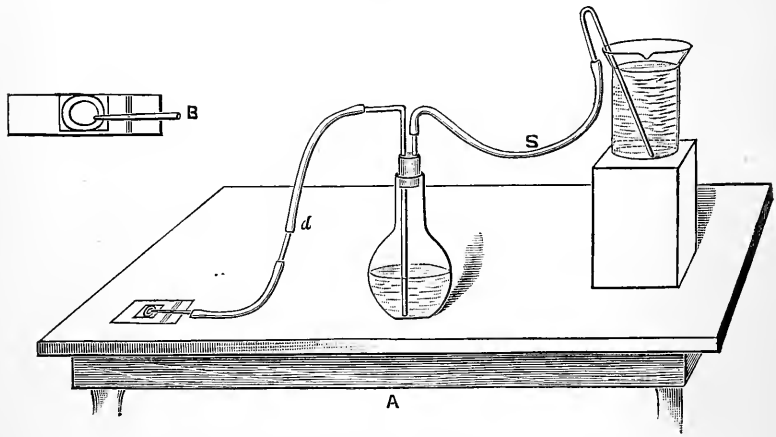
V. CILIARY MOTION MODIFIED BY THE INFLUENCE OF CO_2 AND ANÆSTHETICS.

1. **Appliances.** In addition to the appliances enumerated in the foregoing lesson one needs a ventilating apparatus with ventilated cell slide. Chloroform, ether, absolute alcohol.

Fill the glass flask full of water and displace it with CO_2 gas. Fill the siphon and adjust apparatus as shown in Fig. 11. During any readjustments of the apparatus the siphon may be kept filled and ready for action by putting on a screw clamp at *S*. Through

varying the height of the receptacle into which the siphon dips or through adjustment of the screw clamp or of the spring clamp at *d*, the pressure and the rate of flow of gas are under perfect control. Prepare a specimen of cilia for observation with a low-power microscope. (Great care must be taken to *remove all of the mucus*, otherwise the CO_2 may have little effect on the cilia.) Bring a good specimen into the field, focus the microscope, and observe the rate and character of ciliary movement. Remove screw clamp at *S*.

FIG. 11



Apparatus for forcing a stream of gas or vapor through a microscopic slide chamber. (For description see text.)

2. **Observations.** (*a*) *The effect of CO_2 upon ciliary activity.*

(1) While observing closely the normal action of the cilia, press the spring clamp gently for a few moments. If after half a minute or more no noticeable change takes place in the rate of movement of the cilia, repeat the dose of gas. What is the effect of CO_2 gas upon the activity of cilia?

(2) After the effect of gas has become apparent, clamp the tube at *d;* disjoin at glass tube beyond and gently draw air through the cell, thus ventilating it and restoring practically the normal condition. Do the cilia resume the normal movement?

(3) How many times may the cilia be narcotized to the point of complete cessation of activity and then by ventilation be revived again?

(*b*) *The effect of chloroform gas upon ciliary activity.*

(4) Clamp tube at *S;* remove flask from apparatus; fill flask with water to expel CO_2; empty. (Suspend in the flask a wad of cotton saturated with chloroform or ether.) Make a new preparation of cilia and observe normal movement.

Allow the chloroform gas to flow for a moment into the cell. Note the effect of chloroform upon ciliary activity.

(5) How many times may the cilia be narcotized with chloroform and revived again through ventilation?

(6) Repeat (4) with ether in place of chloroform.

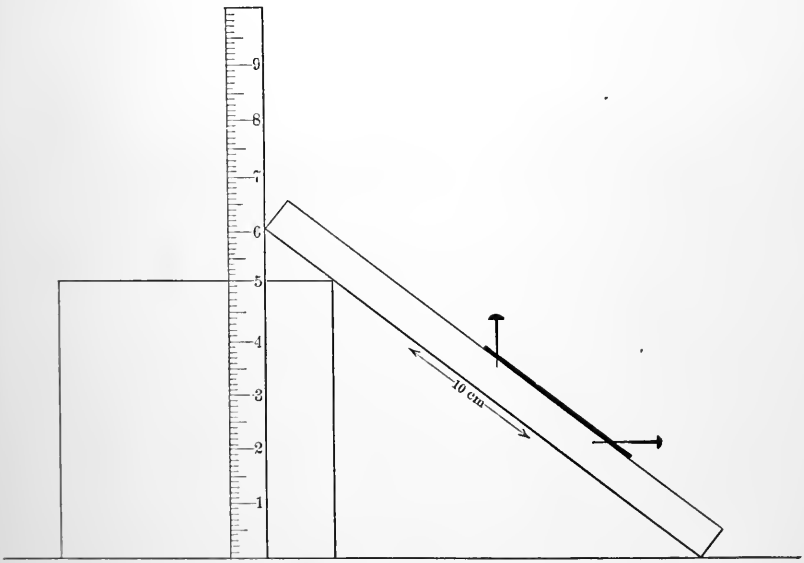
(7) Repeat (5) with ether in place of chloroform.

(c) *Determine the action of alcohol vapor upon cilia.*

VI. TO DETERMINE THE AMOUNT OF WORK DONE BY CILIA.

1. **Appliances.** Physiological operating case; frog board; cork board 10 cm. long by 5 cm. wide; a centimetre rule; a block of wood 4 cm. or 5 cm. in height; a set of weights as follows: 50 mgm. and 100 mgm., 3 mm. square; also 100 mgm. and 200 mgm., 5 mm. square.

FIG. 12



Apparatus for use in determining the amount of work done by cilia. (For description see text.)

2. **Preparation.** Pith a frog and destroy cord. Dissect out œsophagus and stomach as directed in Lesson IV. Fix to cork board so that the long axis of the œsophagus shall be parallel with the long axis of the board.

3. **Operation.** Wash off ciliated surface, remove surplus moisture with filter paper, and place a lead weight gently on the anterior end of the œsophagus.

The incline of the ciliated surface may be changed by resting it, at different angles, against the block of wood as shown in Fig. 12.

4. **Observations.** (1) If the preparation is successful, the piece of metal will be slowly carried up the incline. Should it fail, a thinner piece of lead or a new preparation may succeed. With a given incline, is the small piece of lead carried more rapidly than the large piece?

(2) If W = work done, g = weight in milligrams and h = height in millimetres, then $W = g \times h$ would give the work in milligram-millimetres.

(3) Determine the distance through which the weight is carried in a unit of time (one minute is a convenient unit of time to use), when the incline is placed as shown in Fig. 12.

(4) With the apparatus so adjusted, what is the value of h when the distance which the weight moves is 1 cm.? Does the thickness of the cork board need to be considered?

(5) What is the work per minute, expressed in milligram-millimetres?

(6) What is the work per minute, expressed in gram-centimetres?

(7) What is the work per minute, expressed in ergs? (An erg = 1 dyne $\times$ 1 cm.; 1 dyne = 1 gm. divided by 981 or 1 gm. = 981 dynes; therefore, 1 gram-centimetre—981 dyne-centimetres. To express work in ergs find the gram-centimetres and multiply by 981.)

(8) What is the "activity" of the cilia in *work per second?* Divide ergs per minute by 60 to get ergs per second.

(9) Using the same incline of the cork board, with which weight do you get the greatest activity?

(10) Using the weight which gives the greatest activity, find the degree of incline which yields the greatest activity of cilia.

(11) What significance has the variation of the thickness of the lead weight? Determine the upper limit of thickness.

(12) Would it be possible to determine the amount of work accomplished by each cilium? By each stroke of a cilium?

CHAPTER II.

THE GENERAL PHYSIOLOGY OF MUSCLE AND NERVE TISSUE.

VII. ELECTRIC APPARATUS AND UNITS OF MEASUREMENT.

THE function of muscle tissue is to contract. Muscles contract only in response to stimuli. Stimuli may act upon the muscle tissue —direct stimulation; or upon the motor nerve which supplies the muscle—indirect stimulation. To study the functions of muscles and nerve tissue one requires to have at command various methods of stimulation. It is usual to apply mechanical, thermal, chemical and electric stimulation. Experience has shown that of all these means electricity is the most valuable, because it is subject to the greatest number of variations in strength and in method of application. Before entering upon a study of the response of irritable tissues to electric stimuli it is essential to make a short study of the appliances used. As many of these appliances have been used by the student in the physical laboratory it will be taken for granted that he is familiar with the principles involved in their use.

1. **Appliances.** Two Daniell elements or cells; wires; contact key; Du Bois-Reymond key; mercury key; commutator; 10 per cent. sulphuric acid; copper sulphate, saturated solution; mercury.

2. **Experiments and Observations.** (*a*) **The Daniell Cell.** Note the four parts of the cell. Half-fill the outer receptacle of the cell with the saturated copper sulphate solution. Put the copper plate into the cell; half-fill the porous cup with the dilute sulphuric acid; lower the zinc plate carefully into the cup. The plate is of commercial zinc with its various impurities.

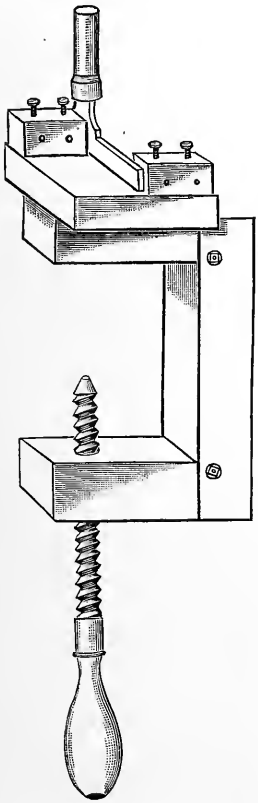
(1) Observe the vigorous chemical action in porous cup. Express the reaction in symbols. It is evident that the zinc will be quickly consumed if allowed to remain in the acid, and this will be the case whether or not the cup and zinc plate be made a part of an electric cell, and whether the cell be acting or resting.

(2) *The amalgamation of the zinc.* (See also Appendix, 4.) Lift the zinc plate out of the acid, dip it into the mercury. The mercury adheres to the zinc, mingles with the surface layer of zinc, forming an alloy; with a brush or an old cloth one may rub the mercury over the whole surface of the zinc plate—the zinc is amalgamated. The impurities of the zinc do not enter into the alloy. In this way only the pure zinc which forms a part of the alloy is presented to the

acid. Chemically pure zinc is acted upon very slowly by 10 per cent. sulphuric acid. Join a wire to the exposed end of each plate; touch the tongue with the free end of each wire separately; touch the tongue with both wires simultaneously. Record results.

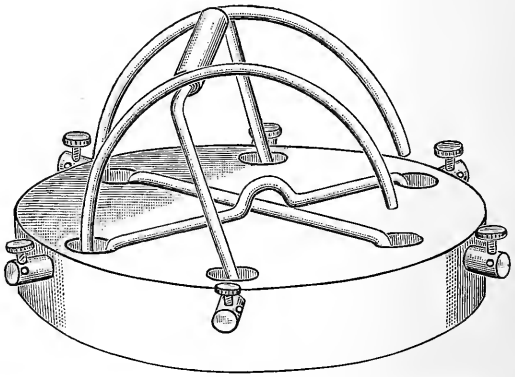
(3) Place the porous cup with the zinc plate in the receptacle holding $CuSO_4$ with the copper plate. Touch the tongue with one wire, then with the other. Touch the tongue with both at once. Bring the two free ends of the wire into contact with the binding posts of a galvanoscope. Note results. Touch the ends of the wires together; if the conditions are favorable a minute spark may be seen on touching and on separating the two poles. What conclusions are to be drawn?

FIG. 13



The Du Bois-Reymond key.

FIG. 14



The pole changer, or the Pohl commutator. (For description see text.)

(4) Define element or cell as used in this connection. Define plate, pole, electrode. The zinc is arbitrarily taken as the positive plate and the copper as the negative plate. The pole which is attached to the negative plate is the positive pole, and that which is attached to the positive plate is the negative pole. The positive pole or electrode of a galvanic cell or of a battery is called the anode, while the negative pole or electrode of a cell or of a battery is called the cathode.

(*b*) **Keys.** (1) Study and describe the simple contact key (Fig. 25, *K*) and the Du Bois-Reymond key (Fig. 13). (2) The two ways of using the Du Bois-Reymond key are shown in the figures: first, as a contact key (Fig. 15); second, as a short-circuiting key (Fig. 16).

(*c*) **The Pole Changer or Commutator.** Most convenient for the physiological laboratory is Pohl's commutator (Fig. 14). This instrument may be used for the following purposes:

Fig. 15

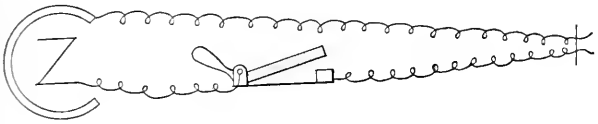


Fig. 16

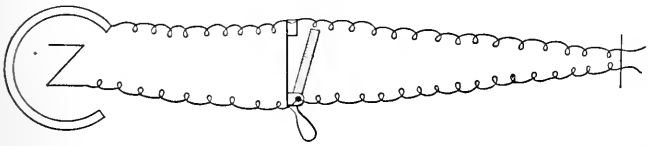


Fig. 17

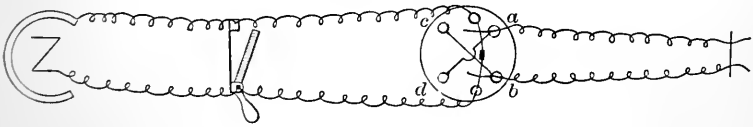


Fig. 18

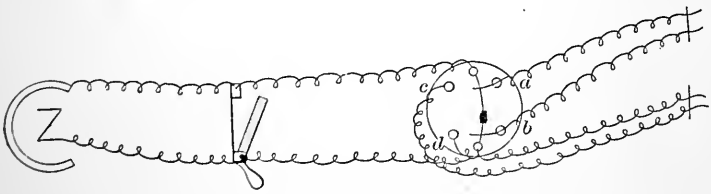
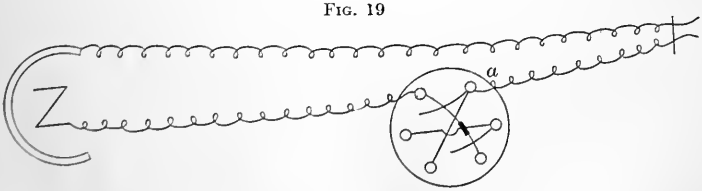


Fig. 19



(1) To change the direction of the current. Set up apparatus with cross-bars in place as shown in Fig. 17. Which is the anode when the bridge is turned toward a *b*? Which is the anode when the bridge is turned toward *c d*?

(2) To change the course of the current. Set up apparatus with cross-bars removed as shown in Fig. 18. What course will the current take when the bridge is turned toward *a b?* What course when the bridge is turned toward *c d?*

(3) Pohl's commutator may be used as a simple mercury key (Fig. 19). Is the current open or closed when the commutator bridge is turned toward *a?* How may the current be opened or broken?

(*d*) **Work Done by the Electric Cell.** The experiments performed show that the galvanic cell may, under proper conditions, liberate energy. This energy is called electricity. But the immediate source of the particular electric energy liberated in the foregoing experiments is the latent chemical energy represented in the plates and liquids of the cell.

Under the conditions produced in the working galvanic cell the latent chemical energy is transformed, and at the same time liberated as electric energy. This liberated electric energy may make itself manifest in the contact spark, in moving the galvanoscope needle, or in lifting the armature of a magnet. In the last case mentioned it would not be difficult to determine the amount of work done, though it might be somewhat difficult to determine the amount of work which a cell is capable of performing in a given time. If one were to weigh the copper plate before and after using the cell, one would find that it had increased in weight. This increase in weight is an index of the amount of chemical action in the cell—of the latent chemical energy which has been transformed into electric energy.

The amount of electrolysis must be, then, an index of the amount of current that is afforded by a cell or battery. For example, if the negative pole of a cell be attached to a silver or platinum cup containing pure nitrate of silver, and the positive pole be attached to a piece of pure silver which is immersed in the silver nitrate solution, it will be found that one ampère of current will uniformly deposit 0.001118 gm. of silver upon the cup in one second of time. This brings us to the question of the units of electric measurements.

(*e*) **Electric Units.** The electric energy available at any point in a circuit—*i.e.*, the current, as it is called—is, according to Ohm's law, equal to the liberated energy—the electromotive force—divided by the total resistance of the circuit. This is expressed in Ohm's formula, $C = \frac{E.\ M.\ F.;}{R}\ C = \frac{E}{R}$. It is impossible for the physicist to make any progress in the study of electric energy without arbitrarily assuming units of measurement for current, for electromotive force, and for resistance.

(1) Current is measured in *ampères.* A current of 1 ampère deposits upon the negative electrode of a galvanic cell or battery is 0.001118 gm. of silver per second, or 4.025 gm. per hour. (See above.)

A concrete idea of the ampère may be gained from the fact that the small-sized Daniell cell produces a current of about $\frac{1}{4}$ ampère when the external resistance is reduced to a minimum.

(2) Resistance is measured in *ohms*. An ohm is that amount of resistance opposed to the transmission of electric energy by a column of mercury 1 sq. mm. in cross-section and 106.3 cm. in length. For general purposes an ohm resistance is that of a pure silver wire 1 mm. in diameter and 1 metre in length.

(3) Electromotive force is measured in *volts*.

A volt is that amount of electric energy which will produce 1 ampère of current after overcoming 1 ohm of resistance.

"The ohm, the ampère, and the volt are thus closely related, and if any two of them be known with reference to any particular electric circuit or portion of a circuit the value of a third may be readily inferred" (Daniell). For if $C=\frac{E}{R}$, then $E=C\times R$ and $R=\frac{E}{C}$. The same relations may be expressed thus: 1 ampère current $=\frac{1 \text{ volt } E.\ M.\ F.}{1 \text{ ohm resistance}}$, or 1 ampère $=\frac{1 \text{ volt}}{1 \text{ ohm}}$.

Therefore (1) volts = ampères × ohms; (2) ampères = volts ÷ ohms; (3) ohms = volts ÷ ampères.

The small Daniell cell has about 1 volt *E. M. F.* and 4 ohms resistance; the current from such a cell is then equal to approximately $\frac{1}{4}$ ampère.

There are numerous other units of measurement used by physicists and electricians, but for our purpose it is not necessary to review these more specialized points.

VIII. BATTERIES.

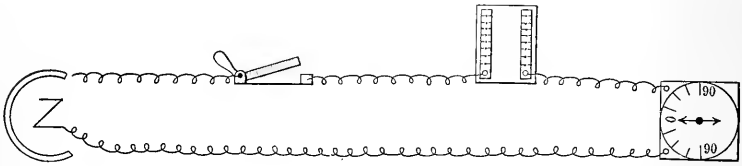
A battery is a group of two or more elements or cells arranged to produce increased or modified effect. If one wishes to use a stronger current than that afforded by one cell, his first thought is to increase the number of cells, or to procure a larger cell. Experimentation will show him that it is not a matter of indifference which of these courses to pursue. In the first place, if he attempts to satisfy the conditions he will find that to increase the size of a cell increases the current only when the external resistance is relatively small, and, furthermore, there are practical limitations to the size of a cell, and these may be much within the requirement which the cell must satisfy. It becomes apparent, then, that he who would use electric energy beyond the most limited field must resort to a battery composed of a number of cells. The problem which first confronts him is, How shall these cells be arranged?

1. **Appliances.** Six Daniell cells; wires; galvanoscope (Fig. 24), composed of a simple magnetic needle mounted over a circle divided

into degrees; rheostat or resistance box, representing at least 100 ohms.

2. **Experiments and Observations.** (1) (*a*) Join up apparatus as shown in Fig. 20. With the plugs all fixed in the rheostat—*i. e.*, with no resistance except that of the wires and battery, and the indicator needle at 0°—open the key and then observe the angle at which the needle comes to rest.

FIG. 20



(*b*) Remove from the rheostat the plug which will throw into the circuit an extra resistance of 10 ohms. Allow the needle to come to rest and note angle.

(*c*) Remove from the rheostat plugs which will represent in the aggregate 100 ohms extra resistance. Note angle of indicator as before.

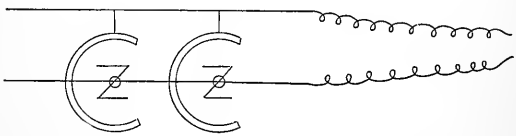
(2) Join up two cells in multiple arc, as shown in Fig. 21. That is, join both copper plates to one copper wire and both zinc plates to another. These wires are to be carried to key, rheostat, and galvanoscope, as shown in Fig. 20.

(*a*) Note angle of needle with no extra resistance.

(*b*) Note angle with 10 ohms extra resistance.

(*c*) Note angle with 100 ohms extra resistance.

FIG. 21



(3) Join up four cells in multiple arc or "abreast," and repeat the observation of angle at the three resistances as above.

(4) Join up six cells in multiple arc and repeat observations with 0 ohm, 10 ohms, and 100 ohms resistance.

(5) Join up two cells in series, as shown in Fig. 22. That is, join the copper of the first cell to the zinc of the second. The first cell will have a zinc uncoupled and the second will have a copper plate uncoupled. These two uncoupled terminal plates of the battery are the ones from which to lead off the wires to the

other apparatus, which should be arranged as shown in Fig. 20. Repeat the observations on the angle of deviation of the needle using the 0 ohm, 10 ohms, and 100 ohms resistance as above.

(6) Join up four cells tandem or in series, and repeat the three observations.

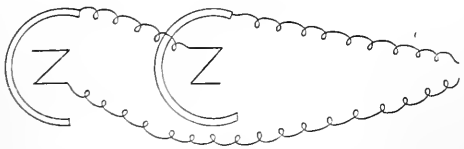
(7) Join up six cells in series and repeat observations.

(8) Tabulate results and draw conclusions:

1. There is a marked difference in the results of the two methods.

2. With low external or circuit resistance the current is indicated by the angle at which the galvanoscope needle stood increased with an increase in the number of cells joined multiple arc or abreast.

FIG. 22.



3. With high external resistance the strength of the current does not seem to be essentially increased by increasing the number of cells joined up abreast.

4. With low external resistance the strength of the current is not increased by adding cells in series.

5. With high external resistance the strength of current increases with an increase in the number of cells joined up in series or tandem.

IX. METHODS OF VARYING THE STRENGTH OF CURRENT.

It has already been shown that the strength of current may be varied by increasing the number of cells or by changing their arrangement in the battery. This method is indispensable, but it has its limitations. If one has a small cell and wishes to decrease the current, he must have a recourse to another method.

From the formula $C=\frac{E}{R}$ it is evident that one may decrease the current by increasing the resistance.

(*a*) The Rheostat.

1. **Appliances.** Resistance box or rheostat; 1 cell; 5 wires; galvanoscope or galvanometer.

2. **Experiments and Observations.** (1) Set up the apparatus as shown in Fig. 20.

(1) With plugs all fixed in rheostat, needle of galvanoscope at 0°, close key and note angle of deviation.

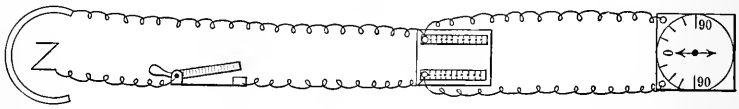
(2) Remove the plug, which will throw into circuit the lowest resistance contained in the rheostat. Note the angle.

(3) Add to the above resistance the smallest possible increment and note angle.

(4) Proceed in this way, tabulating results.

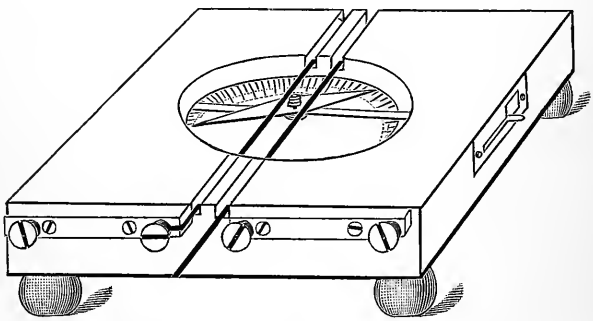
(5) Conclusions.

FIG. 23



(II) Another method of using the rheostat. The rheostat may be used in short circuit, as shown in Fig. 23. From this arrangement of the apparatus it is apparent that when all the plugs are in place the current will be short-circuited by the rheostat. If the resistance of that part of the circuit leading to the galvanoscope —the long circuit—be considerable, the long-circuit current will

FIG. 24



Galvanoscope, composed of a single magnetic needle mounted over a graduated circle. The two heavy copper wires which encircle the compass offer slight resistance to the passage of the electric current.

probably not be sufficient to cause any deviation of the galvanoscope needle; for the current varies inversely as the resistance ($C \propto \frac{1}{R}$), and if the resistance of the short circuit (R') equals zero, then the current of the long circuit (C) will be incomparably less than the current of the short circuit (C')—*i.e.*, $C : C' :: \frac{1}{R} : \frac{1}{R'}$, or $C : C' :: R' : R$; therefore, if $R' = 0$, C must equal 0.

Suppose that the resistance of the galvanometer circuit (R') be only 10 ohms, and suppose we remove from the rheostat the plug that represented 0.1 ohm resistance, then one-hundredth of the current will pass through the galvanometer. If we make the resistance in the short circuit 0.2 ohm, then one-fiftieth of the current will flow through the long circuit.

Problem. In this way we may increase the galvanometer current step by step until the maximum is reached.

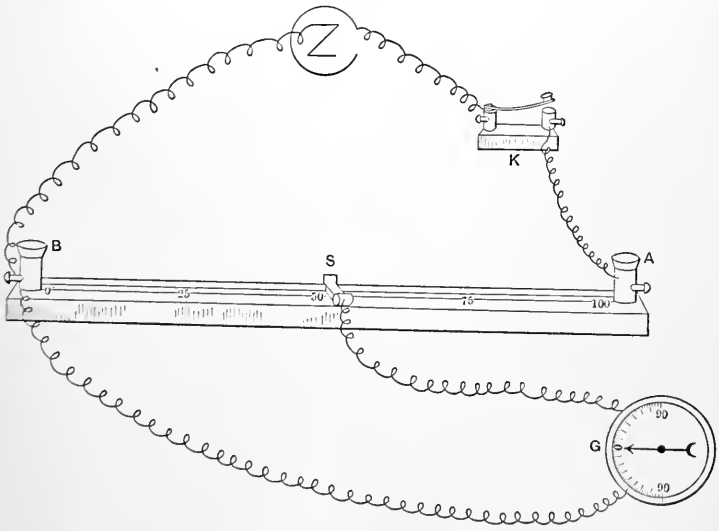
What is the maximum current to be derived when the resistance in the galvanometer circuit (R') equals 10 ohms, the maximum resistance of the rheostat (R) equals 100 ohms, external resistance in circuit between cell and rheostat (r) equals 1 ohm, $E.\ M.\ F.=$ 1 volt, and internal resistance of cell 4 ohms?

(*b*) The Simple Rheocord.

Besides the methods already used for varying the strength of the current one may use the derived current.

The simple rheocord (Fig. 25) may be used for this purpose.

FIG. 25



Rheocord with contact key.

1. **Appliances.** One or more cells; simple rheocord; five wires; galvanometer.

2. **Experiments and Observations.** (1) Set up the apparatus as shown in Fig. 25. From the figure we see that from the cell to post A, thence through the German-silver wire to post B and back to the cell makes a complete circuit. Having reached the

metallic slider (S) the circuit has two paths presented: 1st, from S direct to B; 2d, from S through G and back to B. The total current is divided into two parts: C, which passes along the wire from S to B, and C', the derived circuit which passes through the galvanometer. Suppose the resistance to the last-named current is R' and that to the direct current is R, the relative strength of these two currents is expressed in the following proportion: $C' : C :: R : R'$.

But the resistance of the German-silver wire may be conveniently divided into 100 equal parts (100 r).

If the slider be placed at any position along the wire, say at X cm. from the end, then the formula would be $C' : C :: xr : R'$.

$$C' = \frac{xr}{R'} C$$

Suppose that $R = 1$ ohm ($r = 0.01$ ohm); $R' = 2$ ohms and $x = 0$; *i. e.*, suppose the slider to hard up to B, then $C' = \frac{xr}{R'} C = 0$. This makes it clear that when the slide is in the zero position there will be no current passing through the galvanoscope.

(2) What is the relative strength of the two currents when $x = 10$?

(3) What is the relative strength of the two currents when $x = 50$?

(4) What is the relation of C' to C when $x = 99$?

(5) What is the relation of C' to C when $x = 100$?

From this course of reasoning it is evident that in the simple rheocord we have an instrument with which we can vary a derived current from zero to a maximum. Just what the value of this derived current will be will depend upon the voltage of the cell or battery and the total resistance to be overcome, as well as upon the distribution of that resistance.

(6) Verify the theory just developed, making a table of galvanoscope readings.

X. MUSCLE-NERVE PREPARATION.

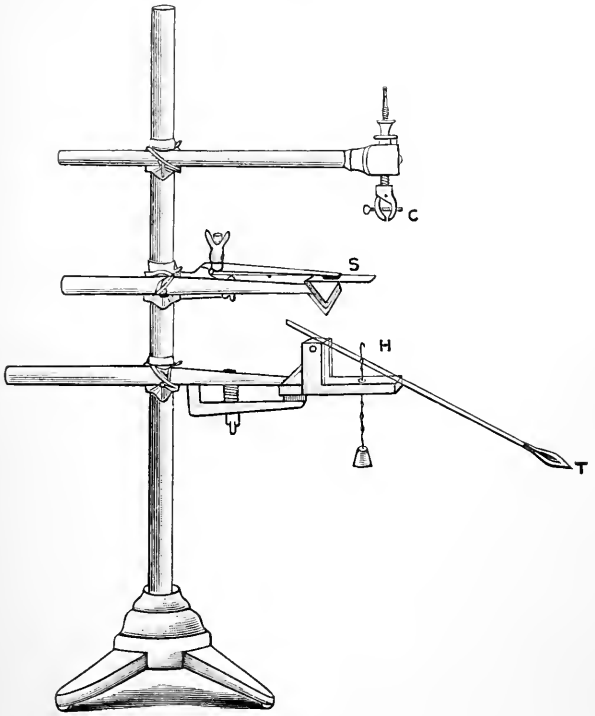
(*a*) The Classic Muscle-nerve Preparation.

1. **Appliances.** Frog board and pins; operating case; glass nerve hooks, like Fig. 28, *A*, made as follows: take a 10 cm. piece of glass rod, heat and draw in center to about $1\frac{1}{2}$ mm. diameter; cool, cut in two, heat the points to smooth them, and bend the end over to form the hook.

Simple myograph or muscle lever (Fig. 26). Watch-glass with salt crystals; 20 cm. of thick copper wire.

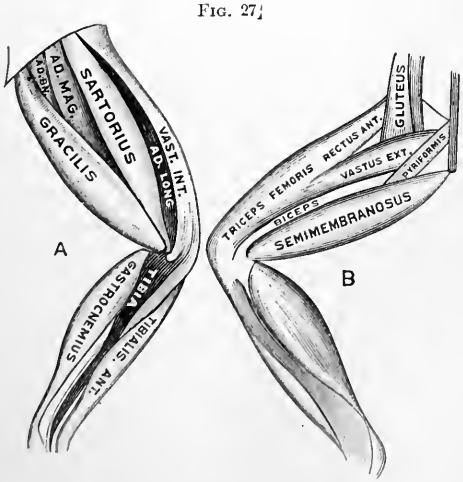
2. **Preparation.** Pith a frog and fix to frog board, with dorsum up. It will be taken for granted that the student is familiar with the anatomy of the frog's leg and thigh. The accompanying cut (Fig. 27) may serve to refresh the memory.

FIG. 26



The simple myograph: *C*, femur clamp; *S*, glass slide on which to rest the nerve; *H*, tendon hook of myograph lever; *T*, tracing point of myograph lever.

FIG. 27

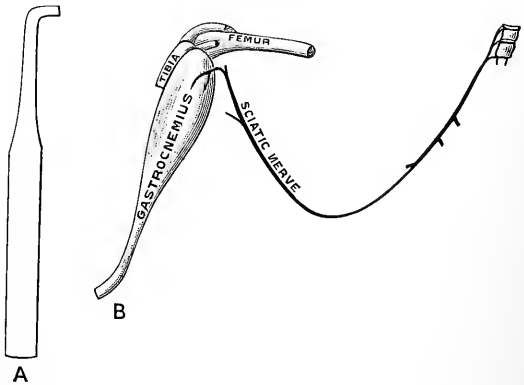


Showing musculature of the frog's thigh and leg: *A*, ventral aspect; *B*, dorsal aspect.

3. **Operation.** To make a gastrocnemius "muscle-nerve preparation."

(1) Make, with scissors, a circular cutaneous incision around the tarsus, corresponding with the lower end of cut *B*. Make a longitudinal cutaneous incision, beginning at the margin of the circular incision where it crosses the external aspect of the tarsus, carry it along the tibia, along the course of the biceps femoris muscle, over the pyriformis to the posterior end of the urostyle, along the whole extent of the urostyle. From the posterior end of the urostyle make an incision posteriorly and ventrally, for 1 cm. or 2 cm. Grasp the free margin of the skin at the point of the circular incision and with a quick traction toward the head of the frog the skin will be removed from the whole field of operation.

FIG. 28



A, a glass nerve hook; *B*, the classic muscle-nerve preparation.

(2) Pass a point of the fine scissors under the glistening tendon of the biceps femoris where it is inserted into the tibia, taking care not to injure any of the neighboring tissues. Sever the tendon. Grasp its free end; lift the biceps up, carefully cutting the delicate connective tissue which joins it to neighboring structures; sever its heads. The removal of the biceps and a separation of the cleft which the biceps occupied reveals three bloodvessels and the large trunk of the sciatic nerve. Which of the bloodvessels is the sciatic artery? Which is the sciatic vein? Which is the femoral vein?

(3) Grasp and lift up the posterior end of the urostyle, sever the iliococcygeal muscles, remove the urostyle.

The sciatic plexuses formed by the seventh, eighth, and ninth pairs of spinal nerves will be revealed.

(4) Pass a glass nerve hook under the sciatic nerve; gently lift it up, severing, with the scissors, the connective tissue. The pyriformis muscle must also be divided. The whole length of the sciatic nerve

may thus be readily dissected out. Care should be taken not to stretch, pinch, or cut the nerve during this process. Lay the nerve upon the gastrocnemius muscle.

(5) Grasp the triceps femoris muscle, pass a blade of the scissors under its tendon; sever, and remove the whole mass of muscles anterior to the femur. In a similar manner remove the muscles posterior to the femur.

(6) Grasp the tendo Achillis, sever it low down where it passes over the calcaneum, lift up the gastrocnemius and sever the tibia and its associated muscles as near the knee-joint as possible.

(7) Sever the femur at the juncture of its middle and upper thirds. The finished preparation has the characteristics shown in Fig. 28. A segment of the vertebral column may or may not be left on.

(*b*) The Indirect Stimulation of the Gastrocnemius.

4. **Observations.** To mount the muscle-nerve preparation in the myograph. Fix the femur in the clamp (Fig. 26, *C*); place a piece of filter paper, wet with normal saline solution, upon the glass nerve-support (*S*); lay the nerve upon the support, make a longitudinal slit in the tendo Achillis, pass the hook of the muscle lever through the slit, and so adjust the height of the clamp as to bring the lever into a horizontal position.

(*a*) **Mechanical Stimulation.** (1) Snip off with the scissors the central end of the sciatic nerve. If the muscle instantly contracts, thereby lifting the lever, the observer will know that his preparation is successful. If it does not respond to the first stimulation it may to a second or subsequent one. If it responds to later stimuli, but not to the first ones, one may conclude that in making the preparation a portion of the central end of the nerve was killed.

(2) What may one conclude if the muscle responds to stimuli applied to a central end of the sciatic nerve, but later fails to respond to stimuli applied farther along the course of the nerve—*i. e.*, nearer the muscle?

(*b*) **Thermal Stimulation.** Make and mount a fresh preparation. Heat the copper wire in a gas flame and touch the end of the nerve with the hot wire. If the preparation has been successful the muscle will respond by a contraction. If the preparation is a good one, save at least two-thirds of the nerve for the subsequent experiment.

(*c*) **Chemical Stimulation.** Cut off the part of the nerve which is dead and lay the central end of the still functional nerve in a saturated solution of common salt. Await results. Record all results.

(*d*) **Electric Stimulation.** While in the operation of making a gastrocnemius preparation after the sciatic nerve has been freed from the other structures in the thigh, slip the glass nerve hook under it so that the handle of the nerve hook will hold the nerve away from

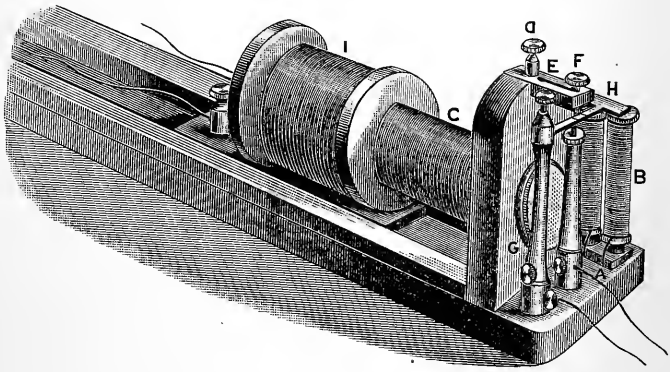
the other tissues. Press the end of a copper wire against the muscles of the thigh; touch the silver probe to the sciatic nerve, then to the copper wire, first separately then simultaneously.

Vary the experiment by using other combinations: silver and steel, copper and steel, etc. Note briefly the original observations of Galvani. Are the observations just made different in any essential respect from the observation which led to the discovery of what we call galvanic electricity?

XI. ELECTRIC STIMULATION AND THE MYOGRAM.

The simplest work in the field of electro-physiology is that which involves the use of the induction shock as a stimulus and the use of a myograph and kymograph to record the result of the stimulus.

FIG. 29



The inductorium.

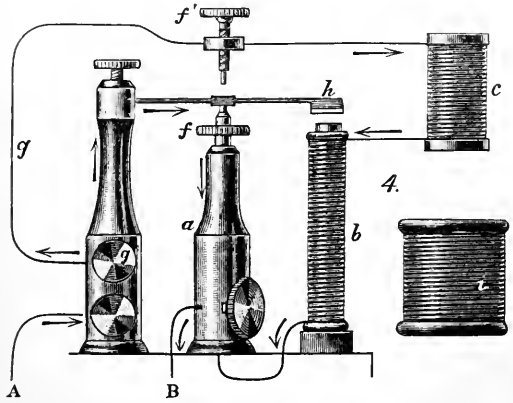
1. **Appliances.** Inductorium; myograph; kymograph; frog; operating case; glass hook; dry cell; contact key with three wires; shielded electrode with two wires; normal saline solution.

2. **Apparatus.** (*a*) **The Frog-board Myograph.** The frog-board myograph is a new form of myograph, so constructed as to permit all experiments usually performed on the gastrocnemius-sciatic preparation without exposing the active tissues to the atmosphere or disturbing the blood supply. The instrument is constructed as follows: An oaken base about one-fourth of an inch in thickness supports a cork plate of equal thickness; the cork plate presents a surface about 10 cm. by 25 cm. (Fig. 31). The lever holder at the end of the plate is constructed of thin sheet steel and slips from side to side in order to bring it opposite either leg of the frog.

The distance from the axis of the elbow lever to the thread-eye is the same as that to the weight; therefore, the weight lifted by the

muscle is the actual weight hung upon the weight link. When the lever passes a little below the horizontal position it comes in contact with the rest. This rest can be used in "after-loading" a muscle. For further description of the instrument see Fig. 31 and its legend.

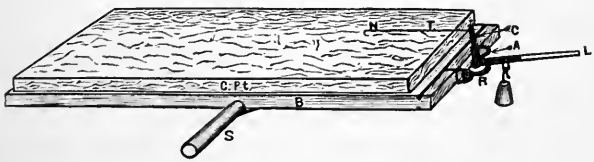
FIG. 30



The Neef hammer.

In the use of the frog-board myograph one proceeds as follows: The frog is pithed and pinned, dorsum up, on the cork plate, with the feet at the lever end. The tendo Achillis is exposed and loosened from the tarsal ligaments; the tendon hook *N* is passed through the tendon and the length of the thread adjusted at *C*. The skin on the thigh is opened to the extent of 2 cm. and the biceps femoris muscle removed, the sciatic nerve carefully separated from the

FIG. 31



Frog-board myograph: *S*, the shaft which is clamped to the upright stand; *B*, the oaken base; *C Pt*, the cork plate to which the frog is fixed; *A*, the lever axis and slide lever holder; *W*, the weight; *L*, the light lever, about 20 cm. in length; *N*, the tendon hook which is joined through the thread *T*, which passed through the eye and under spring the catch *C*; *R*, the lever rest.

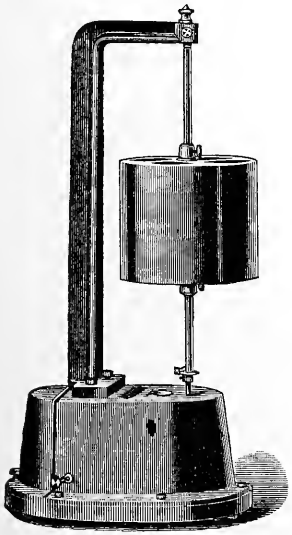
sciatic artery and placed on the insulated electrodes. Stimulation may be made from time to time for a period of several hours before the preparation becomes exhausted.

(*b*) **The Inductorium.** This instrument consists of two spools of wire: a *primary circuit* of few turns of coarse wire and a *secondary*

circuit of many turns of fine wire. It will be assumed that the principle of the inductorium is familiar to the student through his previous work in physics. (See Figs. 29 and 30.)

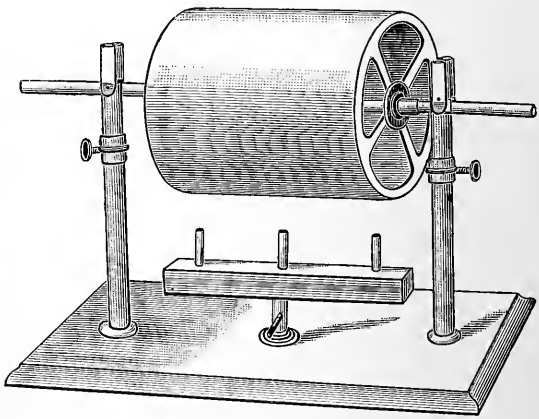
The inductorium used in the physiological laboratory is provided with a vibrating (Neef) hammer which makes and breaks the current with each double vibration of the hammer, the vibration being due to the reciprocal action of an electromagnet and a spring. The instrument must also be provided with a means for either cutting the hammer out of the primary circuit or stopping the vibration of the hammer. The secondary coil or induction circuit must be provided with a short-circuiting key, either as a part of the inductorium or as an extra appliance.

Fig. 32



The kymograph.

Fig. 33



Drum support for use in smoking the kymograph drums.

The secondary coil is movable and may be moved up until it covers the primary coil or moved out along a slide. Some instruments are provided with a long base, permitting the secondary coil to be moved to a considerable distance from the primary coil, while others are provided with a short base and a pivot, allowing the secondary coil to be turned through an angle of 90 degrees after it has been drawn back free from the primary coil. Either arrangement allows one to decrease at will the strength of the induction shocks.

(*c*) **The Kymograph** (Fig. 32). This instrument is the most important one in any physiological laboratory, because with its help graphic records of all movements of tissues and organs and of all pressure changes may be made. The kymograph or *wave-writer* is

thus used in nearly all work in the neuromuscular system, the circulatory system, and the respiratory system.

The instrument consists of a cylinder or drum kept in rotation by clock-work. The rate of the rotation is usually governed by fans of varying sizes, also by adjustments of the propelling mechanism.

To prepare the kymograph for work, remove the cylinder, stretch a sheet of prepared glazed paper tightly upon the surface and place it upon such a stand as that shown in Fig. 33. Set the drum to rotating and bring a gas flame or preferably a triple flame under the drum. In a few moments it will be evenly covered with a film of carbon, which is as sensitive to touch as a photographer's plate is to light.

To fix the carbon tracings and make the record a permanent one see directions in Appendix, 8.

3. **Experiments and Observations.** Pith a frog; mount it upon the frog-board myograph as directed above. Prepare the kymograph for receiving a tracing and adjust it for slowest rotation. Adjust the myograph so that its tracing lever stands horizontal and tangent to the drum with the tracing point lightly touching the side of the drum. Set up the electric apparatus with one dry cell or one Daniell cell so joined in the primary circuit as to avoid the action of the vibrating hammer. Use a contact key in the primary circuit and a short-circuiting key in the secondary circuit.

(1) Determine the *stimulus of liminal intensity* by moving the secondary coil to position of minimum strength; then, while slowly "making and breaking" the current in the primary circuit, move the secondary coil up until the strength of the induction shock is sufficient to cause a contraction of the muscle. The weakest shock which will cause a contraction is the stimulus of liminal intensity sought. Note that this occurs on the break of the primary circuit.

(2) Determine the *stimulus of optimum intensity* by starting the kymograph to rotating slowly; meanwhile make and break the primary circuit while continuing to move the secondary coil from the position of liminal intensity toward the position of maximum intensity. The myograph will trace a series of myograms with the rise and fall of the lever, when the muscle contracts and relaxes. The tracings will present a series of sharp-pointed waves varying in height, showing the varying extent of contraction. At first all the contractions occur on break of primary circuit, then on both break and make of the primary circuit. As the secondary coil is moved toward the maximum position the myograms become higher and higher, finally reaching a maximum height which is not exceeded, however strong the stimulus is made.

The stimulus of optimum intensity is the weakest stimulus which will produce the maximum contraction.

The increase of the strength of stimulus beyond the optimum will only fatigue the muscle and nerve through overstimulation, without producing greater contractions.

4. **Observations.** (1) Take tracings of the contractions produced by a series of "*make-induction shocks*" applied indirectly—*i. e.*, to the nerve. The "*make-induction shock*" is obtained as follows:

(*a*) With primary circuit not interrupted by the Neef hammer, but closed and opened by the contact key, open the short-circuiting key of the induced circuit.

(*b*) Close the contact key of the primary circuit and make induction shock—*i. e.*, a shock in the induced circuit caused by a closure of the battery circuit will stimulate the preparation.

(*c*) Close the short-circuiting key in the secondary circuit.

(*d*) Open or break the primary circuit. An induced break shock occurs in the secondary circuit, but it is short-circuited by the closed Du Bois-Reymond key. If while the drum rotates one makes, in close succession, the changes above indicated—*a-b-c-d*, *a-b-c-d*, etc.—there will be produced a series of contractions, all the result of stimulation by *make-induction shocks*.

(2) Take a tracing of the contractions resulting from a series indirectly applied—*break-induction shocks*.

(3) By leaving the short-circuiting key open one may get a series of contractions due to alternating make-induction shocks and break-induction shocks. Let these be recorded in pairs upon the kymograph.

XII. THE TYPICAL MYOGRAM, COMBINED MYOGRAMS, AND TETANUS.

1. **Appliances.** Inductorium; Daniell cell or dry cell; kymograph; myograph; electrodes; keys; wires.

2. **Preparation.** Pith a frog, make muscle-nerve preparation; mount it on myograph, prepare kymograph for tracing, and adjust for fastest rotation; set up electric apparatus for a series of make-induction shocks or break-induction shocks.

3. **Experiments and Observations.** (1) Start the kymograph drum to rotating. When it has reached the maximum speed, stimulate the preparation with a break-induction shock. The lever point should trace upon the drum a typical myogram. Repeat the experiment several times with the same preparation. Study the characteristics of the myogram.

(2) Trace another myogram while a tuning fork is tracing hundredths of seconds upon the drum and while the instant of stimulating the nerve is traced upon the drum, either through the action of an

electromagnet and tracing lever or through a tracing lever attached to the key of the primary circuit. There will thus be three tracings upon the drum: (*a*) the myogram; (*b*) the time tracing; (*c*) the stimulus tracing, the latter showing the time when the stimulus is made. Note that the myograph lever does not rise until a certain time after the stimulus is given. This period is the *latent period.* What is the length of the latent period?

(3) Trace another myogram, but as the lever is sinking back toward the abscissa stimulate a second time. Note that the result is a double-crested myogram and that the second is higher than the first.

(4) Trace another myogram resulting from a series of stimuli occurring, in rapid succession, if possible about ten times per second. Note that the result is a myogram with a series of crests and that the lever does not fall back to the abscissa between the successive stimuli. Note that the first few crests are progressively higher and higher. This phenomenon is called the "*stair-case series of contractions,*" and is usually observed when a muscle is given a series of stimuli after a period of rest.

(5) Vary the above experiment by increasing the rapidity of stimuli to 20 per second. This may be done through the use of a toothed wheel as a key in the primary circuit, or through modification of the Neef hammer, which causes it to vibrate slowly. Use medium speed of kymograph. Note that the result is a myogram with a serrated crest, the serrations indicating the result of the several stimuli.

(6) Stimulate with a series of induct shocks caused by the rapid making or breaking of the primary circuit through the vibration of the Neef hammer. Use medium-speed drum. Note that this throws the muscle into a condition of typical tetanus.

Trace a series of tetanus curves, each lasting about three or four seconds.

XIII. THE WORK DONE BY A MUSCLE.

(*a*) To determine the amount of work done by a single contraction. (*b*) To determine the total amount of work done by a muscle. (*c*) Reaction changes in fatigued muscles.

1. **Appliances.** Same as Lesson XII.; also 50-gram weight and 20-gram or 30-gram weight.

2. **Preparation.** Arrange electric apparatus for a series of break-induction shocks.

3. **Operation.** Make and mount a gastrocnemius preparation for indirect stimulation.

4. **Observations.** Upon a slow drum record in close order a series of break contractions.

(*a*) *To determine the amount of work done by a single contraction.*

(1) What weight is lifted?

(2) How high is it raised?

(3) What is the ratio between the height of the curve traced by the lever and the height through which the weight was raised?

(4) Let W = work done.

g = weight lifted.

h = height of curve traced by lever.

k = *constant* of the apparatus, in this case the ratio between the lever arms. Then $W = k.\ g.\ h.$

(5) Express the amount of work in ergs.

(*b*) *To determine total work done.*

(6) How many times was the weight lifted before the muscle was fatigued?

(7) Through what average height was the weight lifted?

(8) Has the value of k or g changed?

(9) Give a formula for total height ($H =$).

(10) Give a formula for total work done ($W =$).

(11) Express in ergs the total work done by the muscle.

(12) In the fatigue tracing, did the lever continue throughout the observation to fall back to the original abscissa? If not, describe the general changes in the abscissa.

(*c*) *Reaction changes.*

(13) Apply a piece of neutral litmus paper to the fresh muscle tissue of the frog from which your specimen was taken. Record result.

(14) Apply a piece of litmus paper to a fresh-cut surface of the fatigued muscle. Record results.

(15) What is the reaction of a muscle of a frog after rigor mortis has been established?

(16) What is the reaction of fresh urine?

(*d*) *Secondary fatigue* (Lagrange, p. 60).

(17) Grind a fatigued or exhausted muscle in a mortar and extract with normal saline solution.

(18) Inject this extract into subcutaneous lymph spaces of a frog.

(19) Observe the effect of this injection upon the second or rested frog.

(20) Observe the effect upon the working power of its muscles.

XIV. TO SEND AN ELECTRIC CURRENT INTO A NERVE WITHOUT RESPONSE. FLEISCHL'S RHEONOM.

When one is observing the effects of mechanical and thermal stimuli, he finds that he may apply a mechanical stimulus so slowly that the nerve may be severed without calling forth a response; he

may apply heat to the fresh nerve so gradually that the nerve may be actually cooked without causing a contraction of the muscle which it supplies.

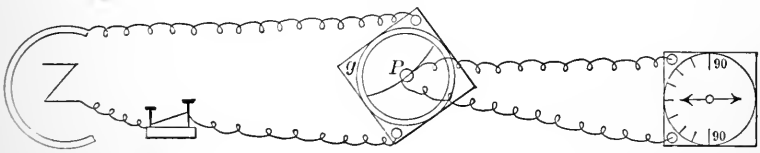
The problem which we have next to solve is to apply an electric stimulus gradually.

1. **Appliances.** Fleischl's rheonom; one Daniell cell; myograph; contact key; galvanoscope; saturated solution of zinc sulphate; five wires; frog; operating case.

The rheonom is constructed as in Fig. 34. Its essential features are: *g*, the non-conducting base with circular groove; *P*, the non-conducting rotatable, central standard; the battery binding posts, having zinc connection with the groove; the rotating binding posts, having zinc limbs dipping into the groove.

2. **Experiments and Observations.** Set up an apparatus as shown in Fig. 34, after amalgamating the zinc tips which dip into zinc sulphate. Fill the groove with zinc sulphate.

FIG. 34



(1) Find and mark the zero position for the rotating limbs of the rheonom—*i. e.*, find the position which will give no deviation of the detector needle when the contact key is closed.

(2) Find and mark the position which the rotating limbs occupy when the detector needle indicates 10°.

(3) Find and mark in succession each higher increment of 10°, until the maximum is reached.

(4) Rotate the limbs so gradually as to cause the detector needle to rotate with slow and regular motion from the zero position to the maximum position and back.

(5) Make a gastrocnemius muscle-nerve preparation, mount it in the myograph; change the wires from the galvanoscope to the electrodes of the myograph; place the limbs of the rheonom in the maximum position; close the key. With the closing of the key the maximum current is instantly thrown into the nerve and serves as a strong stimulus, in response to which the muscle contracts.

(6) Place the limbs of the rheonom in the minimum position; close the key. Inasmuch as the muscle-nerve preparation is much more sensitive to electricity than is the low-resistance galvanoscopy, the muscle will probably respond when the conditions are as above indicated. Theoretically, a zero point exists. Practically it may

be difficult to find it for a muscle-nerve preparation. The finding of a position where there is no response on closing the key is, however, not essential in this experiment.

(7) Keeping the key closed, slowly rotate the limbs of the rheonom from the minimum position to the maximum position. If the conditions are favorable this can be done without calling forth a response.

(8) Without opening the key, slowly rotate the limbs backward from the maximum to the minimum position. One may thus send through a nerve a strong current and may withdraw the same without causing a contraction of the muscle. Keep the key closed.

(9) Quickly rotate the limbs from minimum to maximum; the muscle responds. Quickly rotate from maximum to minimum; the muscle responds.

From the preceding observations one may conclude that response to electric stimulation is elicited not by the simple flow of an electric current through the irritable tissues, but by *a more or less sudden change in the strength of the current.* The opening and closing of a galvanic current, also its sudden increase or decrease, serves as an efficient stimulus, while *the gradual increase or decrease in the strength of the current causes no response.*

XV. TO DETERMINE THE INFLUENCE OF CATHODE AND ANODE POLES.

Many of the phenomena of muscle-nerve physiology were inexplicable until a difference was noted (von Bezold, 1860) in the influence of the anode and cathode. This difference in the influence of the two poles may be best observed by use of the sartorius muscle of a frog.

1. **Appliances.** A double myograph and support; recording drum; Daniell cell; Pohl commutator; Du Bois-Reymond key; non-polarizable electrodes; five wires; electrode clamp and support.

2. **Preparation.** (*a*) **Set Up a Pair of Non-polarizable Electrodes.** (See Appendix, 9.)

(*b*) **A Double Myograph.** A most efficient as well as convenient and economical double myograph may be arranged for this experiment as indicated in Fig. 35.

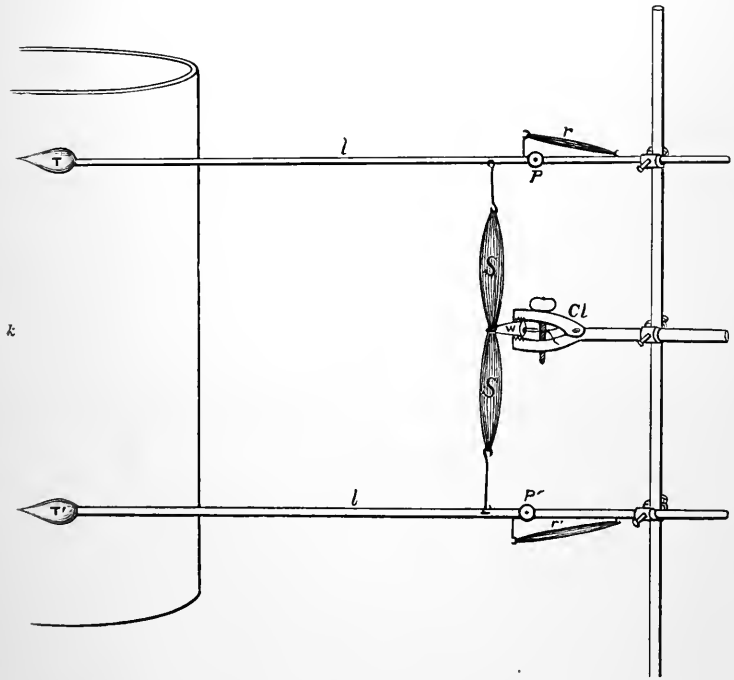
Two common muscle levers, as shown in the figure, are used. These are held in position by common clamps and heavy support. The upper myograph must be reversed and its lever counterpoised by elastic bands. Between the two myographs a small wooden block, with a longitudinal hole for the loop of thread which holds the muscle, is held by a clamp.

3. **Experiment.** (1) Curarize a frog. (See Appendix, 7.)

(2) After the lapse of three hours or more the sartorius muscle may be prepared.

(3) Mount the preparation by passing a loop of coarse thread through the hole in the block *W*, lift the muscle by its tendon of insertion, pass it through the loop, draw the loop gently around the middle of the muscle, and fix by making a single knot around the screw of the clamp. The fine hooks which join the muscles

FIG. 35



Double myograph: *Cl*, femur-clamp holding a wooden wedge (*W*), through which a loop of thread passes. The sartorius muscle *S* is held tightly by the loop of thread which encircles its middle. The two tendinous extremities of the sartorius are hooked to the two levers *l l*. The two levers are pivoted at *P* and *P'*. The muscle is put on a stretch by the two rubber bands *r* and *r'*. The tracing points *T* and *T'* are adjusted to a vertical line on the kymograph *k*.

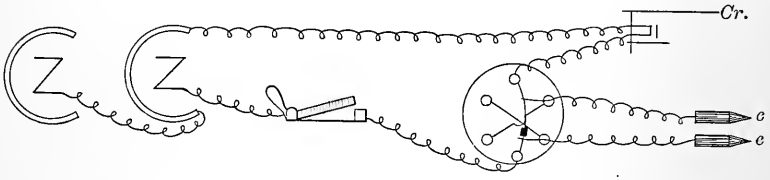
to the levers may now be passed through the tendons, and the proper position of the levers effected by an adjustment of the clamps. The loop around the sartorius may now be drawn as tightly as possible and not actually sever the two portions. The non-polarizable electrodes may be clamped between two pieces of cork and held by an extra support. A "universal" clamp holder is a most desirable accessory to this apparatus.

The electric apparatus should be set up as shown in Fig. 36.

With this arrangement either electrode may be made the anode, the experimenter needing only to reverse the commutator bridge to reverse the position of the anode and cathode.

The recording drum or kymograph should rotate rapidly. The recording points of the myograph levers should be adjusted so that the point of the upper one touches the drum vertically over the point of the lower one. Adjust the marker *Cr* so that it will indicate the time making and breaking the circuit—*i. e.*, so that it will record on the drum the time of making stimulus and the time of breaking stimulus. The recording point of the time marker should, of course, be in the same vertical line with the myograph points. The moist tips of the N. P. electrodes should be so adjusted as to touch the muscle above and below the loop of thread.

Fig. 36



(1) Close the key. If the preparation has been successful the half of the muscle in contact with the cathode pole will respond before the other one.

(2) Break the current. The anode will respond first.

(3) Reverse the direction of the current and repeat (1) and (2).

(4) Vary the strength of the current through use of the simple rheocord and determine whether the results are the same for currents of different strength.

Law I. The make contraction starts at the cathode and the break contraction starts at the anode.

When irritable tissue, muscle or nerve, is subjected to a galvanic current the response to the stimulation begins in the region of the cathode on *making* the current, and in the region of the anode on *breaking* the current.

Would the foregoing observations justify the following statements?

(1) Cathodic contractions, or make contractions, may be caused by a galvanic current which is too weak to cause anodic contractions, or break contractions.

(2) Cathodic contractions, or make contractions, are stronger than anodic contractions, or break contractions.

Law II. With a given strength of current the influence of the cathode pole is more irritating than the influence of the anode.

XVI. ELECTROTONUS (TO DETERMINE THE EFFECT OF A CONSTANT CURRENT UPON THE IRRITABILITY OF A NERVE).

At the beginning of the last century Ritter discovered that the vital properties of irritable and contractile tissues were modified when subject to a constant battery current. The modified condition was called *galvanismus*. During the first half of the last century the subject was investigated by Nobili, Mattencci, Valentin, and Du Bois-Reymond; the last named substituted the word *electrotonus* for galvanismus and further modified the terminology. It remained for Pflüger[1] to rework the whole field, to correct, to elaborate, and finally to formulate laws.

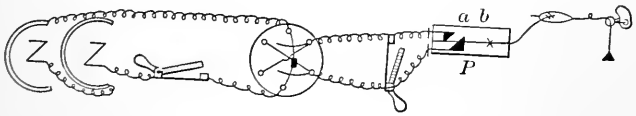
(*a*) Preliminary Experiment.

1. **Appliances.** Muscle signal, or myograph; two Du Bois-Reymond keys; two Daniell cells; commutator; eight wires; salt.

2. **Preparation.** Set up electric apparatus as shown in Fig. 37.

3. **Operation.** Make and mount in the muscle signal a gastrocnemius preparation.

Fig. 37



4. **Observations.** (1) In which position must the bridge of the commutator stand to give a descending current so that the cathode will be nearer to the muscle than is the anode? Mark the opposite side A.

(2) Fig. 37, *P*, represents the glass plate of the muscle signal. So arrange the triangular platinum electrodes that there shall be a distance of about 3 cm. between the electrodes and both electrodes near that end of the plate farthest from the muscle. Lay the nerve over the electrodes and along the glass plate. The segment of nerve which lies upon the glass plate between the electrodes and the muscle must be subject to various stimuli, mechanical and chemical. At a point about 1 cm. from the electrodes, marked *X* in the figure, place upon the nerve trunk as many fine crystals of common salt as would be taken up on the point of a penknife. Moisten these salt crystals with a drop of water. While the salt

[1] Untersuchungen über die Physiologie des Electrotonus, Berlin, 1859.

solution is permeating the sheath of the nerve trunk, adjust the commutator for a descending current. When the muscle begins to twitch, note the effect upon the signal. The contractions become more and more tetanic in character.

(3) Close the commutator circuit, open the short-circuiting key—*i. e., make* the "polarizing" current. If the experiment is successful the tetanus is more marked. Which pole is near the point stimulated?

(4) Close the short-circuiting key—*i. e.,* break the "polarizing" current. Reverse the commutator; make the current. The muscle is put completely or almost completely at rest. Which pole is nearer the stimulus?

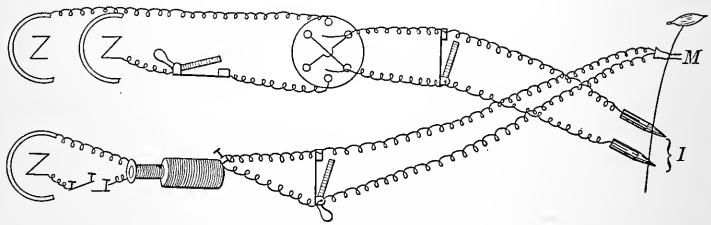
(5) Repeat (3) and (4) several times. It is evident that the irritability of the nerve to the salt stimulus is increased in the region of the cathode pole and decreased in the region of the anode pole. This changed condition of the nerve due to the passage of a constant current is called *electrotonus.* The state of increased irritability in the region of the cathode is called *catelectrotonus.* The decreased irritability in the region of the anode is called *anelectrotonus.*

(*b*) Myographic Record of Anelectrotonus and of Catelectrotonus.

1. **Appliances.** Three or four Daniell cells; three Du Bois-Reymond keys; contact key; two commutators; inductorium; two N. P. electrodes; eighteen wires; kymograph; myograph with moist chamber; two pairs of platinum-wire electrodes to use with induction current.

2. **Preparation.** Arrange apparatus according to plan as shown in Fig. 38.

Fig. 38



3. **Operation.** Make and mount a gastrocnemius preparation in moist-chamber myograph, or frog-board myograph. Adjust electrodes as shown in diagram.

Test apparatus and preparation by sending single make (or break) induction shocks through nerve at *M.* Let there be a typical response to these stimuli. *The secondary coil should be removed to a distance that gives the stimulus of liminal intensity.*

To close the constant current "*polarizes*" the nerve, or, better, *induces electrotonus.*

That segment of the nerve between the anode and cathode is called *intrapolar region.*

Those segments centrally and distally located are called *extrapolar.*

The induced current is called *stimulating current.*

4. **Observations.** (1) Adjust for descending, polarizing current. Stimulate in the region of anode. Note extent of muscle contraction. Induce electrotonus; stimulate again in region of anode. If the experiment is successful the contraction will be found to be decreased or absent.

The nerve is at this point in a condition of *anelectrotonus.*

(2) Stimulate at *M*, or in the region of the cathode. Withdraw the polarizing current. After a few minutes stimulate again at *M*. If the experiment is successful the wave is higher in the former than in the latter case.

The stimulation was made in the region of the cathode and the nerve in a condition of *catelectrotonus.*

(3) Adjust for ascending, polarizing current.

Stimulate at *M*—*i. e.*, in the region of the anode. The contraction is weaker than in the normal nerve, or it may be quite absent. This region is now in a condition of anelectrotonus.

(4) Stimulate in the region of the cathode. The response is probably weak. Withdraw the polarizing current. Stimulate again in the region of the cathode. The response is normal—*i. e.*, it is greater than during the electrotonic condition.

But in *descending extrapolar catelectrotonus* the response was greater than normal. In the experiment just performed we stimulate in the region of *ascending extrapolar catelectrotonus.* Note that the *polarizing current is relatively strong.*

(5) Remove one cell from the battery and repeat (4). If the response to stimulation is still weaker with than without the polarizing current, reduce the strength of the polarizing current still farther by the use of the simple rheocord. Finally, with a *weak polarizing current* the stimulus in the region of extrapolar catelectrotonus causes a *stronger* response than normal.

The response which the muscle makes must be accepted as a measure of the excitation which it receives from the nerve. But the excitation delivered by the nerve depends upon two factors—its *irritability* and its *conductivity.* When the nerve is stimulated in the region of ascending extrapolar or intrapolar catelectrotonus, its increased irritability is of no avail if there is interposed between that region and the muscle a region of decreased conductivity. With strong polarizing current the region of the anode is not only decreased in irritability, but in *conductivity.*

(*c*) Laws of Electrotonus.

(*a*) *The passage of a current through a nerve induces a condition of electrotonus marked by increased irritability in the region of the cathode* (*catelectrotonus*) *and decreased irritability in the region of the anode* (*anelectrotonus*).

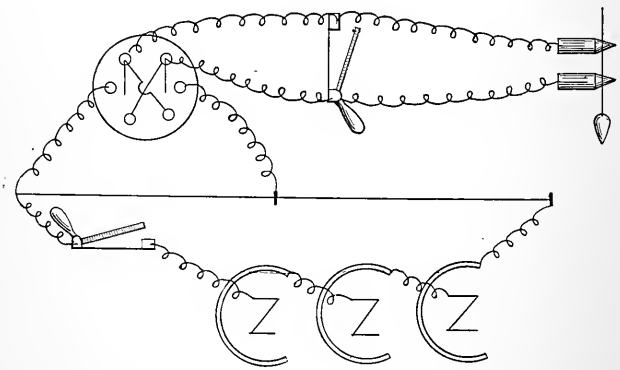
(*b*) *During electrotonus induced by a strong current the conductivity is decreased in the region of the anode. Further—though not derived from the foregoing experiment—"at the instant that the polarizing current is withdrawn the conducting power is suddenly restored in the region of the anode and greatly lessened in the region of the cathode."* —Lombard, in *American Text-book of Physiology.*

XVII. THE LAW OF CONTRACTION.

1. **Appliances.** The simple rheocord; four Daniell cells; frog-board myograph, or myograph with moist chamber, simple key; Du Bois-Reymond key; commutator; two N. P. electrodes.

2. **Preparation.** Set up apparatus with four cells in *series*, simple key as closing key. Commutator with cross-bars; short-circuiting key; the two N. P. electrodes clamped in chamber of myograph or mounted above the frog-board myograph (Fig. 39).

FIG. 39



3. **Operation.** Make and mount a gastrocnemius preparation.

4. **Observations.** (1) Stimulate with make and break of the very weakest descending current. The first response is elicited by the very weak descending current. A slightly stronger current is required to elicit a response with the ascending current.

Record results in such a table as suggested under (5). This table shows what response (contraction or rest) the muscle gives on making

and breaking of the descending current and on making and breaking of the ascending current.

It also shows in a marginal column the gradual increase of the strength of the current through gradual increase of resistance in the rheocord.

(2) Make and break with weak ascending current. If the conditions are typical the muscle will contract on making both ascending and descending current.

(3) Increase gradually the strength of the electrode circuit recording results. After a longer or shorter transitional period in which the result will be characterized by a contraction on the make of both the ascending and descending current, one comes to a strength of current which causes a contraction on both make and break of both descending and ascending current. This is the medium strength for the preparation and the condition in question.

(4) Let the current be increased still farther and by larger increments. After passing another transitional stage one finally reaches a strength of current which causes a contraction on make of descending current and on break of ascending current. This is the "very strong" current for the preparation under observation.

It not infrequently happens that through overstimulation and fatigue of muscle the whole experiment cannot be completed upon one preparation except by increasing the current by larger increments.

(5) Pflüger's law of contraction may be expressed in the following table:

Strength of current.	Descending.		Ascending.	
	Make.	Break.	Make.	Break.
Very weak	c	R	R	R
Weak	cc	R	c	R
Medium	C	C	C	C
Strong	CC	C	C	CC
Very strong	CC	R (or c)	R	CC

(6) But how shall we account for these results?

Let us recall some of the laws which have been demonstrated.

Law I. The make contraction starts at the cathode and the break contraction starts at the anode.

Law II. The make or cathodic stimulus of a constant current is more irritating than the break or anodic stimulus.

Law III. The passage of a constant current through a nerve induces a condition of electrotonus, marked by an increased irri-

tability in the region of the cathode, and a decreased irritability in the region of the anode.

Law IV. During electrotonus induced by a strong current the conductivity is decreased in the region of the anode during the passage of the current and in the region of the cathode after removal or breaking of the current.

These laws account for all typical phenomena observed above.

XVIII. (a) THE CAPILLARY ELECTROMETER. (b) THE METHOD OF USING IT.

In those experiments where we have had occasion to measure the strength of an electric current or the difference of potential between two electrodes we have used the tangent galvanometer. But in all these experiments the strength of current or difference of potential has been considerable, amounting in some cases to that represented by several Daniell cells joined in series with a moderate amount of external resistance.

To detect and measure muscle currents it has been necessary to devise a very delicate and sensitive instrument. The Wiedemann galvanometer has been used for this purpose; but the most simple and satisfactory apparatus is the capillary electrometer.

(*a*) The Capillary Electrometer.

Take a piece of 6-mm. glass tubing and draw two fine capillary tubes; clamp these in burette holders with the capillaries pointing vertically downward. Into one pour a few drops of water; it will pass through the capillary and leave its point drop by drop. Into the second tube pour some mercury—enough to fill the capillary—and stand 2 cm. or 3 cm. above the capillary in the tube. The mercury will not flow through the capillary. Note that the upper meniscus of the water—in the undrawn part of the tube—is concave, while the upper meniscus of the mercury is convex. The water wets the glass and seems to be drawn up for a short distance on the vertical surface of the glass, while the mercury does not wet the glass—there seems rather to be a repulsion. If one looks at the lower meniscus of the mercury with a low-power microscope, he will find it to be convex downward.

Mercury stands up in nearly spherical globules on a glass surface, and water forms nearly spherical globules on an oiled surface. There is no adhesion between the glass and mercury, while there is a strong cohesion between the molecules of the mercury. This accounts for the fact that the mercury forms globules which but for the action of gravitation would be quite spherical. If a drop of liquid be placed

upon a horizontal plane its shape will be modified by three forces: (1) cohesion, (2) adhesion, (3) gravitation. In the case of the globule of water on an oiled surface, or of mercury on a horizontal glass plane, adhesion is practically *nil*, thus leaving the two factors, cohesion and gravitation.

Cohesion tends to draw all the molecules toward a common center and thus brings the individual molecules of the surface into a condition of lateral tension. This condition is technically called *surface tension*. The greater the preponderance of cohesion over the other forces acting upon the liquid the greater the surface tension.

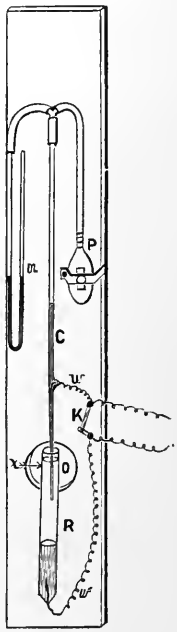
It is surface tension which gives to the mercury in the glass tube a convex meniscus, and keeps it from flowing through a fine capillary of glass.

It must be evident that the relation between the mercury and the glass (adhesion) does not vary. If the position of the meniscus varies it must be through a change in one or both of the other forces mentioned above.

Gravitation measured by the weight of the column of mercury may vary by changing the height of the column of mercury. Through this variation the meniscus may be made to take any desired position.

Experiment has shown that the passage of an electric current through the column of mercury into sulphuric acid modifies the surface tension of the mercury and thus changes its position. As the modification of surface tension varies proportionately with the strength of the electric potential, one may measure this strength by noting the distance through which the meniscus moves.

FIG. 40



Capillary electrometer. (Description in text.)

The observation of the meniscus must be made with a microscope, using the low power. Note that in the instrument (see Fig. 40) the wooden back that supports the instrument is cut away (at *O*) near the capillary in order to permit the microscopic observation of the meniscus to be made with transmitted light.

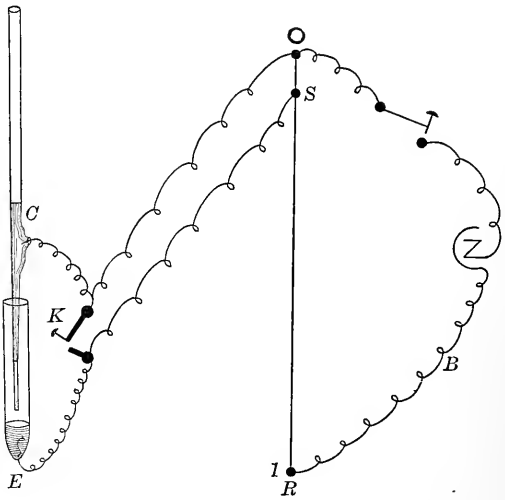
(*b*) The Method of Using the Capillary Electrometer.

To adjust the instrument for use clean the capillary absolutely clean through the use of 20 per cent. H_2SO_4 c. p. and distilled water. Pour into the tube enough mercury to bring the meniscus to the middle of the finest portion of the capillary. Adjust the parts of the electrometer—the pressure bulb *P*, the manometer *m*, and the

reservoir *R*. The reservoir is partly filled with mercury, above which 20 per cent. H_2SO_4 c. p. fills the reservoir to above the capillary meniscus. Note that platinum wires (*w w'*) are fused into the capillary and reservoir passing into the mercury. These wires pass to binding posts and are kept in contact through a short-circuiting key (*K*).

The acid must be in contact with the mercury in the capillary. To effect this press the bulb *P* until the mercury is forced to the tip of the capillary; relieve the pressure and the meniscus will recede drawing the acid after it. Fig. 41 shows how the electrometer is

Fig. 41



Showing method of joining up the capillary electrometer *E*. Note that the positive plate (zinc) is joined through the rheocord *R* to the capillary *C*, while the negative plate (copper) is joined through the rheocord to the reservoir. The battery wires are joined to the zero and 1 meter posts of the rheocord, or to the zero and 10 meter posts. In the former case the slider *S* must be very near, almost touching, the zero post when the first observation of the change of meniscus is made.

to be joined up for use. Certain precautions should always be observed in the use of the electrometer. The two poles of the instrument—the mercury in the capillary *C* and the mercury in the reservoir—should be joined through a short-circuiting key, except when one wishes to test difference of electric potential, when the key may be opened for a few moments. The instrument is so sensitive that only the weakest currents should be allowed to traverse the acid between the poles. The current from a Daniell cell is

much too strong to be permitted to traverse the instrument. In testing the instrument with a Daniell or any similar cell use a rheocord joined as shown in Fig. 41, and make the first test with the slider almost touching the zero post, and subsequent tests with small increments until the movements of the meniscus are considerable in extent, yet not so much as to carry it out of the field of the microscope.

If it is desired to make quantitative tests of electromotive force the electrometer may be graduated. To accomplish this it is necessary to have a micrometer in the ocular of the microscope, so that the position of the meniscus may be accurately determined. It is also necessary to have some means of measuring the force of displacement of the meniscus. This may be done by means of a mercury manometer, shown in Fig. 40 as a part of the electrometer. Pressure exerted on the bulb is measured by the manometer. The amount of pressure required to bring the meniscus back to its original position after the opening of the key *K* is proportional to the electromotive force that displaced the meniscus during the opening of the key.

By testing a series of known values and taking the manometer readings one may easily determine the relation between volts and millimetres of mercury pressure.

XIX. ELECTROMOTIVE PHENOMENA OF ACTIVE MUSCLE.

(A) The physiological rheoscope, or the rheoscopic frog.
(B) Electromotive force detected by the electrometer.

In the process of dissecting out a muscle-nerve preparation (the classical form) one is likely to drop the cut-off central end of the sciatic nerve upon the gastrocnemius muscle. Should this occur a contraction of the muscles is almost sure to occur. Galvani made this observation and cited it as a proof that electricity exists in animal tissues.

A classical experiment well adapted to demonstrate the difference of electric potential in living tissues is that known as the rheoscopic frog.

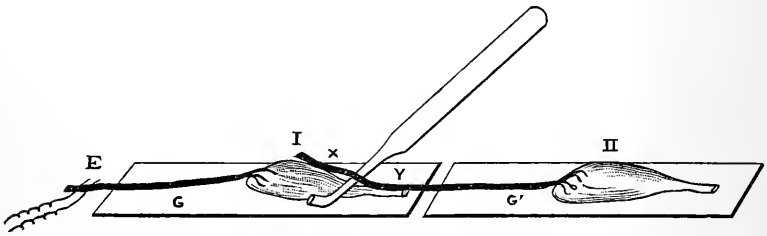
(*A*) The Rheoscopic Frog.

1. **Appliances.** Frog; two glass slides, 1 inch by 3 inches; operating case.

2. **Preparation.** Pith the frog. Make two classical muscle-nerve preparations. Place the two glass slides end to end upon the table (as shown in Fig. 42), with a muscle on each disposed as shown in

the figure. Note that the nerve from muscle *II* touches muscle *I* in two places—at the end and middle. Set up the electric apparatus for single-induction shocks and rest the nerve of muscle *I* upon the electrodes.

FIG. 42



"The rheoscopic frog," an experiment to show the presence of the difference of electric potential in different parts of an active muscle. *I.* The active muscle, stimulated at *E* by induction shocks. *II.* The second preparation, which can be thrown into contraction only through some influence exerted at the points of contact (*X* and *Y*). Note that the muscles lie upon glass plates (*G* and *G'*), and that a glass nerve hook rests upon *I* in order to ensure two separate points of contact of the nerve from *II*.

3. **Observations.** (1) Rule a table as follows:

Strength of stimulus.	Response.			
	Muscle I.		Muscle II.	
	Make.	Break.	Make.	Break.
Very weak	Rest.	Contract.	Rest.	Rest.
Weak	?	?	?	?
Medium	?	?	?	?
Strong	?	?	?	?
Very strong.	?	?	?	?
Tetanizing	?	?	?	?

(2) Stimulate muscle *I* as indicated in the table and record the response in the proper column.

(3) What portion of preparation *I* is traversed by the electric current?

(4) Does any portion of the stimulating current traverse that part of muscle *I* between the points *X* and *Y*.

(5) What causes the contractions of muscle *II?* Preparation *II* is called a rheoscopic preparation or a physiological rheoscope. If the contractions are caused by electricity one should be able to detect it through the use of the galvanometer or electrometer.

(*B*) **Electromotive Force Detected by the Electrometer.**

1. **Appliances.** A large frog; non-polarizable electrodes; capillary electrometer.

2. **Preparation.** Pith the frog; prepare electrodes, using kaolin wet with normal saline solution for the tips. Join the electrodes to the binding posts of the electrometer. Make a muscle-nerve preparation, lay it upon a glass plate, and prepare to stimulate with induction shocks as in the case of muscle *I* above.

3. **Observations.** (1) Place the electrode which is joined to the capillary upon the tendon of the muscle; the other electrode upon the belly of the muscle. Adjust the meniscus in the middle of the field of the microscope. Open the short-circuiting key of the electrometer while watching the meniscus. It will be displaced. Its displacement suggests a difference of electric potential between the tendon and the belly of the muscle. Such a difference of potential is usually to be observed, and it is called the "demarcation current." It is believed to be due to the injury to the muscle tissue incident to its preparation. It is also called the current of injury.

(2) After the meniscus has come to rest stimulate the muscle with a single induction shock. The meniscus will move quickly, but in the direction opposite to that of its first motion. That is, its current of action is greater than its current of injury, and in an opposite direction. Describe phenomena in notes.

(3) Bring the muscle into action through other stimuli than electricity and note results.

PART II.

SPECIAL PHYSIOLOGY.

CHAPTER III.

THE CIRCULATION OF THE BLOOD.

I. THE CAPILLARY CIRCULATION AND THE MOVEMENTS OF THE HEART.

A. To Observe the Capillary Circulation.

1. **Appliances.** Frog; microscope, with low-power and high-power objective; cork board 10 cm. wide by 20 cm. or 30 cm. long and ½ cm. thick; pins; operating case; normal saline solution; watch-glasses; two 100 c.c. beakers.

2. **Preparation.** Pin the frog out, dorsum up, upon a cork board, and bring one hind foot over a hole 1 cm. in diameter cut in the corner of the board with a cork borer. By tying a thread to the second and third toes the web between these holes may be stretched over the hole in the board. Care should be taken not to stretch the web too tightly and thus impede the circulation.

Fix the cork board with the frog upon the stage of the microscope in such a manner as to bring the stretched web over the middle of the stage. Illuminate the web and focus under a lower power. Keep the web moist.

3. **Observations.** (1) Observe the movement of corpuscles within bloodvessels of varying size and irregular course. Make a drawing of the field of observation showing the relative size, the course, and anastomoses of the bloodvessels.

(2) Observe whether the motion is equally rapid in all vessels; if not, observe whether the slower currents are in the larger or the smaller channels. Determine which of the vessels are arterioles, which capillaries, and which venules.

(3) Have you seen evidence of intermittent force acting upon the corpuscles? If so, describe its influence. Determine whether this intermittent force makes itself evident in all the vessels; if not, in which class of vessels is it present?

(4) Do the corpuscles change shape? If so; under what circumstances?

(5) Enumerate all the observed structural and functional features which differentiate arterioles from venules.

B. To Observe the Action of the Frog's Heart.

1. **Preparation.** After the capillary circulation has been observed, the frog may be pithed and stretched upon a cork board, ventrum up. Make a median incision through the skin from the pelvis to the mandible; make transverse incisions and pin out the flaps. Raise the posterior cartilaginous tip of the sternum; insert a blade of the fine scissors under it and divide it transversely, about ½ cm. anterior to the tip. Raise the anterior segment of the sternum at the point of the transverse incision; insert the blade of the strong scissors under it and divide it longitudinally in the median line. Withdraw from the board the pins which fix the anterior extremities; make gentle lateral traction upon the fore-feet until the slit sternum is sufficiently separated to afford a convenient working distance and to expose the whole heart.

2. **Observations.** (1) Note rate of systole.

(2) Note sequence of contraction of auricles, ventricles, and bulbus.

(3) Note change in shape of different parts.

(4) Note the change in color and the position of the different parts of the heart during the cycle of changes that come with each heart beat.

(5) Carefully excise the heart, including the sinus venosus and the bases of the posterior and two anterior venæ cavæ, also the bases of the two aortic trunks. Place the excised heart in a watch-glass. Observe whether the pulsation continues. If so, what is your conclusion regarding the relation of the heart movements to the central nervous system?

(6) If the pulsation continues, note whether or not the rate of pulsation has been notably changed by the excision.

(7) Bathe the heart with a few drops of normal solution. Note any change in the rate of the beat.

(8) Hold the watch-glass in the palm of the hand and note whether there is any change in the rate of the beat.

(9) Float the watch-glass on ice-water and note any resulting modification of rate.

(10) If the heart seems vigorous (otherwise procure a fresh one), carefully sever the sinus venosus with the fine scissors. Does the sinus continue to beat? Does the heart continue to beat? Interpretation.

(11) If the heart beats, sever the auricle from the ventricle through the auriculo-ventricular groove. Note results.

(12) If the auricles beat, divide them. If they continue to beat, do they follow the same rhythm?

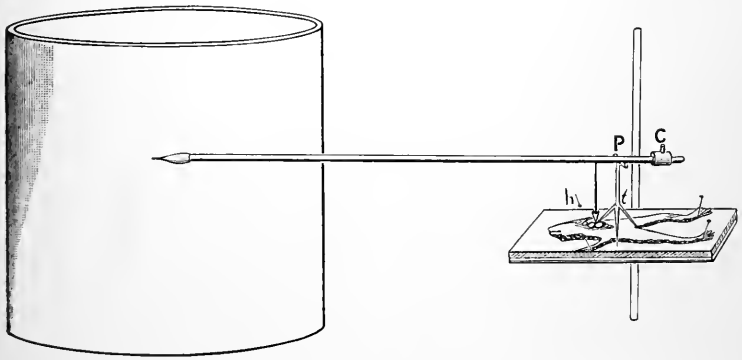
(13) If the ventricle becomes quiescent, stimulate it either mechanically or with a single induction shock. How does it respond to a single stimulus? Continue to subdivide the heart until the parts refuse to respond to stimuli.

(14) Repeat the experiments and see if the results are the same on subsequent trials. Note results and give your interpretation.

C. To Make a Graphic Record of the Frog's Heart Beat.

1. **Appliances.** Large frog; kymograph; heart lever. (For description of heart lever see Appendix, 10.) Frog-board myograph or similar apparatus; chronograph with a chronographic system, adjusted to record seconds upon the kymograph; cover-glass; normal saline; operating case.

FIG. 43



Frog-heart lever: *t*, tripod to support lever; *p*, the pivot; *c*, counterpoise; *h*, frog's heart, on which the cork point rests.

2. **Preparation.** Pith a frog without destroying its spinal cord. Take great care not to cut a vertebral artery during the pithing operation. Should a hemorrhage occur plug the opening with absorbent cotton. Hemorrhage depletes the circulatory system, and the action of the heart is weakened.

In the operation to expose the beating heart, take care not to cut any large vessel, for the reason just given. Pin out the frog, ventrum up, upon the frog-board myograph. Expose the heart as described in the previous lesson. Open the pericardium carefully, thus exposing the heart to direct observation. Place some resistant object—a cover-glass, for example—under the ventricle. So adjust the heart lever that the wedge-shaped cork foot of the long arm of the lever will rest upon the groove which marks the line of juncture between the auricle and the ventricle. (See Fig. 43.) If the weight

of the lever seems to be too great for the heart, move easily; the long arm may be made relatively lighter by adjusting the counterpoise upon the short arm. If the tracing point of the long arm has a sufficient excursion to make a good tracing, bring the kymograph to a position where the point will lightly touch the carboned surface of the drum. The lever should be nearly tangent to the surface of the drum, and so arranged that the rotating surface of the drum turns away from the tracing point of the lever rather than toward it.

All tracings should be accompanied by a time tracing or *chronogram.* Study the chronographic system and make drawings of the plan, showing all electric connections. Study the chronograph or time marker, and make a diagram showing its construction.

3. **Observations.** (1) Note whether the curve is a simple one or composed of a major wave, with crests superimposed upon it. In either case closely observe the phases of the heart cycle and determine the relation of each part of the cycle with each part of the tracing. If the tracing has a single crest, more delicately counterpoise the lever and more carefully adjust the narrow foot of the lever to the auriculo-ventricular groove and repeat the experiment.

(2) Take tracings of the auricle alone. Compare these with those of the auriculo-ventricular groove and determine the causes of variation.

(3) Without altering the counterpoise take a tracing of the ventricle and compare it with the two preceding curves and account for all the differences.

(4) By adjusting the foot of the lever between the ventricle and the bulbus it is possible to get a ventriculo-bulbar tracing which differs from the auriculo-ventricular in having the superimposed crest following the ventricular crest, while in the auriculo-ventricular tracing the superimposed crest precedes the ventricular.

(5) If the conditions of the experiment are favorable it is possible to get an auriculo-ventriculo-bulbar tracing. To get this the lever foot must be placed in the auriculo-ventricular groove so that it rests upon the auricles, ventricle, and bulbus. A typical tracing may be recognized by the high central ventricular crest flanked by two superimposed crests made by the auricles and bulbus.

(6) If a time tracing be added by means of the chronograph one may determine the time relations of the different phases of the heart cycle.

D. To Observe the Movements of the Mammalian Heart.

1. **Appliances.** Dog or rabbit; operating case, supplemented by hæmostatic forceps, heavy scissors and scalpels, clippers, heavy linen thread; hand bellows with tube and respiration cannula (see

Appendix, 11); animal holder; morphine solution with hypodermic syringe; chloroform or ether; tannic acid; absorbent cotton; porcelain-lined trays for instruments; cotton, calipers, and rule.

2. **Preparation.** The dog of medium or large size is to be preferred for class demonstrations, while rabbits or small dogs may be used for laboratory work by students. When rabbits are used anæsthetize with ether. When dogs are used anæsthetize with chloroform after having given ½ to 1 grain of morphine hypodermically fifteen minutes before the use of the chloroform.

Make a litre of one-half saturated solution of tannic acid to be used as an hæmostatic.

3. **Operations.** (1) **To Induce Artificial Respiration.** The opening of the thorax causes the lungs to collapse, and if artificial respiration were not instituted the animal would die in convulsions in a few minutes. The successful induction of artificial respiration involves the opening of the trachea, insertion of respiration cannula, and the maintenance of respiratory movements of the lungs through the use of the bellows.

Clip the hair from the ventral surface of the neck; make a median cutaneous incision; with forceps and fingers separate subcutaneous tissue, fascia, and muscles over the middle of trachea, and clear one to two inches of the trachea; cut a longitudinal ventral slit into the trachea and insert tracheal end of respiration cannula, ligating it firmly in place.

The animal will now breathe through the cannula. When the thorax is opened—but not before—the bellows should be attached to the cannula through the medium of a rubber tube at least one foot in length, and the bellows should then be brought into rhythmical action, causing the lungs to fill eighteen to twenty times per minute in the case of a dog (twice as fast for the rabbit).

After the introduction of the cannula and before the bellows is attached apply the anæsthetic to the distal end of the cannula. When the bellows is attached the anæsthetic must, of course, be applied to the intake valves of the bellows.

(2) **To Expose the Heart.** After the introduction of the respiration cannula, make a median incision over the sternum from anterior tip to posterior end of the xiphoid appendix. Strip the skin back laterally as far as the junction between the ribs and the costal cartilages. Saturate with tannic acid solution strips of absorbent cotton large enough to cover all cut surfaces.

With strong scalpel cut through the thoracic wall at the junction of the first left rib with its cartilage, carrying the incision quickly back along the thorax parallel to the sternum until all cartilages are cut. The cut-off ends of intercostal arteries will bleed freely, but this can be stopped in a moment by folding a strip of absorbent cotton wet with tannic acid over the cut-off ends of the ribs. A

strip of dry cotton and a towel may be placed outside of the tannic acid cotton.

Before proceeding farther, note that the left lung is collapsed. Begin artificial respiration, continuing the rhythm observed in the animal.

Carry the incision transversely across the thorax just posterior to the end of the sternum; catch the cut-off internal mammary arteries with hæmostatic forceps; carry the incision forward along the right side to correspond with the incision already made on the left side, and stop the hemorrhage in the same way.

The sternum may be covered with absorbent cotton and a towel and tipped forward out of the way.

The heart is now clearly exposed within its pericardium, and its relation to other structures of the thoracic cavity may be carefully noted before the pericardium is removed.

4. **Observations.** (1) Note position of heart with relation to lungs, spinal column, diaphragm, œsophagus, trachea, and large bronchi.

(2) Note character of pericardium and its attachments. Remove the pericardium by making a free longitudinal incision with scissors and slipping the heart through the incision. Note character of inner surface of pericardium; of outer surface of heart; presence of liquid in the pericardium.

(3) Note sequence of contraction of the chambers of the heart.

(4) Note change of shape of the heart during several phases of a cardiac cycle.

(5) Note change of position of the heart apex during phases of a cardiac cycle.

(6) Hold the beating heart in the hand and note the change in the tension of the heart muscle during phases of cardiac cycle, comparing diastole with systole.

(7) With calipers and rule measure carefully changes in the diameters of the heart, comparing end of diastole with the end of systole and observing the lateral diameter and the dorsoventral diameter.

(8) Is there a change in the anteroposterior diameter—base to apex? If so, when does this change occur?

(9) "Push the anæsthetic" to the limit and note that the animal's heart continues to beat. The same amount of ether or chloroform administered under ordinary conditions would cause the death of the animal through the stopping of respiration. But the respiration being carried on artificially, the amount of chloroform which can be taken is much increased. In the case of the dog, it will be hardly possible to kill with chloroform so long as respiration is kept up. If the respiration be stopped the animal will die very soon in convulsions.

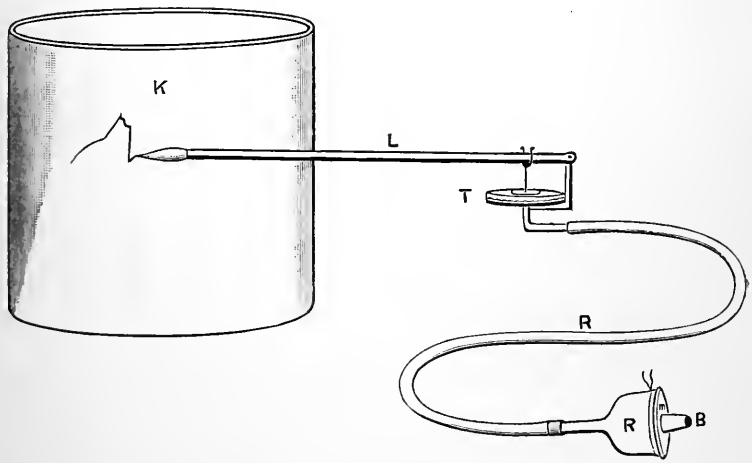
To terminate the experiment open the right ventricle. The thoracic cavity will quickly fill with blood and the animal will die a quick and painless death, free from any convulsions.

II. THE APEX BEAT AND THE HEART SOUNDS.

1. **Appliances.** A cardiograph, consisting of a receiving tambour and a recording tambour (Fig. 44).

The receiving tambour should be about 4 cm. in diameter and not less than 1 cm. deep. The tambour membrane should be of dentists' rubber-dam and should be stretched tightly enough to give it a resistance about equal to that of the relaxed biceps muscle. Upon the middle of the membrane a small cork (1 cm. long) is glued.

FIG. 44



The cardiograph: *R*, receiving tambour provided with a rubber membrane (*m*) and a cork button (*B*) to be placed on the apex beat. The receiving tambour is joined through the rubber tube *R* to the tracing tambour *T*, whose lever (*L*) records the movements of the thoracic wall upon the kymograph *K*.

The recording tambour should be 3 cm. to 5 cm. in diameter and not more than 3 mm. in depth. The tracing lever should be at least 20 cm. long and provided with a delicate celluloid or parchment tracing point. The recording tambour should be mounted on a light chemical stand and held by a universal clamp holder.

The two tambours should be joined through a piece of pressure tubing two feet in length.

For construction of tambours see Appendix, 12.

Besides the cardiograph one will need a chronograph, a kymograph, and a stethoscope.

Study the new instruments and make drawings and diagrams showing their construction.

2. **Preparation.** Let a student remove the clothing from the chest. Find the apex beat. In which intercostal space is it located? How far is it to the left of the middle of the sternum? Is the location of the apex beat the same for all members of the class? In recording the location of the apex beat refer to the bony landmarks of the chest rather than to the nipple.

To take a cardiogram place the button (cork) of the receiving tambour upon that point of the thorax most affected by heart beat. The movements of the apex of the heart will be transmitted and magnified by the cardiograph. Trace a cardiogram upon the kymograph.

3. **Observations.** (1) Take several cardiograms from the same individual, being careful so to adjust the apparatus as to gain the maximum excursion of the lever. What features have all of these tracings in common? What features seem to be accidental and non-essential? What are the causes of the essential features? What are the sources of the non-essential features?

(2) Take cardiograms of several individuals. Do all of them possess the features which seemed essential in the first series, taken from one individual? If not, how would you account for the difference.

(3) With a stethoscope, whose construction you have carefully described in your notes, listen to the heart sounds while the cardiograph is tracing the record of the heart movements. Note that two sounds are audible and that there is a notable pause following the shorter, sharper sound; let us call the sound which succeeds the pause the first sound.

(4) With what part of the cardiogram does the first sound seem to correspond? With what part of the cardiogram does the second sound seem to correspond? Give reasons for this correspondence.

(5) As far as the data will admit, enumerate causes for the first sound; for the second sound; for the essential features of the cardiogram. Can one locate on the cardiogram that crest or feature which corresponds to the auricular systole? The ventricular systole? The recoil of the ventricles? The closure of the semilunar valves? The opening of the semilunar valves?

(6) Giving full attention to the auscultation of the cardiac region of the chest with the stethoscope, note carefully: (*a*) The point where the first sound is most distinctly heard. Locate this point with reference to the thoracic skeleton. (*b*) The point where the second sound is most distinctly heard. Locate same with reference to skeleton.

(7) Compare the two sounds as to duration, intensity, pitch, and quality.

III. THE FLOW OF LIQUID THROUGH TUBES UNDER CONSTANT PRESSURE.

The problems presented by the circulation of the blood through the bloodvessels involve some of the general principles of hydraulics.

The supply of blood to the various glands and other active tissues of the body is analogous to the supply of water to the buildings of a city.

The blood-circulatory system differs from the water-circulatory system in possessing elastic tubes instead of inelastic ones, and an intermittent initial force instead of the constant force furnished by the "head" of water in the reservoir or stand-pipe.

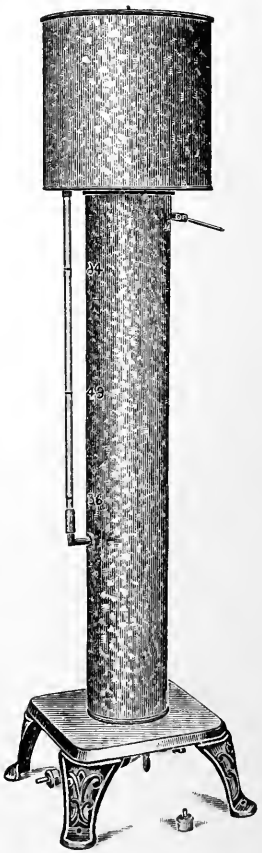
It will be profitable for the student to make a few simple experiments in hydraulics in order to make himself familiar with those physical laws which he will apply later.

1. **Appliances.** Each table is provided with a reservoir (Fig. 45) consisting of a galvanized-iron reservoir about 10 cm. in diameter and 70 cm. in height, with a supply tank above. At the bottom of the reservoir there is a faucet, to which may be screwed a 6-mm. nozzle or a 3-mm. nozzle, thus varying the radius of the outlet stream.

The reservoir is supplied with a gauge which indicates the height of the water above the middle of the outlet nozzle.

Each table will need besides the reservoir three 6-mm. T-tubes and five 6-mm. glass tubes 50 cm. long; also ten rubber connectors, a screw clamp, and centimetre rule. Provide a large flask or jar for catching discharge and a 500-c.c. graduated cylinder for measuring the discharge.

FIG. 45



Reservoir for use in experiments in hydraulics, and illustrating principles underlying circulation of the blood.

2. **Preparation.** We have thus a means of varying the radius of the outlet and the height of the water above the outlet. These are the two factors upon which the quantity of the discharge depends, viz., area of a cross-section of the stream and velocity of flow of the stream. The velocity of flow is determined by the law of Torricelli: "The rate at which a fluid is discharged through

an orifice (or nozzle) in a reservoir is equal to the velocity which would be acquired by a body falling freely through a height equal to the distance between the orifice and the surface of the liquid."

Make out a table which will show for each of the first five seconds of a falling body the distance traversed (d); the velocity (v); the total height at the end of each second respectively (h); and derive from this table the value of velocity in terms of g (g = acceleration of gravitation, 32 + ft. or 981 cm. per second) and of t (t = time in seconds).

(1) $$V = gt.$$

(2) $$H = \frac{gt^2}{2}.$$

Eliminate t from these two equations and express the value of velocity (V) in terms of the acceleration of gravitation (g) and the height (h).

(3) $$V = \sqrt{2gH} = \sqrt{2 \times 981\ H} = 44.3\sqrt{H}.$$

How does the velocity vary in terms of height? The velocity varies as the square root of the height.

(4) $$V \propto \sqrt{H}.$$

Given the height of the water in the reservoir (h) and the radius of the nozzle (r), to compute the discharge (D).

(5) $$D = \text{area} \times \text{velocity}.$$

(6) $$D = \pi r^2 \times 44.3\sqrt{h} = 44.3\ \pi r^2 \sqrt{h} = 139.2\ r^2 \sqrt{h}.$$

The discharge will vary as the product of the square of the radius multiplied by the square root of the height.

(7) $$D \propto r^2 \sqrt{h}.$$

3. **Observations.** To test the influence of the radius and the pressure upon the discharge one uses the law (expressed in $D \propto r^2 \sqrt{h}$). given above. Note that the discharge varies with two different factors.

It is a fundamental principle governing all experimental work, that, where one is studying a quantity which varies with two or more factors, he makes all but one of the factors constant and allows the quantity in question to be modified by only one variable factor at a time.

We will, therefore, make the radius constant by using the small nozzle while we observe the discharge as modified by varying height.

(1) Take the discharge in c.c. through 3-mm. nozzle at $h = 36$ cm.; $h = 49$ cm.; $h = 64$ cm.:

$$D : d :: \sqrt{H} : \sqrt{h}.$$

(2) Take the discharge in c.c. through 6-mm. nozzle at $h = 36$ cm.; $h = 49$ cm.; $h = 64$ cm. In these observations maintain a constant height in the reservoir by letting water flow in from the supply tank.

Use a time unit of ten seconds. Repeat each observation at least three times.

(3) From the above results compare influence of a varying radius when the height is constant—*i. e.*, discharge at $h = 36$, through 6-mm. nozzle; through 3-mm. nozzle.

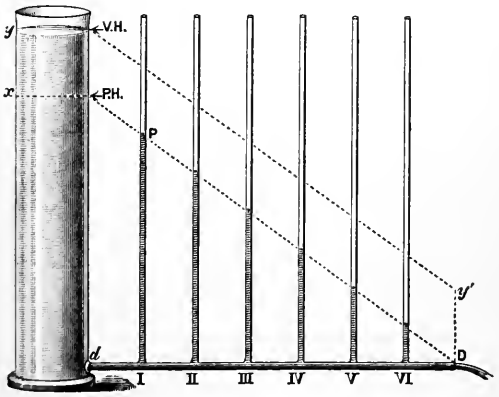
$$D : d : : R^2 : r^2.$$

(4) Having tested the two variables separately, test the two combined variables.

$$D : d : : R^2 \sqrt{H} : r^2 \sqrt{h}.$$

(5) To determine the relation of discharge to resistance: Attach to the larger nozzle one length of 6-mm. tubing. Note the discharge in, say, ten seconds. Attach a second length of 6-mm. tubing, taking care that the tubing is approximately horizontal. Note the discharge in the same length of time. What is your conclusion? Why does the discharge decrease when the length is increased?

FIG. 46



Reservoir with piezometers.

(6) To measure the pressure at various points along the course of the discharge tube: (*a*) Insert a 6-mm. T-tube with an upright limb not less than 50 cm. in length between the two 6-mm. discharge tubes. Is the height of the water in the upright (piezometer) as great as in the reservoir? (*b*) Add another T-tube to the end of the second 6-mm. discharge tube; how high does the water rise in the second piezometer? Comparing the height of the water in the reservoir and the two piezometers, what are your conclusions as to the pressure in different parts of the discharge tube?

(7) By leaving out the 50 cm. tubes and setting the T-tubes end to end thus (⊥⊥⊥⊥⊥⊥) a set of piezometers similar to those shown in Fig. 46 can be set up and new observations made.

IV. THE FLOW OF LIQUIDS THROUGH TUBES UNDER THE INFLUENCE OF INTERMITTENT PRESSURE.

A. The Influence of Intermittent Pressure.

1. **Appliances.** A glass tube of about 6-mm. lumen and about 100 cm. long; a thin-walled elastic tube of about the same lumen as the glass tube and about 100 cm. long; a double-valved, strong rubber bulb (about 7.5 cm. long); very thick-walled rubber tubing for joining up the apparatus; a 2-litre jar and a flask or water receptacle; heavy linen thread; a wide capillary and a fine capillary or a piece of glass tubing 10 cm. long for constructing the same; 500-c.c. graduated cylinder; piece of 8-mm. rubber tubing about 50 cm. long.

2. **Preparation.** Join the large elastic tube to the entrance valve of the bulb. Couple the glass tube closely to the exit valve of the bulb. Make all joints as close as possible, and tie tightly with thread. Draw a coarse and fine capillary tube from the 10-cm. piece of glass tubing. Fill the jar with water and immerse the tube from the entrance valve in the water.

Clasp the bulb in the hand and make rhythmical contractions at the rate of ten to fifteen in ten seconds. This process will, of course, pump water from the jar into the flask held at the distal end of the long glass tube. One person should pump the bulb and the greatest care should be taken to exert the same force and use the same rate in the several observations.

3. **Observations.** *a. Intermittent force and inelastic tubes.*

(1) Does the stream of water which is ejected from the exit tube flow in a constant or in an intermittent jet?

(2) Attach a wide capillary and repeat. What is the character of the stream? Measure the discharge in ten seconds.

(3) Attach a fine capillary and repeat. Measure the discharge in ten seconds.

b. Intermittent force and elastic tubes.

(4) Disjoin the glass tubing from the bulb and join the 100-cm. elastic tube. Work the bulb as directed above and observe the character of the flow. Measure the quantity of discharge.

(5) Join on the coarse capillary and repeat, noting the change in the character of the jet and the amount of discharge.

(6) Replace the coarse capillary with the fine capillary and repeat. Sum up results and formulate conclusions.

B. The Pulse or Impulse Wave.

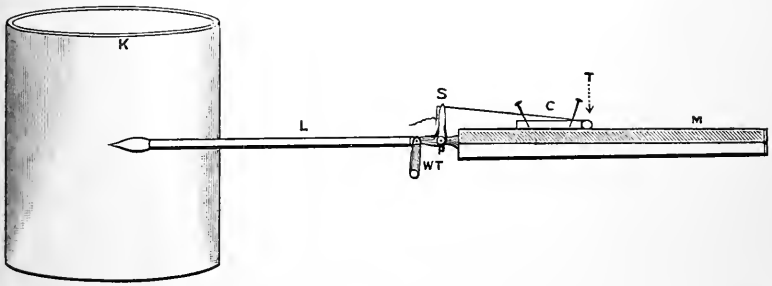
By putting the finger upon the rubber tube while the bulb is in action the pulse may be felt. To trace this upon the kymograph

lay the rubber tube across the frog-board myograph and pass a thread from the proximal end of the board around the pulsating tube and thence to the thread-eye of the tracing lever as shown in Fig. 47.

A block or cork will hold the tube in place. Pulsations of the tube will be transmitted to the thread and in turn to the lever and may be traced upon the kymograph.

Observations. (1) If the finger be held upon this elastic tube while the bulb is being rhythmically squeezed a series of impulses or pulsations will be felt by the finger. Place one finger upon the elastic tube near the bulb; another finger near the capillary. Let the bulb be pumped with sudden but infrequent contractions. May one note the difference in the time of pulsation felt by the two fingers? If so, which is felt first, and why? What is the cause of the pulsation?

FIG. 47



Myograph in use as a pulse-writer: *K*, kymograph; *L*, tracing lever; *S*, short arm of elbow lever; *M*, section of frog-board myograph; *T*, cross-section of rubber tube; *C*, block of cork against which the tube rests; *WT*, weight-link; *P*, pivot.

(2) To get a tracing of this pulse, pass the rubber tube across the cork board as shown in the figure; adjust to kymograph and take tracing. Vary the character of the bulb contractions as follows, taking one complete rotation of the drum for each variation:

(*a*) Slow initial contraction of bulb and slow relaxation.
(*b*) Slow initial contraction of bulb and quick relaxation.
(*c*) Quick initial contraction of bulb and slow relaxation.
(*d*) Quick initial contraction of bulb and quick relaxation.
(*e*) Same as (*d*) with slow rhythm (1 contraction per second).
(*f*) Same as (*d*) with rapid rhythm (3 contractions per second).

(3) Make a careful study of these tracings and determine:

(*a*) The characteristic and essential features.
(*b*) The accidental and non-essential features.
(*c*) The cause of the essential features.
(*d*) The cause of the non-essential features.

V. THE LAWS OF BLOOD PRESSURE DETERMINED FROM AN ARTIFICIAL CIRCULATORY SYSTEM.

Having tested by experiment some of the laws of governing the flow of liquid through tubes under the influence of intermittent pressure, we come to the point where we may attempt to reproduce experimentally a set of physical conditions so nearly like those which exist in the animal body that we shall be able to draw conclusions from our experiments that shall hold good for the animal circulatory system.

The last preceding exercise demonstrated (1) that the continuous and even flow of liquid through the capillaries is made possible by the elasticity of the arterial walls; (2) that the pulse is caused by a varying pressure within the elastic artery; (3) that the varying pressure is due to the alteration of systole and diastole of the heart; and (4) that the pressure within the arteries is largely influenced by the size of the capillary through which the fluid must pass—*i. e.*, by the peripheral resistance.

Blood pressure is then the product of two factors: Cardiac force $\times$ peripheral resistance ($P = H \times R$); but cardiac force is in turn due to the product of two factors: Rate $\times$ strength; ($H = r \times s$); therefore: *Pressure is the product of heart rate* $\times$ *heart strength* $\times$ *peripheral resistance* ($P = r \times s \times R$).

We have here to deal with these three variables. Applying a principle set forth in a previous exercise (to the effect that "when a value which is being tested by experiment is affected by two or more variable factors only one of these must be allowed to vary in any one experiment") one will so arrange his experiment that these three factors of pressure will *vary one at a time.*

1. **Appliances.** An artificial circulatory system may be constructed as follows: A rubber bulb such as used in the preceding exercise, to which is attached a capacious entrance tube. To the exit tube attach the 100-cm. soft-rubber tube used before. This will serve as the main artery, at the end of which a T-tube may be inserted, one limb passing to the arterial manometer. Beyond the T-tube is another rubber tube leading to a Y-tube. From each limb of the Y-tube lead off a smaller elastic tube, one branch being a small, thin-walled tube supplied with a screw clamp, while the other passes to a large calcium chloride tube which has been filled with sponge to represent the capillary system of minute tubes. (See Fig. 48.)

After traversing the capillary system the liquid is collected at a Y and returns to the heart through a tube which is nearly twice as large as the artery. In this vena cava is inserted a T-tube, to which is attached the venous manometer.

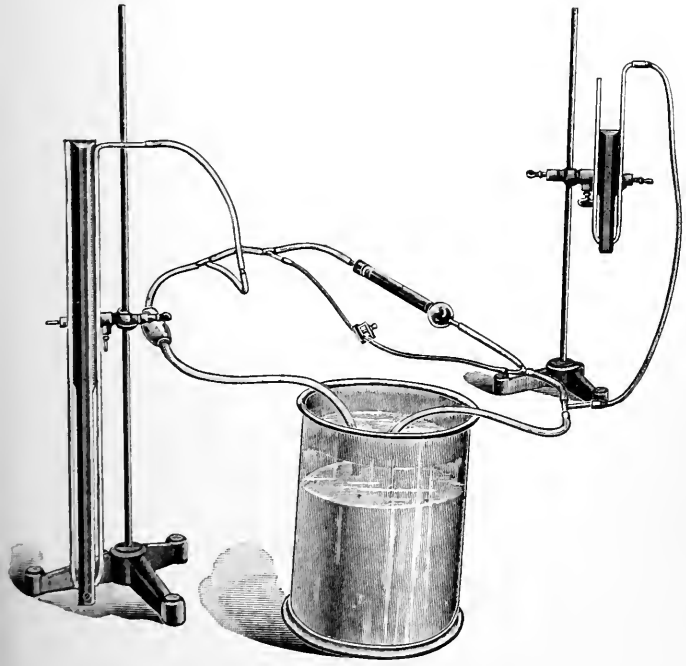
Between the Y-tubes the blood may be opposed by high resistance or, with the screw clamp open, by the low resistance.

The bulb may be pumped weak or strong, fast or slow, while the peripheral resistance may be high or low. We have, therefore, a contrivance through which we are able to vary one factor at a time.

The arterial manometer should have limbs not less than 50 cm. in length, while those of the venous manometer need not be more than one-half that length. These manometers may be held by clamps to chemical stands which are on or beside the table.

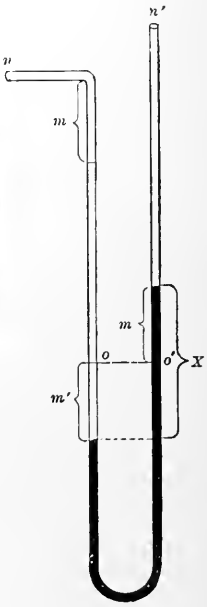
2. **Preparation.** Set up an artificial circulatory system as shown in Fig. 48.

FIG. 48



Artificial circulatory system, described in detail in text.

FIG. 49



Mercury manometer.

After the system is set up make a study of the mercury manometers, the instruments with which the pressure is to be measured.

The specific gravity of mercury is approximately 13.6. What is the gas pressure at *n* that will cause a rise of 4 cm. of mercury in the distal tube? (See Fig. 49.)

What is the water pressure at *n* that will cause a rise of 6 cm. of mercury in the distal tube?

What is the water pressure at *n* that will cause a rise of *m* cm. of mercury in the distal tube?

After the system has been freed from air and is at rest, do the proximal and distal columns of mercury in the arterial manometer

stand at the same lever? If not, why? What allowance, if any, should be made for this?

3. **Observations.** (1) By experiment fill out the following table:

Observation.	Heart activity.		Peripheral resistance.	Arterial manometer.	Venous manometer.
	Strength.	Rate.			
1	Weak	Slow	Low	..... mm.	....., mm.
2	Weak	Slow	High	..... mm.	..... mm.
3	Weak	Fast	Low	.... mm.	..... mm.
4	Weak	Fast	High	..... mm.	.... mm.
5	Strong	Slow	Low	.... mm.	.... mm.
6	Strong	Slow	High	..... mm.	.... mm.
7	Strong	Fast	Low	..... mm.	.... mm.
8	Strong	Fast	High	..... mm.	..... mm.

(2) Trace the pulse upon the kymograph as indicated in the foregoing lesson.

(3) What are the principal factors which control blood pressure?

(4) State concisely just what effect these factors have upon blood pressure.

(5) What combination of conditions yield the highest arterial pressure?

(6) What set of conditions yield the lowest arterial pressure? The highest venous pressure? The lowest venous pressure?

VI. THE RADIAL PULSE AND THE SPHYGMOGRAM.

1. **Appliances.** A sphygmograph; tracing slips; a fish-tail gas jet or kerosene lamp, and a holder in which to place the slips while they are being smoked (Fig. 50).

FIG. 50

Holder for smoking slips for the Dudgeon sphygmograph.

2. **Preparation.** That the sphygmograph is so little used by the general practitioner may be attributed to the fact that hurry of business or some other cause has hindered him from making himself thoroughly conversant with the adjustment and use of the instrument, with its limitations and with the interpretation of the tracings.

To Adjust the Sphygmograph. (1) Let the observer stand with his right foot on a chair. This brings his thigh into a horizontal position.

(2) Let the subject stand at the right of the observer, resting the dorsal surface of the left forearm upon the observer's knee.

(3) Let the observer with pencil or pen mark the location of the radial artery.

(4) Let the observer wind the clockwork which drives the tracing paper; adjust the latter in readiness for tracing; rest the instrument upon the subject's arm with its foot on the radial artery and adjust the position, tension, and pressure in such a manner as to obtain the maximum amplitude of swing of the tracing needle. Take the tracing. Fix in damar-benzole solution.

3. **Observations.** *a. The Location, etc., of the Radial Artery.* (1) What are the relations of the radial artery at the distal end of the radius?

(2) How may the relations vary?

(3) Is there any variation, among the members of the division, in the location of the radial artery?

(4) May excessive muscular development affect the ease with which the artery may be located and its pulsations studied?

(5) May excessive deposit of adipose tissue hinder the observations of the pulse?

(6) May faulty position of subject or of his clothing affect the pulse?

b. The Digital Observation of the Radial Pulse. (7) Feel the pulse with the side or back of the finger, then with volar surface and tip of each finger of each hand, and note the finger or fingers with which the feeling is most acute. It will be wise to always use these fingers in all tactile examinations. Their acuteness of feeling will increase with practice. One may thus acquire the educated touch —*tactus eruditus.*

(8) How much may be learned of the pulse by means of the touch alone? Observe and note: (*a*) rate; (*b*) rhythm; (*c*) volume; (*d*) strength; (*e*) compressibility; (*f*) may anything else be determined by this method?

c. The Sphygmogram. (9) Take at least three pulse tracings of each individual in the division. (*a*) Compare the tracings taken from one individual; if they differ, determine the cause of the difference. (*b*) Compare the tracings of different members of the division. Determine, if possible, the cause of the differences.

(10) Do variations of the relations of the artery affect the sphygmogram? Does the adjustment affect the sphygmogram? Does the elasticity of the artery affect the tracing? How does strength or rate of heart beat affect it?

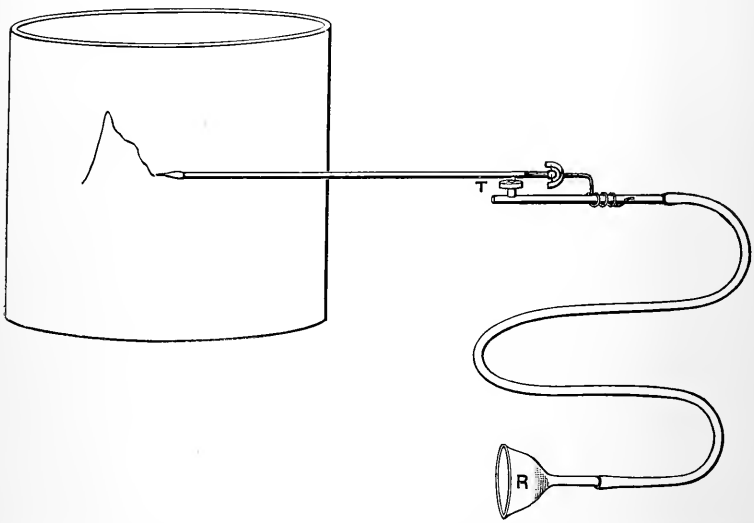
Make a list of the facts regarding the condition of the circulatory

system which may be determined with the help of the sphygmograph. Make a list of the precautions to be observed in the use of the sphygmograph.

The Carotid Pulse.

One will frequently experience difficulty in taking the radial pulse; in fact, not more than one person in three or four is a favorable subject for this observation. The reason for this is not far to seek. Anything beyond a moderate development of the musculature of the forearm is accompanied by such development of the tendons and of the styloid process of the radius that the artery is quite inaccessible for the sphygmograph-foot as usually constructed. A moderate deposit of subcutaneous fat also obscures the radial pulse and makes the use of the sphygmograph most unsatisfactory.

FIG. 51



Porter's carotid sphygmograph: *R*, receiving tambour in form of open bell, that is pressed against the throat over the common carotid; *T*, tracing tambour with small, thin membrane and light lever.

The simple sphygmograph devised by Dr. Porter, of Harvard, enables one to overcome some of the difficulties mentioned above. This instrument consists (1) of a very small and delicately adjusted recording tambour with high magnification and a delicate tracing point; (2) of a receiving tambour not over 2 cm. or 3 cm. in diameter and at least 1 cm. deep, connected with the recording tambour through a 50-cm. piece of pressure tubing, which is provided with a side vent closed by a clamp. For the receiving tambour a small thistle tube may be used. (See Fig. 51.)

To trace the carotid pulse, place the open mouth of the receiving tambour, over which no membrane has been stretched, over the course of the carotid artery beside the larynx, taking care that the side vent of the pressure tube is open while the adjustment is being made. Close the vent, and if the adjustment has been successful the lever will show the carotid pulse. Trace it upon the kymograph.

Compare the carotid sphygmogram with the radial sphygmogram. Account, if possible, for any essential difference.

One may trace a radial sphygmogram with the same instrument by stretching a rubber membrane rather tightly across the mouth of the receiving tambour cementing a bone collar-button to the middle of the membrane then placing the head of the collar-button upon the radial artery.

VII. TO DETERMINE THE ARTERIAL BLOOD PRESSURE IN AN ANIMAL.

1. **Appliances.** Dog or a large rabbit; mercurial manometer, with manometer tambour. (See Appendix, 13.) In lieu of the manometer tambour (Fig. 52) one may use the ivory float with tracing point in the distal limb of the manometer; kymograph; chronograph; physiological operating case; glass arterial cannulæ; half-saturated solution of sodium carbonate, sodium sulphate, or magnesium sulphate; clippers; dog board or rabbit holder; absorbent cotton; one-half grain of sulphate of morphine; hypodermic syringe; chloroform and ether.

2. **Preparation.** The manometer should be filled with mercury with at least 10 cm. in each limb. Attach to the proximal limb of the manometer a piece of pressure tubing 6 or 8 inches in length, to which attach one limb of a T-tube. To the opposite limb of the T-tube attach another piece of pressure tubing not less than a foot in length, into the end of which the glass cannula can be placed. To the side limb of the T-tube attach a rubber tube, which may be carried upward to an inverted flask filled with the non-coagulant (Na_2CO_3, Na_2SO_4 or $MgSO_4$). The tube leading from the reservoir should have a screw clamp near the T-tube. The reservoir may be supported near the top of the same stand which supports the manometer.

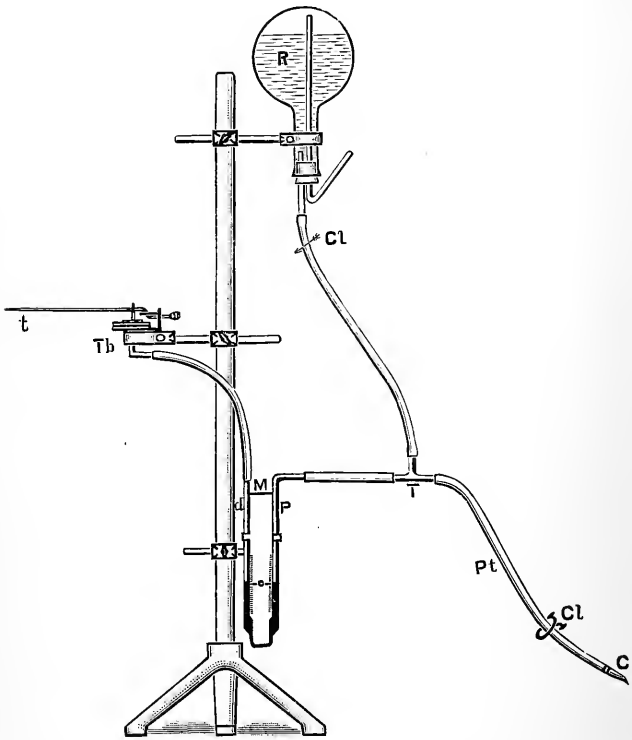
If a dog is used for the experiment he should be given from $\frac{1}{4}$ to $\frac{1}{2}$ grain of morphine twenty minutes before the anæsthesia with chloroform. If a large rabbit is used anæsthetize with ether.

3. **Operation.** After fastening the animal to the holder, clip the throat, make an incision from the upper end of the sternum over the right sternothyroid muscle to the middle of the neck, cutting

through the skin and subcutaneous tissue to the surface of the muscle. The external jugular vein lies close to the incision externally.

Sponge the oozing vessels until hemorrhage is checked, then using forceps and fingers dissect away the fascia until you reach the external margin of the sternothyroid muscle. Separate this muscle to the inside, making an opening down to the carotid artery, where pulsation may be felt near the trachea. Lifting the sheath which contains the carotid artery and the vago-sympathetic nerve, taking care not to

FIG. 52



The manometer tambour: *M*, manometer; *Tb*, tambour; *R*, reservoir filled with one-half saturated solution Na_2CO_3; *T*, T-tube; *Pt*, pressure tube; *Cl*, clamp; *C*, cannula; *P*, proximal limb of manometer; *D*, distal limb of manometer; *t*, tracing point of tambour lever.

wound the internal jugular vein, which lies in close relation to these structures, tear open the sheath of the artery and nerve and separate out the carotid artery to the extent of one or two inches.

Choose a glass cannula not larger than the carotid; place the large end of the cannula in the pressure tubing attached to the manometer. Open the screw clamp and allow the tubes to fill with the solution clear to the point of the cannula. Close the screw

clamp to stop the flow of the solution from the reservoir and do not open the clamp after this except to clear the cannula of a clot; and this cannot be done, of course, while the cannula is in the artery. Ligate carotid artery at the upper end of the incision. Clamp the lower portion of the carotid with the seraphin forceps, place the finger under the artery, make a longitudinal incision in the middle of the exposed portion of the carotid. Insert the point of the cannula into the lumen of the artery, tie the cannula in place, and remove the seraphin forceps.

4. **Observations.** As soon as the seraphin forceps have been removed the blood will rush into the cannula and tube for a distance of 4 to 8 cm., the mercury will rise in the distal limb of the manometer to a corresponding degree.

(1) Measure this rise in the distal limb of the manometer. What is the blood pressure in centimetres of mercury per unit area?

(2) Note that the mercury rises and falls in the manometer with a rhythmical motion. Attach the manometer tambour or adjust the float and watch the movements of the tracing point. Feel the pulse of the animal and note whether the movements of the tracing point correspond to the heart beats.

(3) Bring the kymograph into position, adjust the tracing point of the blood-pressure apparatus, also the chronograph, and take a tracing. What is the rate of heart beat?

(4) Are the respiratory movements evident in the tracing? If so, what is the influence of inspiration upon blood pressure? What is the influence of expiration? Account for the influence of respiratory movements upon blood pressure.

(5) What causes the blood pressure to rise during inspiration? Modification in blood pressure must be due either to the rate or strength of the heart beat or to the condition of peripheral resistance.

(6) If a line were drawn through the lowest point of the individual cardiac waves, this waving line would represent the influence of respiratory movements upon blood pressure. If the lowest point of these respiratory waves were joined by a line, would this line be a straight one or would it be a long, undulating curve? If such a curve is observed, it may be recognized as the Traube-Hering curve. This curve represents a gradual rise and fall of the blood pressure under the influence of changing peripheral resistance, which in turn is controlled by the vasomotor nerve centres.

VIII. THE SPHYGMOMANOMETER AND PULSE PRESSURE.

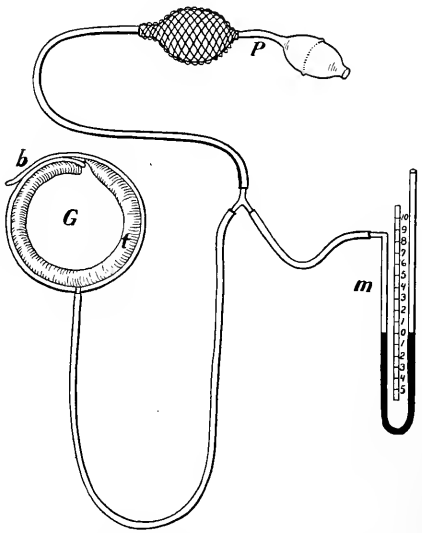
Various clinical instruments have been devised for the purpose of determining blood pressure in the human subject in health and in disease. The most satisfactory of these devices involves the use

of the mercury manometer in measuring the pressure in a pneumatic arm-girdle so adjusted as to suppress or to modify the pulse. An accurate determination of blood pressure is occasionally of very great importance, and it goes without saying that methods used on the lower animals are not applicable in the case of man because they involve the opening of an artery.

The only *appliance* needed is the sphygmomanometer (Fig. 53) and the only *preparation* is for a member of the class to remove clothing from one arm.

Observations. (1) Let the subject lie upon his back on the table in an easy and comfortable position, and absolutely relaxed and quiet for five minutes. During this period the girdle may be

FIG. 53



The sphygmomanometer: *G*, arm-girdle with inflatable rubber tube (*t*) within and sole-leather belt (*b*) without; *P*, pressure bulbs; *m*, mercury manometer.

fastened about the right arm. While one observer is counting the pulse at the left wrist, another may feel the right pulse. A third observer may watch the manometer while he gradually pumps air into the girdle until the pulse is shut off at the wrist. Read the manometer, relax the girdle pressure until the pulse reappears. Read the manometer. The mean between the two readings as thus made is taken to represent the *pulse pressure*. Record the pulse rate as counted on the left pulse. Record the pulse pressure as determined by the sphygmomanometer.

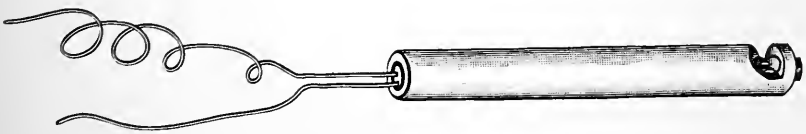
(2) Let the subject lie on his right side. Take observations as outlined above and record pulse rate and pulse pressure.

(3) Let the subject lie on his left side. Record results.
(4) Let the subject sit. Record results.
(5) Let the subject stand. Record results.
(6) Let the subject take vigorous exercise for five minutes. Take observations of pulse rate and pulse pressure while the subject stands.
(7) From the tabulated results of the above observations write in a list the postures and conditions which give an increasing series of pulse pressures.
(8) Prepare a similar list of the postures and conditions which give an increasing series of pulse rates.
(9) Are these two series alike in the order in which the conditions are named?—*i. e.*, do the conditions which give high rate give also high pressure? Account for what you discover.
(10) If pulse rate increases, under what conditions could pulse pressure fall ($P = Hr \times Hs \times R$)?

IX. TO DETERMINE THE INFLUENCE OF THE VAGUS NERVE UPON THE ACTION OF THE HEART.

1. **Appliances.** Operating case; a pair of curved, blunt-pointed shears, or, better, a pair of barber's clippers; a rabbit board; a large sheet of heavy paper; cotton; ether; thread; one dry cell; inductorium; shielded electrode (Fig. 54); seven wires; stethoscope; a rabbit; contact key; short-circuiting key.

FIG. 54



A shielded electrode of hard rubber, bearing copper or platinum wires.

2. **Preparation.** Let six or eight students be divided into three or four groups of two each.

Let the group "a" be responsible for the anæsthesia. Use the sheet of heavy paper to make a conical hood, whose spiral turns may be held in place with sealing-wax or pins. Place a wad of cotton loosely in the mouth of the cone.

Let the group "b" perform the operation. Tie the rabbit back downward upon the holder; fix the nose in special holder; with the barber's clippers remove the hair from the ventral side of the thorax and neck; make hands and instruments *clean;* place instruments in shallow basin of warm water; cut two or three ligatures of thread and place them in the instrument basin.

Let the group "c" arrange the electric apparatus for stimulation

of the nerves. Fill the cell; join up with contact key in the primary circuit, and a short-circuiting key in the secondary circuit. Test the apparatus to see if everything is in order. Group "d" should keep all the records of pulse or other observations.

3. **Operation.** **Group "a."** (1) Pour 1 c.c. or 2 c.c. of sulphuric ether upon the cotton in the cone; place the cone over the rabbit's nose; observe and note carefully the three stages of anæsthesia.

(2) Carefully note the rate of the heart before beginning anæsthesia, and the influence of anæsthesia upon rate and strength of heart and respiration.

(3) Keep the cotton moist with ether; watch the respiration and pulse, and be careful not to give the animal too much and thus interrupt the experiment.

Group "b." Wash the clipped surface of the throat. After the rabbit is completely anæsthetized, make with scissors a median incision through the skin, beginning at the anterior end of the sternum and cutting anteriorly for about 5 or 6 cm.; divide the subcutaneous connective tissue over the middle of the trachea. Carefully separate from the median line on either side laterally the subcutaneous connective tissue with the associated adipose tissue.

How many pairs of muscles come to view? What two muscles approach the median line to form the apex of a triangle at the anterior end of the sternum? Observe a pair of thin muscles lying dorsal to the muscles just mentioned, and joining them in the median line to form a thin muscle sheet covering the trachea on its ventral side. What muscles are these?

Carefully lift up the median edge of the sternomastoid muscle and separate with the handle of a scalpel or a seeker the delicate intermuscular connective tissue. A bloodvessel and several nerves come into view.

Is the bloodvessel an artery or a vein? How many large nerves accompany the bloodvessel?

Take hold of the sheath of the vessel; lift it up and note in the connective tissue accompanying the bloodvessels two nerves, one large and one small. When the artery is in its normal position, what relation do these two nerves sustain to it? Which of the two nerves is external and which is dorsal to the bloodvessel? Which is in close relationship with the artery? The larger of the two nerves is the vagus or pneumogastric.

In preparing the nerve for stimulation one should neither grasp it with the forceps nor with the fingers. It may be separated from the delicate connective tissue in which it lies by use of a blunt seeker. Far better than any metallic instrument is a small glass rod drawn to a point, curved and rounded in the Bunsen lamp. Prevent the tissues drying up by occasionally pressing them lightly with pledgets of cotton moistened with normal salt solution. Adjust the electrode

carefully upon the vagus and see that no unnecessary tension is allowed to be exerted upon the nerve. It is usually necessary to hold the electrode in place during the observations.

4. **Observations.** *a.* **Anæsthesia.** (Observations of Group "a.")

(1) Are you able to make out the different stages of anæsthesia?

(2) How many stages did your animal manifest?

(3) Give the characteristics of each stage?

(4) What effect did the ether have upon the rate of heart beat?

(5) What effect did ether have upon respiration?

b. **The Stimulation of the Vagus.** (Observations of Groups "*c*" and "*d.*")

(6) Stimulate moderately one vagus. Note with a stethoscope any change in the rate of the heart.

(7) Cut both vagi high up in the neck. Note the rate of heart beat at intervals of five minutes for thirty minutes, allowing the rabbit to partially recover from the anæsthesia.

(8) Stimulate one vagus. Compare the result with that obtained under experiment (6).

(9) Will very strong stimulation bring the heart to a standstill?

(10) If the heart were brought to a complete standstill by the stimulation, will it start up spontaneously when the stimulus is removed? Will the rate be the degree of acceleration observed in experiment (7)?

(11) Sum up the observations into a concise statement as to the influence of the vagus upon the heart.

NOTE. Dispatch the rabbit with chloroform.

X. TO DETERMINE THE INFLUENCE OF THE CARDIAC SYMPATHETIC NERVES UPON THE ACTION OF THE HEART.

The appliances should be the same as for the preceding exercise.

Let the students who work at one table continue the same grouping that was arranged in the preceding exercise, but rotating in the work: Group "a" to operate; group "b" to arrange electric apparatus and stimulate nerve; group "c" to note pulse rate and keep records; group "d" to give anæsthetic.

The operation should be similar to that of the vagus experiment.

Find the cardiac branch of the cervical sympathetic in the lower part of the neck, where it is in close relation with the carotid artery and the internal jugular vein. The most certain way to recognize it is through its function. Carefully separate out the nerve trunks in the region described; with the glass nerve hook lift up any nerve except the vagus and stimulate moderately.

Stimulation of the cardiac sympathetic distinctly increases the pulse rate.

Find the corresponding nerve of the opposite side, verifying your choice by observing the effect of stimulation.

Cut both cardiac sympathetic nerves and observe the rate of the heart beat at intervals of five minutes through a period of thirty minutes.

XI. THE INFLUENCE OF THE VAGUS AND THE CARDIAC SYMPATHETIC UPON THE ARTERIAL BLOOD PRESSURE.

1. **Appliances.** Dog or large rabbit; animal holder; mercury manometer, with float or tambour and with flushing flask of non-coagulant; with tubing and cannulæ, as described in Appendix A, 12; a kymograph, inductorium; Daniell cell; two Du Bois-Reymond keys; operating case; chloroform, ether, morphine; hypodermic syringe.

2. **Preparation.** Let eight students in four groups of two each have charge of (*a*) anæsthesia, (*b*) operations, (*c*) electric apparatus and stimulation, (*d*) pressure tracings.

3. **Operations.** (*a*) Anæsthetize the animal in accordance with directions given in previous exercises for the dog and rabbit, respectively.

(*b*) Remove the hair from the throat; make a cutaneous incision in the median ventral line from the anterior end of the sternum to the anterior end of the larynx. Remove the subcutaneous tissue and expose the sternomastoid and sternothyroid muscles. Let one operator expose the carotid artery of one side while the other operator exposes the vagus and sympathetic of the other side.

(*c*) Adjust the shielded electrode for stimulation. Insert the cannula into the artery.

(*d*) Make the tracing of arterial blood pressure in accordance with directions given in a previous exercise.

While the tracing is in progress stimulate the vagus with a moderate tetanizing current for a period of two to five seconds. Repeat the stimulation at intervals of ten to twenty seconds for ten minutes.

Adjust the electrode for stimulation of the cardiac sympathetic. Stimulate.

4. **Observations.** (1) What is the average blood pressure measured in centimetres of mercury in the animal under observation before stimulation of a nerve?

(2) What influence does stimulation of a vagus nerve have upon the arterial blood pressure?

(3) Is the effect clearly marked on the pressure tracing?

(4) What influence does stimulation of the cardiac sympathetic have upon arterial blood pressure?

(5) Is the effect clearly marked on the pressure tracing?

(6) In the case of which nerve is the influence of stimulation the more pronounced?

(7) In one animal cut both vagi nerves and note the influence on blood pressure for a period of one hour after the section.

(8) In another animal cut both cardiac sympathetic nerves and note the influence on blood pressure.

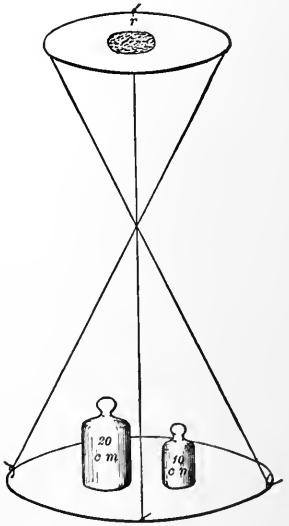
XII. THE BLOOD PRESSURE IN THE TISSUES.

A. To Determine Capillary Blood Pressure.

1. **Appliances.** A set of metric weights from 1 to 100 grams; a common-sized watch-crystal; a $\frac{7}{8}$-inch round cover-glass, No. 3; sealing-wax; linen thread; dividers; millimetre scale.

2. **Preparation.** To make an apparatus for determining capillary pressure mark upon the edges of the watch-crystal and cover-glass, points distant from each other 120° of arc, cut three equal pieces of thread from 10 to 12 cm. in length; fasten the ends to the points marked in the circumference of the glasses with melted sealing-wax. If the threads are of equal length, and if the cover-glass is held in a horizontal plane, the watch-crystal suspended by the threads should be parallel to the cover-glass, and, therefore, in an horizontal plane. If the cover-glass is given a half-turn to right or left, the three threads will cross as seen in Fig. 55. A thread should be tied around where this cross occurs and the knot secured with sealing-wax. Weigh this apparatus and mark upon the watch-glass its weight in grams.

FIG. 55



Apparatus for determining the capillary pressure.

Hold the left hand with palm upward, fingers slightly flexed. Hold the apparatus with the cover-glass horizontal and place the middle of the cover-glass on the tip of the ring finger, the watch-crystal hanging below. The weight suspended on the finger would be simply the weight of the apparatus.

3. **Observations.** Place sufficient weight upon the watch-glass scale-pan to nearly exclude capillary circulation from the flattened circular area where the cover-glass presses upon the finger. If the capillary circulation is completely excluded from this area the skin

will look quite white. A sufficient weight should be put on to make the area distinctly paler, but not white.

(1) What is the diameter of the area from which the capillary circulation is excluded?

(2) What is the area expressed in square millimetres?

(3) What weight was added to the apparatus?

(4) What is the total weight resting on the computed area?

(5) What is the weight in milligrams resting upon each square millimetre of surface?

(6) How high would a column of water be in milligrams that would represent this same pressure per square millimetre?

(7) How high would a column of mercury be that would represent this same pressure?

(8) What is the capillary pressure in the volar surface of the ring finger in the different members of the class?

(9) Is the capillary pressure modified by a variation of the position of the arm?

(10) Is the capillary pressure modified by variation in the posture of the subject: lying, sitting, standing?

B. The Plethysmograph.

This instrument is designed to determine the tissue pressure in contradistinction to the arterial pressure in larger arterial trunks.

When an arm, leg, or finger is thrust into a case just large enough to accommodate the member, any change in the volume of the tissues will change the amount of space between the limb and the case, and this change in volume may be easily traced with a recording tambour.

Such a case is really a modified receiving tambour and is called a plethysmograph. One of these adapted to the finger is shown in Appendix, 12.

1. **Appliances.** Plethysmograph; recording tambour, smallest size for finger, medium size for arm; kymograph.

2. **Preparation.** Pass the finger or the bare arm through the rubber collar of the receiver. The collar should fit the arm above the elbow, or the index finger around the first phalanx tightly enough to prevent any escape of air between the tissue and collar, but not tightly enough to prevent ready return of venous blood.

The tube leading from the plethysmograph to the recording tambour should have a side vent, which should be left open while the adjustment of the apparatus is in progress.

Closing the vent, one should find that the tracing lever of the recording tambour rises and falls rhythmically, showing a rhythmic change in the size of the limb.

3. **Observations.** Trace a plethysmogram while holding the limb as still as possible. Breathe regularly and deeply.

(1) Are the cardiac contractions visible in the tracings; and, if so, does the part get larger or smaller in cardiac systole?

(2) Are the respiratory movements evident; and, if so, does the part get larger or smaller during inspiration. Account for results.

(3) While the arm is enclosed in the plethysmograph, slowly contract the flexor muscles of the forearm. Does the volume increase or diminish on contraction? Account for results.

XIII. THE ACTION OF ATROPINE UPON THE HEART.

1. **Material.** Two dogs; atropine sulphate; morphine sulphate; chloroform (or ether); mask.

2. **Preparation.** Make up following solutions: a strong solution of atropine, 0.4 grm. to 10 c.c.; morphine, 0.6 grm. to 10 c.c. Simply restrain dog "*a*." Fasten dog "*b*" to board. Give hypodermically 0.03 grm. of morphine to dog "*b*," then anæsthetize him. Set up inductorium so as to obtain tetanizing current.

3. **Experiments and Observations.** (1) Expose the vagus of dog "*b*." Stimulate it with weak induced current, using shielded electrode.

(2) Count the pulse; then give 5 mg. atropine hypodermically.

(*a*) Count the pulse at short intervals after the injection of atropine for at least thirty minutes, or until its rate is markedly affected.

(*b*) What is the effect of atropine on the rate of the pulse? Could atropine produce this effect by acting on the vagus centre? On the vagus fibres? On the heart muscle direct?

(3) After the pulse rate has been markedly affected by atropine, stimulate vagus as before, using shielded electrodes.

(*a*) What is the effect on the rate of the heart's action?

(*b*) Compare this result with that obtained in experiment (2).

(*c*) Had atropine acted solely by depressing the vagus centre, would we have found a difference in results in stimulating the vagus nerve before and after its exhibition?

(*d*) Had atropine acted on the accelerator apparatus, would there be a difference in such results?

(*e*) If now, on stimulating the heart muscle directly, you obtained a normal physiological effect, to what elements have you limited the possible action of atropine?

(*f*) Basing your opinion on the experiments you have performed, to what elements have you limited the possible action of atropine?

(4) Further general observations.

(*a*) Note condition of visible mucous membranes with regard to their secretions.

(*b*) If dog can be kept until next day, note size of pupils.

XIV. THE ACTION OF PILOCARPINE UPON THE HEART.

1. **Material.** One rabbit; one dog; hydrochlorate of pilocarpine; sulphate of morphine; sulphate of atropine; chloroform.

2. **Preparation.** Make solution of pilocarpine, 50 grm. to 10 c.c.; atropine, 0.02 grm. to 10 c.c.; morphine, 0.6 to 10 c.c.

Do not fasten the rabbit to the holder. Fasten the dog to the dog board, after giving preliminary hypodermic injection of 0.03 grm. of morphine.

3. **Experiments and Observations.** (1) Give, hypodermically, 5 mg. per kg. pilocarpine to rabbit. Record weight, pulse, and temperature. Note secretions and size of pupils.

(*a*) Record symptoms as they arise, especially as regards:

(I) Secretions.
(II) Pulse rate.
(III) Size of pupil.
(IV) Temperature.
(V) Weight.

(*b*) Formulate the total effect of pilocarpine upon the animal.

(2) After morphinizing the dog, fasten it firmly to the dog board and lightly anæsthetize; expose both vagi. Count the pulse. Give a subcutaneous injection of 0.03 grm. pilocarpine. After salivation has become profuse count the pulse again.

How does pilocarpine affect the pulse rate?

(3) Now sever the vagi.

(*a*) How does the severing of the vagi affect the normal animal?

(*b*) How does it affect an animal poisoned by pilocarpine?

(*c*) Could pilocarpine alter the effect produced by severing vagi if it acted on the proximal side of the point at which the vagi were cut? On a point beyond that at which they were cut?

(*d*) Could the pilocarpine alter the effect normally produced by severing the vagi, by acting on the cardiac sympathetic?

(*e*) Enumerate the possible points at which pilocarpine may act to produce the effects observed.

(4) Give the same dog 5 mg. atropine, hypodermically.

(*a*) Is the rate of heart beat altered?

(*b*) Where does atropine act to produce alteration in rate of heart beat?

(*c*) Does atropine antagonize the action of pilocarpine in this experiment?

(*d*) To what elements have you limited the probable action of pilocarpine?

(5) General observations.

(*a*) Compare the action of pilocarpine with that of atropine throughout the range of action observed.

(*b*) Is atropine a *physiological antagonist* of pilocarpine?

XV. THE ACTION OF DIGITALIS UPON THE HEART.

1. **Material.** Tincture digitalis; sulphate of morphine; sodic chloride; chloroform; two dogs; one frog; sodic carbonate (one-half saturated solution).

2. **Preparation.** Make solution of morphine, 0.6 grm. to 10 c.c. Pith frog. Morphinize dogs, using 0.03 grm., and chloroform them previous to operation. Set up induction coil so as to obtain tetanizing current, having contact key in primary circuit. Prepare kymograph for tracing.

3. **Experiments and Observations.** (1) Fasten a dog firmly to the dog board and lightly anæsthetize. Expose the vagus. Count the pulse. Using shielded electrodes and separating secondary from primary coil, find a current just weak enough not to affect heart when applied to vagus. Now inject subcutaneously 0.3 c.c. tincture digitalis per kilo animal. After waiting at least twenty minutes, in the mean time using no anæsthetic except a repetition of the morphine if necessary, and keeping the wound closed after moistening with saline solution, stimulate the vagus with same current that before the exhibition of digitalis was unable to affect the heart.

(*a*) What is the function of the cardiac fibres of the vagus?

(*b*) What result is produced by the stimulation of these fibres in the normal animal?

(*c*) Does digitalis increase or decrease the influence of the vagus? (Maximum effect occurs after two hours.)

(*d*) With the stimulus applied to the vagus fibres, the cardiac fibres carrying impulses centrifugally, could this altered excitability be due to central action of the digitalis?

(2) After morphinizing dog, fasten firmly to dog board and lightly anæsthetize; expose carotid artery.

Insert the cannula of the manometer tambour apparatus into the artery. There must be no air-bubbles in the apparatus at any point.

The anæsthetic should be discontinued as soon as the cannula is inserted into the artery. Take normal tracing and read pressure as indicated in the manometer. Now give the dog, hypodermically, 0.3 c.c. tincture digitalis for each kilo of weight.

(*a*) Watch effect on elevation of mercury meniscus, making tracings at short intervals.

(*b*) What factors enter into arterial pressure?

(*c*) How does a "high-pressure" tracing differ from a "low-pressure" tracing?

(*d*) What effect has digitalis on arterial pressure?

(3) Having firmly fastened a pithed frog to frog board with web stretched over a hole in the board, focus the microscope upon a certain arteriole in the field, and measure its diameter with an eye-

piece micrometer. Now inject into dorsal lymph spaces 0.3 c.c. tincture digitalis and measure same arteriole at intervals of ten minutes. Keep the web moist with normal saline solution.

(*a*) What change occurs in the diameter of the arteriole?

(*b*) What effect would you expect this to have on arterial pressure?

(*c*) Would its action on the arterioles help to account for its effect on arterial pressure?

(4) *Comparisons.* Compare digitalis and atropine with regard to (*a*) their effects on the rate of the heart beat; (*b*) their effects on the irritability of the vagus.

XVI. THE ACTION OF ACONITE UPON THE CIRCULATION.

1. **Material.** Tincture aconite; sulphate of atropine; one dog; one frog; sphygmograph.

2. **Preparation.** Make solution of atropine, 0.02 grm. to 10 c.c. Pith frog. Do not fasten the dog to dog board.

3. **Experiments and Observations.** (1) Give 1 c.c. tincture aconite hypodermically to the dog. Record symptoms as they arise. (1 c.c. often not fatal.)

(2) Fasten the pithed frog on its back to the board. Count the heart beats, exposing heart if necessary. Now give two drops tincture aconite subcutaneously. What effect has aconite on the pulse rate? (To obtain satisfactory results, observations must be made at short intervals, for from thirty to sixty minutes.)

(3) Take a sphygmographic tracing of the radial pulse of a student. Note the pulse rate. Administer by mouth 0.2 c.c. tincture aconite and 0.06 c.c. every ten minutes until action on pulse is noticeable. Repeat tracing and counting of pulse at short intervals.

(*a*) How does aconite affect blood pressure?

(*b*) How is the rate of the heart's action affected?

(*c*) What subjective sensations are produced?

(4) *Comparisons.* Compare aconite and pilocarpine with regard to their action on the gastrointestinal system.

XVII. THE ACTION OF ADRENALIN UPON THE CIRCULATION.

1. **Materials.** White rabbit; adrenalin.

2. **Preparation.** Make solution of adrenalin 0.01 grm. to 10 c.c. of normal saline solution. Weigh the rabbit and fasten it to the holder, and with probang made of absorbent cotton on probe apply solution to cornea; note local effect on peripheral circulation.

3. **Operation.** Anæsthetize rabbit, expose carotid, and insert cannula into artery and take blood pressure. Ligate external carotid;

inject toward heart enough of the solution to make 2 grm. per kilo animal; ligate below needle point and withdraw needle.

4. **Observations.** (1) What is the effect of adrenalin applied locally?

(2) What is the effect upon the peripheral circulation of adrenalin injected intravenously?

(3) Is the rate or apparent force of the heart modified by adrenalin?

(4) Is the blood pressure modified; if so, how may the change be accounted for?

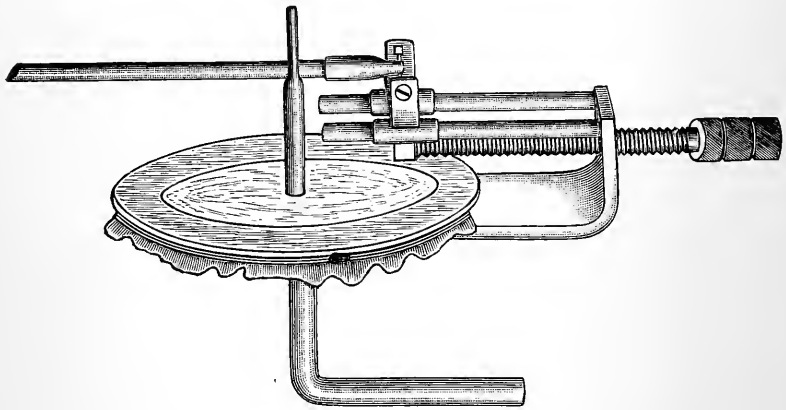
CHAPTER IV.

RESPIRATION.

I. THORACIC MOVEMENTS. INTRATHORACIC PRESSURE.

1. **Appliances** necessary for these exercises are: Kymograph; physiological operating case; clippers; stethograph; thoracic cannula. (See Appendix, 12.) The stethograph consists of two tambours; the recording tambour is the same as used in other analogous experiments (Fig. 56), while the receiving tambour, joined to the recording tambour through a $\frac{1}{2}$-metre length of No. $\frac{1}{8}$ pressure tubing, is provided with a cork button which may be placed upon the rabbit's thorax and receive and communicate its movements to the air in the tambour system.

FIG. 56



Recording tambour. (Described in Appendix, 12.)

2. **To Study the Movements of the Rabbit's Thorax.** The problem is to take a graphic tracing or stethogram of the movements of the thoracic walls, and from this tracing to determine the rate and the character of the movements, particularly the latter.

To record a stethogram, fasten the rabbit upon its board and hold the button of the receiving tambour upon the thorax, tracing the movement of the lever upon the kymograph.

Study the characteristics of this curve.

Anæsthetize the rabbit with ether. How does the stethogram vary as the anæsthesia progresses? Is the stethogram of full anæsthesia different in any essential feature from the normal one?

3. **To Study the Intrathoracic Pressure.** Locate an intercostal space to the right of the sternum and opposite its middle point. Make an incision 1 cm. long, parallel with the intercostal space and 1 cm. from the sternum. Dissect through the intercostal muscles, taking care not to cut the pleura. Insert into the wound the point of the glass cannula, previously provided with a rubber tube which is clamped, and press it carefully through the pleura into the right pleural cavity.

Join the rubber tube to a recording tambour and unclamp. Slowly and gently manipulate the cannula until there is evident communication through the lumen of the cannula and tube from the pleural cavity to the tambour.

So adjust the cannula that the recording lever makes the maximum excursion. Bring the levers into such a relation to the kymograph that the tracing point of the stethograph lever shall be vertically over that of the lever which is to record intrathoracic pressure, and 2 cm. to 3 cm. from it.

Trace upon the drum a stethogram and chronogram as well as an intrathoracic pressure record, taking care that the tracing points of the recording tambours are in a vertical line.

(1) Does the rhythm of varying pressure correspond to the rhythm of the respiratory movements?

(2) If so, does that necessarily establish between them the relation of cause and effect?

(3) What change of pressure is indicated by the rise of the pressure lever?

(4) What movement of the pressure lever corresponds to a rise of the stethograph lever?

(5) What is the condition of intrathoracic pressure during inspiration? During expiration?

(6) Stop the entrance of the air into the respiratory passages by closing the rabbit's nostrils. What effect does this have upon the respiratory movements?

(7) Is the intrathoracic pressure affected by the experiment? If so, explain the effect.

(8) If two phenomena correspond perfectly in their cycles, and if a variation of one is always accompanied by a variation in the other, can there be any reasonable doubt that they sustain to each other the relation of cause and effect?

(9) Is one of the phenomena in question the cause of the other? If so, state which is the cause and establish your position.

(10) Clamp the rubber tube of the pressure apparatus. Replace the recording tambour with a water manometer. Unclamp. Is the pressure during inspiration positive or negative, and how much?

(11) Is the pressure during expiration positive or negative, and how much?

(12) If the whole apparatus were filled with water instead of air and water, would it make any essential difference in the result? What effect do the variations of the intrathoracic pressure have upon the circulation?

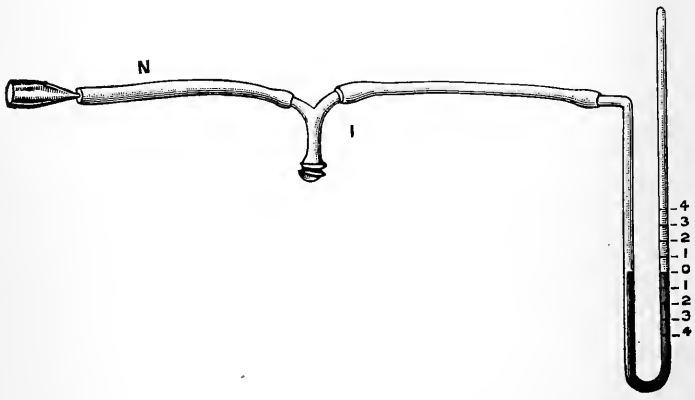
II. RESPIRATORY PRESSURE. ELASTICITY OF THE LUNGS. PNEUMATOGRAM.

A. Respiratory Pressure.

1. **Appliances.** Operating case; clippers; rabbit board; ether; ether cone; absorbent cotton; rabbit stethograph; kymograph; a small mercury manometer, to the proximal limb of which is attached a thick-walled rubber tube, a piece of glass tubing for a mouth-piece, a screw clamp; chronograph; two recording tambours; rabbit.

2. **Preparation.** Fix the rabbit to the operating board and anæsthetize; clip the ventral surface of the neck. Join up the manometer as shown below.

FIG. 57



Tracheal cannula, with manometer attached.

3. **Operation.** Make a longitudinal incision over the trachea, through skin and connective tissue. Part the sternothyroid muscles in the median line and expose the trachea. Separate the trachea from the œsophagus and other surrounding tissues for 3 cm. below the larynx. Carefully pass a strong linen ligature under the trachea. Make a median ventral slit in the trachea anterior to the ligature. Pass through the slit the limb of the Y-tube marked *I* (Fig. 57). Ligate.

4. **Observations. Respiratory Pressure. The Pneumatogram.** (1) After the ligature is tied how does the rabbit breathe? Are the

thoracic and abdominal movements of respiration accompanied by other respiratory movements?

(2) With tube *N* (Fig. 57) open, is there any variation of the mercury during respiration?

(3) With a screw clamp slowly close tube *N*. As the resistance to the flow of air increases, what change is noted in the manometer?

(4) Quickly clamp tube *N* at end of expiration and carefully note the manometer reading. Is it positive or negative?

(5) Clamp tube *N* at the end of inspiration. Is the pressure positive or negative?

(6) You have been determining certain facts regarding respiratory pressure. Are the causes of the changes of respiratory pressure the same as the causes of the changes of intrathoracic pressure?

(7) In what way does respiratory pressure differ from intrathoracic pressure?

(8) Disjoin the manometer and join its tube to a recording tambour and trace a pneumatogram, with stethogram and chronogram.

(9) Compare the pneumatogram with the tracing of intrathoracic pressure. Account for all differences.

(10) While dispatching the rabbit with chloroform trace a pneumatogram of chloroform narcosis. Describe its characteristics. Does the heart continue to beat after the respiration has ceased?

B. Elasticity of the Rabbit's Lungs.

(1) After the death of the rabbit open the thorax freely, taking care not to wound the visceral pleura. The lungs will collapse. Why?

(2) Replace the manometer; gently blow into the mouth-piece until the lungs have been inflated to their normal size. Measure carefully the rise of mercury in the distal column. What degree of positive respiratory pressure will the elasticity of the lungs alone cause?

(3) What is the significance of the elasticity of the lungs in respiration?

C. The Cardio-pneumatogram.

Remove the tube *N* from the Y-tube; join it to a recording tambour.

(1) Let a member of a division sit in perfect repose, and, while the drum of the kymograph rotates very slowly, hold the mouth-piece between the lips. Hold the nose and suspend all respiratory movements for a period. Let some member of the division count the pulse of the experimenter. Trace the cardio-pneumatogram.

(2) Is there a relation between the rhythm of the pulse and the waves of the tracing? If so, account for this relation.

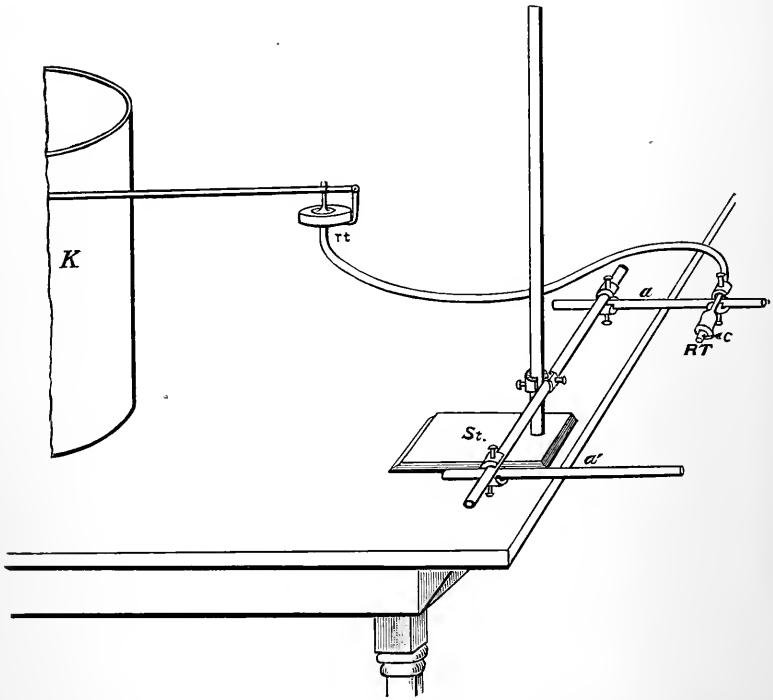
(3) Account for the essential features of the cardio-pneumatogram.

III. TO STUDY THE MOVEMENTS OF THE HUMAN THORAX.

1. **Appliances.** Stethograph (see Appendix, 14); chest pantagraph (see Appendix, 15); chronograph and kymograph.

2. **Observations.** *With the stethograph* (Fig. 58). (1) How much may be learned of man's respiratory movements by simple inspection? Make a careful enumeration and record.

FIG. 58



The human stethograph: *St*, stand with heavy base, supporting a thoracic frame constructed of gas-pipes and clamps; *a* and *a'*, horizontal parallel arms, to be adjusted on either side of the thorax; *a'*, to touch the thoracic wall; *RT*, receiving tambour, constructed as described in the Appendix; the movements of the cork *c*, which touches the thoracic wall, are transmitted to the recording tambour *rt*, thence traced on the kymograph *K*.

(2) Take a stethogram of the lateral diameter in the nipple plane.

(3) Take a stethogram of the dorsoventral diameter of the thorax over the middle of the sternum in the nipple plane. Compare.

(4) Adjust the stethograph and make a record (a stethogram) of the changes of the lateral diameter of the thorax at the ninth rib.

(5) Take a lateral ninth-rib stethogram while the subject reads a paragraph, sighs, coughs, and laughs. Account for the peculiarities.

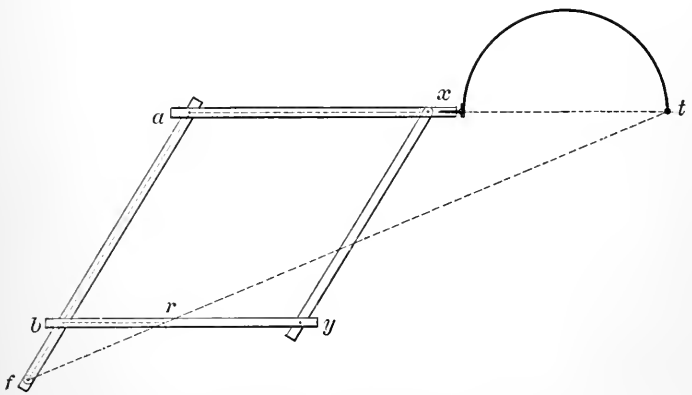
(6) Take a lateral ninth-rib stethogram after the subject has taken vigorous exercise. What changes are to be noted?

(7) Compare the stethogram from several individuals. Determine the essential features and give causes of these.

(8) Seek the causes of the differences which exist between stethograms of different individuals. May they be accounted for by stature, condition, occupation, or habit?

3. **Observations.** *With the chest pantagraph.* The purpose of this instrument is to record the outline of any horizontal section of the thorax, though it could be used as well for tracing the periphera

FIG. 59



The chest pantagraph. For measuring and recording *chest contours*. The instrument is constructed of brass or of wood with brass or steel semicircle. The joints *a*, *b*, *x*, and *y* move easily in the plane of the instrument. The semicircle, forty inches in diameter, rotates at *x* around the diameter *t x*. The point *f* is fixed to a table. With *f* a fixed point all movements of *t*, the tracing point, are accompanied by corresponding movements of *r*, the recording point. The triangle *f r b* and *f t a* are similar triangles in all positions of the instrument *f b* : *f a* : : *f r* : *f t*; but $\frac{f\,b}{f\,a} = \frac{1}{5}$; therefore the distance *f r* is always 1/5 the distance *f t*.

of the abdomen, of the head, or of the limb. To use the pantagraph for the purpose here intended, let the subject sit beside a table adjustable as to height. Make such adjustment as to bring the circumference of the thorax to be observed even with the upper surface of the table. Fix the point *f* of the instrument to the table. Let the observer locate, with pen or pencil, upon the side of the subject distal from the table, a point which shall serve as a starting point. (See Fig. 59.)

When the point (*t*) of the instrument rests upon this point of the subject's thorax the instrument should be well extended, somewhat more than represented in the figure. Fix a sheet of paper to the table under the recording pencil at *r*. To take a graphic record of the contour of the thorax proceed as follows:

(*a*) Let the observer place the tracing point (*t*) upon the "starting point" on the distal side of the thoracic perimeter.

(*b*) Sweep the tracing point quickly around one-half the perimeter to a point approximately opposite to the starting point.

(*c*) Rotate the curved arm of the instrument upon its axis (*t x*) through 180 degrees.

FIG. 60

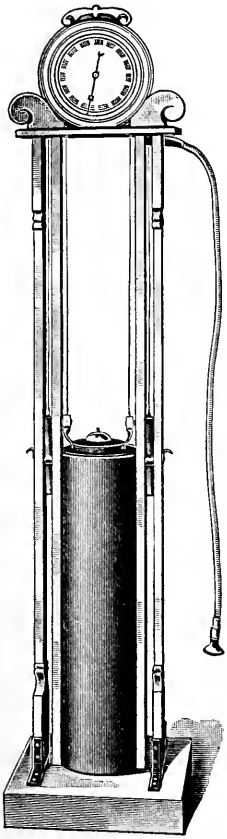


FIG. 61

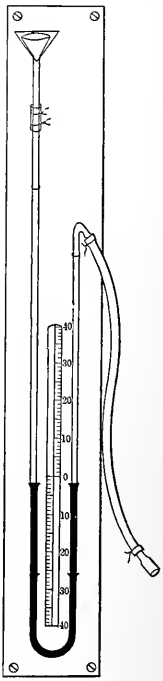


FIG. 60.—The water spirometer. The outer receptacle contains water. The inner inverted reservoir receives the air through the mouth tube, at the right, and is raised.

FIG. 61.—Pneomanometer.

(*d*) Sweep the tracing point around the other one-half of the perimeter to the starting point.

The movements of the tracing point (*t*) in the horizontal plane have been faithfully recorded upon the sheet of paper by the recording pencil at *r*. It is hardly necessary to remind the student that the subject must remain motionless during the observation.

(1) Take a thoracic perimeter with the chest in repose. Measure different diameters of the tracing and multiply by five to reduce to actual measurements.

(2) Take a tracing at end of forced expiration; at end of forced inspiration. Compare diameters.

(3) Make a series of these tracings for different individuals. Compare.

(4) Do different individuals of the class represent different types of contour, as *broad*, *medium*, and *deep?*

(5) Which type of chest is capable of adding the greatest area of contour by expansion?

IV. LUNG CAPACITY (CHEST MEASUREMENTS, RESPIRATORY PRESSURE). RECORDING OF ANTHROPOMETRIC DATA.

1. **Instruments.** Spirometer (Fig. 60); pneomanometer (Fig. 61); meter tape; steel calipers; standard, with horizontal arm for measuring height; scales for taking weight.

2. **Observations.** (1) Test with spirometer the lung capacity of each member of the division. May differences in lung capacity be accounted for by difference in stature, condition, occupation, or habit?

(2) Take with the meter tape the girth of chest over the nipples in a plane at right angles with the axis of the thorax.

(*a*) With chest in normal repose.

(*b*) At the end of forced expiration.

(*c*) At the end of forced inspiration.

(3) Take the girth of chest over the juncture of the ninth rib with its cartilage, holding the tape in a plane at right angles with the axis of the thorax.

(*a*) With the chest in repose.

(*b*) At the end of forced expiration.

(*c*) At the end of forced inspiration.

(4) With the calipers measure the dorsoventral diameter at the level of the nipple, holding the calipers in a plane perpendicular to the axis of the thorax.

(*a*) Normal; (*b*) after forced expiration; (*c*) after forced inspiration.

(5) Take the lateral diameter in the nipple plane.

(*a*) Normal; (*b*) after forced expiration; (*c*) after forced inspiration.

(6) Take the lateral diameter at the ninth rib.

(*a*) Normal; (*b*) after forced expiration; (*c*) after forced inspiration.

(7) Test with pneomanometer the force of inspiration and expiration. Let each member of the division test with the pneomanometer the maximum positive pressure which he is able to produce in the respiratory passages during expiration.

(8) Test with the same instrument the maximum negative pressure which each individual can produce during inspiration.

(9) Does the face become red in either of these tests? If such is uniformly observed, account for it.

(10) The preservation of data. Experience has shown that when data are to be preserved for subsequent use in comparison of one class of individuals or cases with another, it is very much more economical in time to record the data upon cards.

For the above data one may use such a card as is appended below.

In addition to the measurements above given record upon the cards the weight, the height, the bodily condition of the individual, and especially whether the individual has lived in a hilly or in a flat country, and whether he has been active or inactive.

Name . . . . . Address . . . . . .

Place of residence: level, hilly, or mountainous altitude . . . . . .

. . . . . .

Previous occupation . . . . . .

Habits: Exercise, sports, character, amount . . . . . .

. . . . . .

Parents { Father's weight . . . . . height . . . .
Mother's weight . . . . . height . . . .

Which parent do you resemble physically? . . . . . .

Which parent do you resemble temperamentally? . . . . . .

Age . . . . . Weight . . . . Emaciated, thin, spare, stout, obese.

Height . . . . Dwarfish, short, medium, tall, very tall, ...

Lung capacity . . . . Respiratory pressure { Inspiration . . . . .
Expiration . . . . .

Girth of Chest, Nipple Plane:

1. Repose . . . 2. Inspiration . . . . 3. Expiration . . .
4. Expansion . . . . . Per cent. . . . . .

Diameter of Chest, Dorsoventral:

1. Repose . . . 2. Inspiration . . . . 3. Expiration . . .
4. Expansion . . . . . Per cent. . . . . .

Diameter of Chest, Lateral:

1. Repose . . . 2. Inspiration . . . . 3. Expiration . . .
4. Expansion . . . . . Per cent. . . . . .

Examiner . . . . . .

Date . . . . . .

V. THE EVALUATION OF ANTHROPOMETRIC DATA.

A large proportion of the problems that the medical man has to solve involves the finding of averages of a large number of observations. This is sure to be true in all anthropometric problems. In the course of the preceding lesson valuable anthropometric data were collected and recorded upon cards. The value of this material is purely potential. Before the data will furnish a basis for drawing conclusions it is necessary to subject them to a process of evaluation. This process consists, first, in grouping; second, in getting the average or the median value for each measurement; and, third, in graphically representing the averages. In the previous lesson the observer noted upon each card whether the subject had lived in a hilly or flat country; further, whether he had lived a physically active or inactive life. This gives one an opportunity for four groups when the cards for the whole class are collected.

Group I. Active men from a hilly country.
Group II. Active men from a flat country.
Group III. Inactive men from a hilly country.
Group IV. Inactive men from a flat country.

Until recently it has been customary simply to write the data for any group in columns and "strike an average" of each column. If there are only 10 to 20 or 30 individuals in each group this method does not entail the unnecessary expenditure of much energy, but it is not reliable, for one "giant" or "dwarf" in any group would vitiate the whole result. If there are 100 or 1000 individuals in a group, then the use of the old method of finding the arithmetical average is exceedingly wasteful of both time and energy. It must be added, however, that when the number of observations is large the chances are that there will be as many dwarfs as giants, thus making the average approximate closely the median value. It is the latter we are seeking, viz., the median value; this may be defined as *that value which is so located in the whole series of observations in a single measurement of any group, that there are as many below it as above it—i. e.,* that the number of values which it exceeds equals the number of values which exceed it.

Let us take a concrete case. In a group of 316 seventeen-year-old boys certain physical measurements were recorded upon individual cards. Let us take, for example, the girth of the head recorded in centimetres and tenths. Instead of writing in a column the 316 head-girths, each expressed in three figures, adding and averaging, let us adopt the new method, first suggested by the Belgian astronomer and anthropologist, Quetelet, and later elaborated by Galten, the London anthropologist. Arrange the cards in piles, placing in one pile all of the cards having girth of head 51 cm., in another pile all

having 52 cm., and so on. In the case in question it was found that the 316 cards were quickly distributed, falling into the following groups:

Girth of head . . .	51	52	53	54	55	56	57	58	59	60
No. of observations . . (*i. e.*, of cards.)	1	7	17	41	70	74	60	29	10	7

The problem is to find the value of the median measurement or the median value. There are 158 values below the median value and as many above it.

1. *To Locate the Median Observation.* This is equivalent to saying —find in the lower series of numbers (1–7–17, etc.) the 158th observation from either end. It must be located in the pile of cards which number 74. This group may be called the *median group.* But where in this group is the median observation located? In order to determine this, add the groups to the left of the median group, these may be called the minus groups, the values which they represent being less than that of the median group: 1, 7, 17, 41, 70 = 136.

To this sum we must add 22 observations from the median group to make 158. The median observation is then located in the median group, 22 points from the left.

2. *To evaluate the median observation* we must take it for granted that the 74 observations of the median group are evenly distributed over the distance between 56 cm. and 57 cm. That being the case, the median value would be 56 and $\frac{22}{74}$ cm.

If it is desired to reduce this simple process to a mathematical formula, that can readily be done:

Let n = the total number of observations (316).
m = the number of observations in the median group (74).
l = the sum of the minus groups at the left (136).
r = the sum of the plus groups to the right (106).
a = the minimum value of the median group (56 cm.).
d = the arithmetical difference in the minimum values of the groups (1 cm.).
M = the median value to be determined.

Then $M = a + \frac{d\left(\frac{n}{2} - l\right)}{m}$ or $M = 56 + \frac{1\left(\frac{316}{2} - 136\right)}{74} = 56\frac{22}{74} = 56.3$ cm.

After one has found the median value for each measurement in each group, these may be tabulated and the values compared. When the table of median values is large it is almost necessary to carry the work of reduction a step farther and represent these values graphic-

ally in a chart. Another opportunity will be used for giving the methods used in the graphic representation of statistical tables.

The table which results from the data collected in connection with the previous lesson is not so large but that the observer can practically comprehend the whole at a glance.

Our grouping enables us to answer the following questions:

1. Has general physical activity any essential influence in the development of the respiratory organs and function?

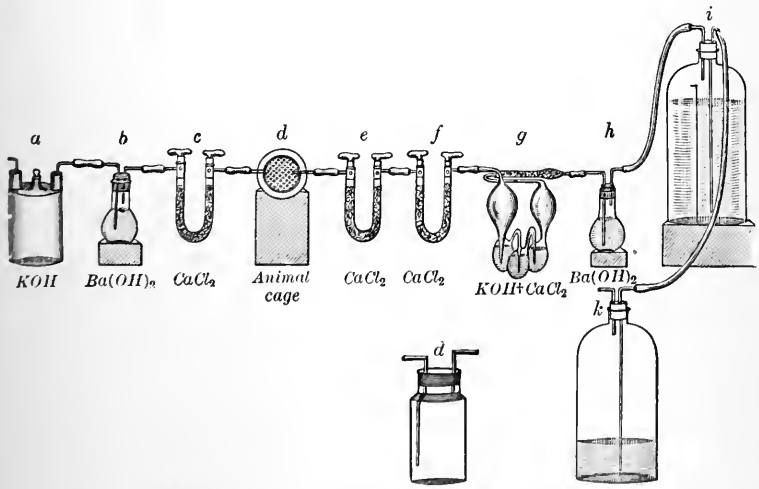
2. Is the climbing of hills in early life a factor in the development of the respiratory organs and function?

If both of these questions may be answered affirmatively, then one would expect to find that the median values of Group I. (active individuals from a hilly country) uniformly exceed the values of Group II., and that those of Group III. uniformly exceed those of Group IV., but that the median lines of Group II. may or may not exceed those of Group III.

VI. QUANTITATIVE DETERMINATION OF THE CO_2 AND H_2O ELIMINATED FROM AN ANIMAL'S LUNGS IN A GIVEN TIME.

1. **Appliances.** A 4-ounce Woulffe bottle with three necks and with delivery tubes and stopper ground in the necks (Fig. 62, *a*);

FIG. 62



Apparatus for the estimation of CO_2 and H_2O in exhaled air.

three 5-inch calcium chloride tubes, with side tubes and perforated glass stoppers, opening and closing the flow of gas (Fig. 62, *c*, *e*, *f*);

Geissler's potash bulbs, with $CaCl_2$ tube ground on (*g*); two small flasks (*b*, *h*) with rubber stoppers, double bored, with delivery tubes fitted as shown in figure; a 1-litre or 2-litre bottle with very wide mouth to use as animal cage, fitted with delivery tubes and with a cork impregnated with paraffin; siphon apparatus, as figured, consisting of two 8-litre bottles with paraffined corks and tubes; analytical balances; laboratory balances (correct to 0.01 grm.); drying oven; chemicals, KOH, $Ba(OH)_2$, $CaCl_2$; any small animal whose weight in grams does not exceed one-fifth the volume of the animal cage expressed in cubic centimetres.

2. **Preparation.** (1) Fill the calcium chloride tubes; put them into the drying oven, where they are to be kept at a temperature of 100° to 120° C. for several hours; cool in a desiccator and weigh upon the analytical balances the tubes *e* and *f*, recording the weight in milligrams.

(2) Fill the Woulffe bottle and the Geissler's bulbs with a strong solution (50 per cent. or more) of KOH. Fix into position, upon the Geissler bulb, its filled and desiccated $CaCl_2$ attachment, and fit to each end a rubber connecting tube; clamp with strong serre-fine forceps and weigh upon the analytical balances.

(3) Fill the flasks *b* and *h* with a strong solution of $Ba(OH)_2$. These flasks serve simply to show whether or not the CO_2 gas has all been absorbed by the KOH through which it has just passed.

(4) Pieces *e*, *f*, and *g* should be fixed to a light wooden rack, by which they may be moved; if this is not convenient clamp them to supports.

(5) Join up the apparatus *a*, *b*, and *c*.

(6) Fill siphon apparatus.

(7) Weigh the animal cage without the animal.

3. **Operation.** (1) Put the animal into the cage; fasten the stopper in so that it will not leak air.

(2) Join the animal cage with *c* and with siphon apparatus at *i*, leaving out for this preliminary operation the apparatus *e*, *f*, *g*, and *h*. Start the siphon and note the rate of flow per minute. The level of the water in the lower bottle should be probably 1 metre below that in the upper bottle. Notice whether the animal seems to be respiring normally. If so, it may be taken for granted, after ten minutes, that the ventilation is sufficient. If it seems insufficient, one has only to increase the difference of level in the two siphon bottles.

(3) Disjoin the animal cage and weigh the cage with the contained animal upon the laboratory balances. Note the time; join the animal cage in circuit again, attaching it to *e*, and attaching *h* to the siphon apparatus at *i*. Start the siphon. The greater resistance to be overcome will necessitate a greater difference in the level of the two bottles in order to ventilate at the same rate as before. To

test joints place the finger over the distal tube of the Woulffe bottle (*a*); if the joints are all right the siphon stream will stop after a few moments. When the water in the upper bottle is lowered nearly to the end of the siphon, clamp the tube joining *h* to *i*, set the empty bottle upon the floor and the full bottle on the higher level, join the tube at *k*, and unclamp. This whole change need only occupy a few seconds. If it is desired to make a determination of the amount of oxygen which the animal consumes in a given time, the air that passes out of the ventilating apparatus after the second change may be caught and tested.

(4) It is evident that in the afferent apparatus (*a*, *b*, and *c*) one has a means of robbing the air of CO_2 and H_2O, thus furnishing the animal with pure dry air. It is further evident that in the afferent apparatus one has a means of collecting absolutely all of the CO_2 and H_2O given off from the animal during the experiment. Further, the weights before and after will show just how much of these excreta have been passed into the collecting apparatus.

(5) Note the time (one hour or more); clamp siphon tube; turn the stoppers off *e* and *f*, clamp *x* and *y;* disjoin *d* and weigh it.

(6) Weigh *e*, weigh *f*, weigh *g*.

4. **Observations.** (1) How much has the animal lost in weight during the period of observation?

(2) How much water left the animal cage during the period of observation?

(3) What was the source of this water?

(4) Did the animal micturate or defecate during the time of the experiment? If so, is this to be looked upon as a source of error in the experiment? Would such an occurrence tend to increase or decrease the amount of water caught in the $CaCl_2$ tubes *e* and *f*? Would it interfere in any way with the experiment? If so, how may such a source of error be avoided or corrected?

(5) How much CO_2 left the animal cage during observation?

(6) What is the total amount of CO_2 and H_2O collected?

(7) Does the amount of these excreta collected equal the loss in weight of the animal? What should the relation of these two quantities be? Explain in full.

(8) What is the respiratory quotient?

(9) Formulate several problems which may be solved with this method.

VII. TO DETERMINE THE AMOUNT OF OXYGEN CONSUMED BY AN ANIMAL IN A GIVEN TIME.

1. **Preparation.** The oxygen is determined by a volumetric method, using two or more gas burettes and a solution of potassium pyrogallate.

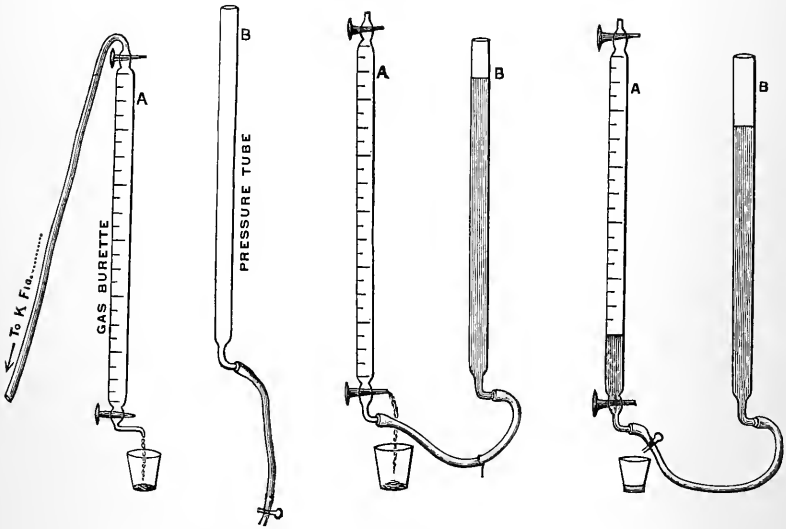
The solution of potassium pyrogallate is prepared by mixing two parts of 25 per cent. aqueous solution of KOH and one part of 5 per cent. aqueous solution of pyrogallic acid.

Comparison must be made between the oxygen content of the expired air and that of the atmosphere at the time of the experiment. If it is desired to calculate the *respiratory quotient* it will be necessary to make the oxygen analysis from the air that traversed the animal cage in the previous experiment when the CO_2 was being determined.

If it is not desired to compute the respiratory quotient it will be necessary only to have it traverse the animal cage, drawn through by the ventilating apparatus.

The air should come into the cage from out of doors (brought in through glass or rubber tubes from the window).

FIG. 63



Position 1. Position 2. Position 3.

Gas burettes to determine oxygen.

2. **Operation.** These two constituents of the pyrogallate should be mixed in the pressure tube of the gas apparatus just before the analysis is made. To collect samples of air for analysis, one fills the gas burette (Fig. 63, *A*) with water by suction. Connection is then made between the exit tube at *k*, of the respiration apparatus used in the previous experiment (see Fig. 62), and the upper end of the gas burette as shown in Fig. 63, position 1; the respired air flows in, displacing the water. The stopcocks are now turned so that no air can escape from the burette. The rubber tube of the pressure tube *B*, which has been filled with the potassium pyrogallate, is now

connected to the lower end of the gas burette. After all the air has been expelled from the connections, turn the three-way stopcock in such a position as to permit the pyrogallate to flow up into the gas burette, coming in contact with the air to be analyzed. The pressure tube should now be elevated as high as the connecting rubber will permit and the potassium pyrogallate solution allowed to run into the burette *A*. The clamp on the connecting tube should now be applied to it close to the lower end of the burette.

This operation made positive pressure in the burette, thereby causing a more rapid absorption of the oxygen. The burette should now be taken by the experimenter and its ends alternately raised and lowered. At frequent intervals he should loosen the clamp on the connecting rubber tube and raise the pressure tube, thus permitting potassium pyrogallate solution to take the place of the oxygen as it is absorbed. This procedure should continue ten minutes, after which the clamp on the connecting rubber tube should be loosened. The burette and its pressure tube should be allowed to remain ten minutes longer, at the end of which time the solution in the burette should be brought to a level with the solution in the pressure tube by elevating or lowering the tube. This causes the air in the burette to be under the atmospheric pressure existing at that time. The reading for the amount of oxygen is now taken.

To calculate the amount of oxygen consumed by the animal, one subtracts the amount of oxygen found in the respired air from that found in the normal air. At least one sample should be analyzed from each 10 litres of respired air, the average being used to obtain the result.

VIII. THE RESPIRATORY QUOTIENT.

The respiratory quotient being the ratio between the volume of carbon dioxide exhaled and that of oxygen consumed $\left(\text{R.Q.} = \frac{\text{vol. } CO_2}{\text{vol. } O_2}\right)$, it may be computed from data given in Exercises VI. and VII., or it may be directly determined in the following manner:

1. **Appliances.** Ventilating apparatus (Fig. 64); animal cage; $CaCl_2$ tube; Geissler bulbs; two barium hydrate flasks; 25 per cent. solution of KOH; 5 per cent. solution of pyrogallic acid; two gas burettes with pressure tubes; guinea-pig or small rabbit.

2. **Preparation.** Pass one end of the glass tube out through hole in window sash; to inner end attach a rubber tube to whose other end is joined a $Ba(OH)_2$ flask, followed by cage, $CaCl_2$ tube, Geissler bulbs, barium flask, and ventilating apparatus, as shown in the figure. Weigh animal cage. Note temperature of room.

3. **Operation.** Put the animal into the cage; take weight. Start the ventilation, noting the time. While the first pressure bottle is

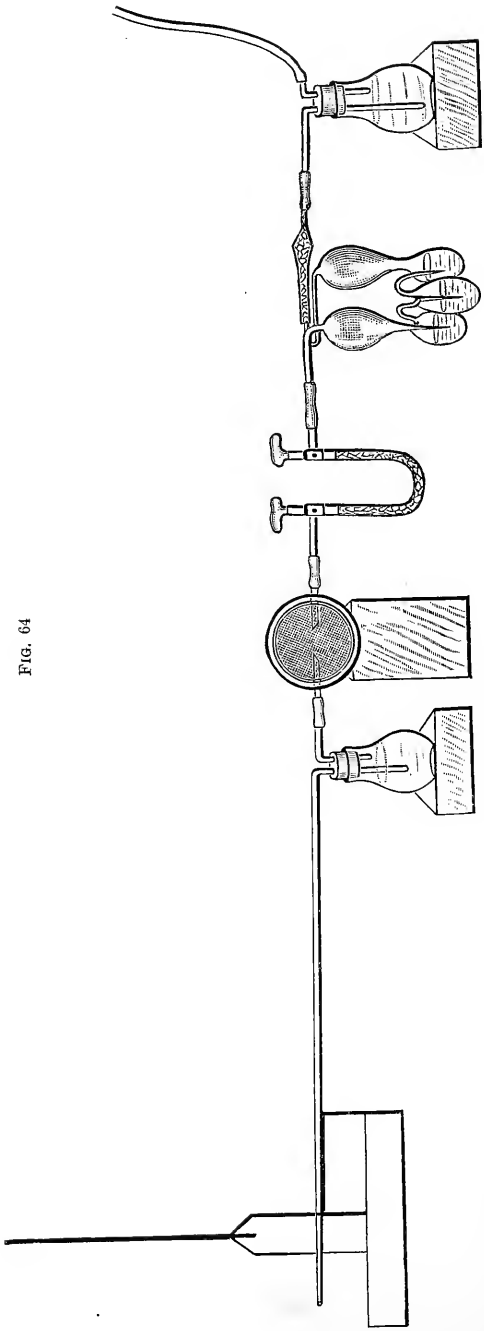


FIG. 64

Apparatus for determining CO_2 alone. (For further description see text.)

emptying take a specimen of out-of-door air and determine its oxygen, taking care to let it reach room temperature before measuring it.

After the second change of the ventilating apparatus take 100 c.c. of air from every 8 or 10 litres of air that traverse the cage and determine the oxygen. Note very carefully the amount of air that traverses the animal cage and keep the ventilating stream as regular as possible.

At the end of the experiment take a second 100 c.c. of air from out of doors and determine its oxygen.

4. **Observations.** (1) How many cubic centimetres of oxygen has the animal consumed during the experiment, measured at the room temperature and the pressure read from the barometer?

(2) Reduce the volume of oxygen, as determined under the conditions given above, to the volume which it would represent if measured under standard conditions of 0° C. and 760 mm. pressure.

(3) How many milligrams of CO_2 were caught by the Geissler bulb?

(4) How many cubic centimetres of CO_2 at 0° C. and 760 mm. barometric pressure would be equal to number of milligrams determined under (3).

(5) What is the respiratory quotient? $\text{R. Q.} = \frac{\text{vol. } CO_2}{\text{vol. } O_2} = \ldots\ldots$

(6) Is the subject of the experiment a carnivorous, omnivorous, or herbivorous animal?

(7) What has been the diet of the animal during the last three days before the experiment?

(8) How long before the experiment had the animal eaten?

(9) Determine the influence of diet on respiratory quotient.

(10) Determine the influence of fasting on respiratory quotient.

IX. RESPIRATION UNDER ABNORMAL CONDITIONS.

1. **Appliances.** Six small animals—*e. g.*, rats or guinea-pigs; six wide-mouthed bottles or jars, which may be sealed; scales or large balances; CO_2 generator; water-bath; operating case; dissecting boards.

2. **Preparation.** Determine the weight of animals "a," "b," and "c." Choose a receptacle whose cubic contents is not over twice as many cubic centimetres as the weight of animal "a" in grams. Choose second and third receptacles whose contents represent about 10 c.c. to 1 grm. of animals "b" and "c," respectively.

3. **Operation.** I. **Preliminary.** (*a*) Put animal "a" into the small jar "a;" count respirations; close the jar.

(*b*) Put animal "b" into jar "b." Before closing count respirations; close air-tight.

(*c*) Fill jar "c" one-third full of water and displace the water with

CO_2. Put animal "c" into the jar, taking care to allow as little loss of CO_2 as possible; close; count respirations.

(*d*) Fill jar "d" full of water and displace with CO_2. Put animal "d" into jar, taking care to allow as little loss of CO_2 as possible; close jar and count respirations.

(*e*) Put an animal into a jar; cover the mouth of the jar with a towel; insert into the jar the end of a rubber tube through which illuminating gas (a mixture of CO with various other gases) may be let into the jar. Let the gas in in little momentary puffs every five minutes, noting the effect upon the animal.

II. Post-mortem Examination. After an animal dies fix it to the dissecting board and open the abdominal and thoracic cavities; take great care not to cut a large bloodvessel; pin the flaps out so that all of the organs will be exposed in place.

4. **Observations.** (*a*) **Respiration in Small Closed Space.** (1) Make a careful record of number of respirations and general condition of animal "a" in the normal state, and at the end of every five minutes after the closure of the jar.

What changes in rate or depth of respiration have been noted?

(2) Note all abnormal signs and symptoms.

(3) On post-mortem examination record the condition of heart, large bloodvessels, lungs, liver, kidneys, and the general appearance of the tissues.

(4) Compare the conditions with those found in a normal animal, prepared by the demonstrator.

(*b*) **Respiration in a Larger Closed Space.** (5) Note all symptoms of animal "b" every five minutes after confinement in the jar.

(6) Make a post-mortem examination; record in detail the condition of the organs as in the case of animal "a."

(7) Compare animal "b" with normal animal.

(8) Compare animal "b" with animal "a."

(*c*) **Respiration in an Atmosphere of One-third CO_2.** (9) Note all symptoms at intervals of five minutes.

(10) Compare these observations with corresponding ones from animal "a" and "b." What are your conclusions?

(11) Make a post-mortem examination; make a record as before.

(12) Compare appearances in animal "c" with those in the normal animal; with those of animal "a"; with those of animal "b."

(13) Make a generalized statement of the facts discovered in the experiments.

(14) What is the cause of death when an animal is enclosed in a small space?

(15) What is the cause of death when an animal is enclosed in a large space?

(16) Have the relations which you have discovered any bearing upon the future development of animal life upon the earth?

(*d*) **Respiration in CO_2** ("**Choke-damp**"). (17) Lower a lighted candle into a jar of CO_2. Record results.

(18) What happened to the animal when it was lowered into an atmosphere of CO_2?

(19) Record post-mortem appearances.

(*e*) **Respiration in an Atmosphere of One-third Illuminating Gas (CO +)**. Record all symptoms.

Record post-mortem appearances.

How does death in an atmosphere of CO compare, as to symptoms, with death in an atmosphere of CO_2.

Compare it in turn with other forms of death as induced in this and the previous chapter.

Compare the post-mortem appearances in this case with those in preceding cases.

X. TO DETERMINE THE INFLUENCE OF THE PHRENIC NERVE. THE NORMAL PHRENOGRAM.

1. **Appliances.** Operating case; clippers; rabbit board or dog board; rabbit or dog; ether or chloroform; anæsthesia cone; tambours, arranged as used to record the rabbit stethogram; beaker with warm water; inductorium; one dry cell; two keys; vagus electrode; seven wires; a piece of glass rod 10 cm. which has been rounded at one end and sharpened at the other.

2. **Preparation.** Fix the animal to the board; anæsthetize; clip the anterior median region of abdomen. Set up electric apparatus with short-circuiting key in secondary coil and with Neef hammer in primary circuit.

3. **Operation.** From the posterior extremity of the xiphoid appendix make a median incision through the abdominal walls. The incision should be just large enough to admit the glass rod, and should be located in the rabbit 1 cm. from the tip of the xiphoid and in the dog 3 cm. from the xiphoid.

Clamp with the serre-fines any small vessels which may be oozing.

The rounded end of the glass rod is passed through the abdominal wall and held against the diaphragm *A*. The point is inserted into the cork button of the receiving tambour. (See Fig. 65.) Any contraction of the diaphragm presses the round end backward and the rod is forced posteriorly, slipping back and forth through the hole in the body wall, the point is pressed back, and the lever of the recording tambour rises. Trace a *phrenogram*.

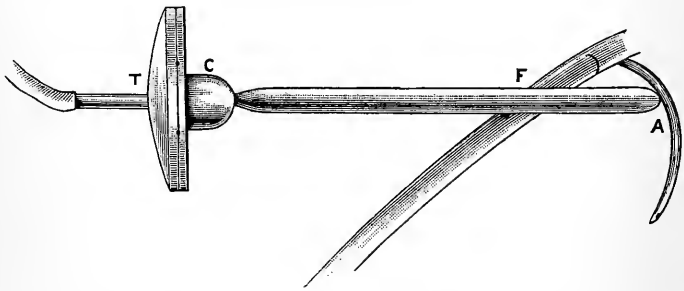
In the mean time let another member of the division dissect out the left phrenic nerve.

This operation to expose the phrenic nerve is the most difficult

operation yet attempted in any of these exercises. The nerve is very small and lies deeply buried in the neck not far from the spinal column and in close relation to other nerves, making it difficult so to describe its relations that the operator will be certain when he has found it. The only sure course is to test it by stimulation, and if it causes a contraction of the corresponding side of the diaphragm the operator may be certain he has found either the main trunk or one of its three roots.

The cutaneous incision should be on the course of the sternomastoid muscle, just dorsal to the course of the external jugular vein. The cutaneous incision should be ample, extending to the clavicle at least 5 cm. long anteriorly in the rabbit and correspondingly long if the dog is used.

FIG. 65



Phrenograph for taking tracings of the movements of the diaphragm: *T*, tambour joined to recording tambour and fitted with a cork button (*C*); a glass rod passes through a slit in the abdominal wall at *F* and rests against the diaphragm at *A*.

Dissect through the subcutaneous tissues and separate the skin flaps widely, pressing the external jugular toward the median line. The superficial layer of muscles consists of the sternomastoid on the median side and the cleidomastoid laterally in the rabbit (the cephalohumeral in the dog).

Divide the connective tissue that separates these two muscles and pass to the deeper layer. On the median side one sees the carotid, the internal jugular, and the nerves that lie in close relation to them; drawing the cleidomastoid outward one exposes the roots of the brachial plexus, emerging from between the deep muscles of the neck and passing downward and backward toward the axilla.

Very careful dissection of the delicate connective tissue which lies over the roots of the brachial plexus will reveal a fine nerve thread crossing these roots very near to the line where they first come into view, and passing posteriorly it gradually draws nearer to the median line as it passes under the clavicle (under the subclavian artery in the dog). This nerve is the phrenic. Carefully dissect it out as near the clavicle as possible, lift it gently on the nerve hook, and place it

in the groove of a shielded electrode. Stimulate gently. If you have dissected out only the phrenic and posterior to its three tributaries, the stimulation will be followed by a tetanic contraction of the corresponding side of the diaphragm. If, however, one has taken up with the phrenic a communicating thread, passing from one root of the brachial plexus to another, the stimulation will be followed by a tetanic contraction, not only of the diaphragm, but also of some of the muscles of the front leg of the side operated upon. As this will disturb the result the error must be corrected. The nearer the clavicle one can get the nerve the more unlikely he is to get nerve fibres belonging to the brachial plexus.

4. **Observations.** (*a*) **Tactile Observation of the Diaphragm.** (1) In what condition is the diaphragm during inspiration? Expiration?

(2) In what position is the diaphragm during these two phases of respiration?

(3) What parts of the diaphragm make the greatest change of position during inspiration?

(4) What causes the diaphragm to arch anteriorly during normal expiration? Are the conditions changed during the present observations?

(5) Are the diaphragmatic movements synchronous with the costal movements?

(*b*) **The Normal Phrenogram.** (6) Take a phrenogram. What may be learned from it?

(7) Without varying the adjustment of the phrenograph take a tracing while repeatedly interrupting the respiration by holding the nostrils. What does the phrenogram show? What is the interpretation?

What effect upon intrathoracic pressure would holding the nostrils have?

(*c*) **The Phrenic Nerve and its Function.** (8) Describe minutely the relations of the nervus phrenicus in the neck.

(9) Cut the nerve while tracing a phrenogram from the left side of the diaphragm. Note the result.

(10) Take a phrenogram from the right side of the diaphragm. Does it differ essentially from the normal?

(11) While taking the left phrenogram stimulate the distal end of the left phrenic nerve. Interpret the result.

(12) While taking a right phrenogram stimulate the distal end of the left phrenic nerve. Interpret the result.

(13) Dissect out and cut the right phrenic nerve. Does the diaphragm cease to move? If it moves, is its movement active or passive? Does it move backward during inspiration and forward during expiration? If so, what causes it to make these movements? If the movements are reversed, what has caused the change?

Account for the phenomena. Kill the animal with chloroform.

CHAPTER V.

NORMAL HÆMATOLOGY.

INTRODUCTION.

The examination of the blood, like that of the urine, gives a positive diagnosis in a number of diseases. It assists the diagnosis in many diseases and is often of much value negatively. It is important, then, to be familiar with the characteristics of normal blood. The examination of the normal blood consists of an actual study of the blood by use of the microscope and the determination of many of its properties by the use of various instruments, which will be described in the text. The accurate use of the instruments can be learned only by experience. While the instruments are delicate and easily broken, yet the technique of their use is easily mastered by the student if he is careful, accurate, and persevering. The technique once acquired can be quickly regained in later years, although it may apparently be forgotten for the time being. Speed in the tests can be obtained only by continuous practice. Theoretically all these instruments are accurate, but because of the minute quantity of blood used, slight inaccuracies will be multiplied in the final results and may be large or small according to the experience and carefulness of the observer. By knowing where these errors are possible and avoiding them by the best-known methods, and by adopting a definite method of use of each instrument, these inaccuracies can be largely eliminated and good comparative results obtained. In the use of blood instruments the observer must constantly avoid manufacturing results. There is always the tendency to read into the test a preconceived result. This is best governed by control tests and by repeated tests. When one can repeat a test three or four times with the same individual's blood and obtain approximately the same results he is quite proficient.

Reference Books.—Clinical Examination of the Blood, by Cabot. Clinical Pathology of the Blood, by Ewing. Clinical Hæmatology, by Da Costa. Histology of the Blood, by Ehrlich and Lazarus. Text-book on Physiology, by Hall. Works on Histology and Physiology.

GENERAL DIRECTIONS.

All blood instruments must be perfectly clean and dry if the best results are to be obtained. The various pipettes are cleaned by the use of hydrogen peroxide and distilled water; they are then dried

by the use of alcohol to remove the water, followed by ether, which will evaporate quickly and remove the alcohol.

Hydrogen peroxide oxidizes organic matter; alcohol and ether coagulates.

The cleaning fluids (hydrogen peroxide and water) are used by filling the pipette and rolling it for a few minutes between the thumb and fingers and then blowing or drawing the fluid out.

In the use of the drying fluids (alcohol and ether) do not blow the fluids out of the pipettes, as the moisture of the breath will defeat the object which one is seeking. Having filled the pipette with alcohol or ether, draw it into the rubber tube, remove the rubber tube from the pipette, blow the fluid out of the rubber tube, replace it upon the pipette, then *draw air through the pipette.* After using alcohol in this way, followed by ether, one may be assured that the pipette is absolutely dry.

For the student's work, secure the blood from the lobe of the ear or the side of the tip of the third finger. The ear is better, as it contains fewer nerves, gives more blood, and will continue to bleed for a longer time. The ear or finger should be lightly washed with a towel moistened with distilled water, then dried with the towel to remove any dirt or loose epithelial cells. The needle used should be a fair-sized glover's needle. It is a three-sided needle, the sides of which are so ground that each has a fine saw-edge and will cut and not crush the tissues as a saddler's needle will. The needle should be kept clean with distilled water and hydrogen peroxide, and sterilized with alcohol.

The puncture should be made by holding the lobe of the ear between the thumb and finger and pricking lengthwise of the ear in its lowest part. The needle should enter about one-quarter of an inch, and should be thrust in quickly while the thumb and finger hold the ear, and when withdrawn it should be given a half-turn and be quickly removed. The first drop of blood should always be wiped away to moisten the skin with blood, and also because it clots quicker than the following drops.

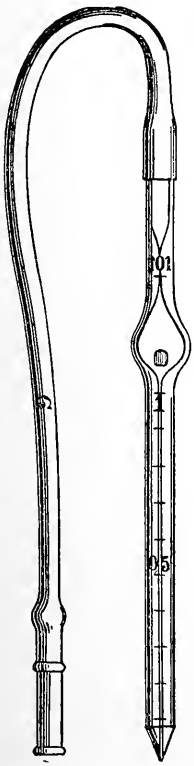
The blood should gradually ooze out of itself. It should never be forcibly squeezed out by pinching, as that will give an abnormal specimen; but the ear may be gently pressed an inch or so above the puncture, to make the blood flow more freely.

To fill a pipette by suction, take the lobe of the ear between the thumb and finger of the left hand, standing behind and to the right when using the right ear and in front and to the left when using the left ear. Place the tip of the pipette upon the thumb that is behind the ear hold the pipette with the right hand near its upper extremity, with the marks showing in front; then, by turning the thumb, insert the capillary point into the drop of blood and do not allow it to touch the skin of the ear; the column of blood drawn into

the capillary must be accurate and complete. It must not remain short of or go beyond the mark desired, and air must not be allowed to enter the pipette. If any of these errors take place the pipette must be recleaned, dried, and filled again.

When properly filled, any blood adhering to its outer surface must be completely removed before proceeding farther. It is better to have a large drop of blood at first than to use two or three small drops, as there is less liability of getting air into the capillary and of the blood clotting.

FIG. 66



The Thoma-Zeiss blood-counter. The pipette for use in counting the red corpuscles.

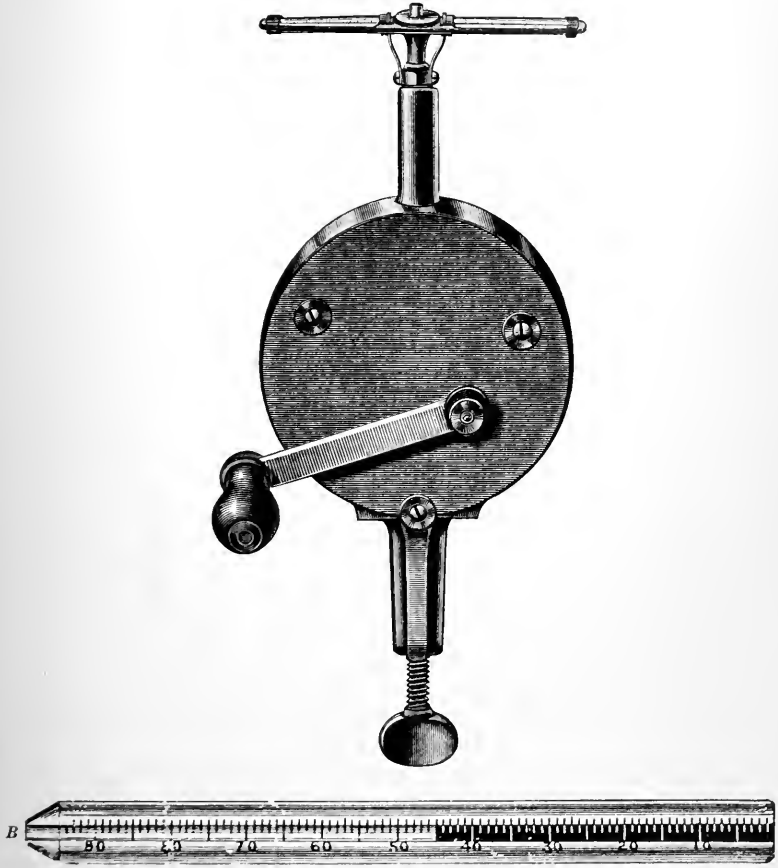
I. THE COUNTING OF THE BLOOD CORPUSCLES.

Introductory. In health the number of red cells in the blood is quite constant. The variations that occur are quite small and are due to normal processes. In the male there are about 5,000,000 red cells in each cubic millimetre. In the female there are about 4,500,000 red cells. Any deviation from normal health quickly causes a diminution in the number of red cells. In fact, simple unhygienic surroundings or habits are sufficient to speedily reduce the number of red cells without other demonstrable pathological conditions.

The life of the red cell is probably of about two weeks' duration. There are approximately in the normal male's blood 200,000,000,000 red cells. Then according to the length of the lifetime of the cells about 14,000,000,000 red cells die and must be disposed of each day. A corresponding number must be manufactured each day in order to keep the number within its normal limits. It will be readily seen that such an immense process, which depends upon perfect elimination as well as assimilation, can be disturbed very easily. It is important that this fact about the blood be thoroughly understood. Even though the physician may not estimate the number of red cells in every case, yet he must recognize the fact that every disturbing element in the normal body must disturb the number of red cells contained therein. There are, then, two objects to be gained by actually counting the

red cells and estimating their number. First, to gain a clear idea and understanding of the number of red cells in normal blood, and, second, to be able readily and accurately to estimate the number of red cells per cubic millimetre in any given clinical case.

FIG. 67



The hæmatocrit. The attachment at the upper end of the vertical shaft is made to rotate at a speed of 7000 to 10,000 per minute by means of the gear-work of the body of the instrument. Each arm of the rotating attachment is provided with a capillary tube which is graduated into 100 divisions. If the tube be filled with blood and rotated for two or three minutes at the speed above mentioned the corpuscles will be thrown to the outer end and the volume per cent. may be read off on the tube. *B*, an enlarged view of tube with centrifugalized blood.

There are three methods of estimating the number of red cells per cubic millimetre.

1. **The Thoma Hæmacytometer.** An instrument by which, with accurate dilution, the corpuscles may be actually seen and counted in a known space (Fig. 66).

2. **The Oliver Hæmacytometer.** This instrument depends upon the transmission of a transverse line of light from a candle through a flat glass tube. The blood stops this light until a certain dilution is obtained. The tube is graduated to read in the number of cells per cubic millimetre of the blood used according to the dilution.

3. **The Hæmatocrit.** By this instrument is obtained the volume of the corpuscular elements in the blood by centrifugation. From this the number of red cells per cubic millimetre may be estimated except in some special cases (Fig. 67).

A. To Count the Red Blood Corpuscles.

Appliances. Microscope with one-fifth-inch objective and mechanical stage; Thoma corpuscle counter, consisting of the ruled counting slide and the diluting pipettes; glover's needle; three small beakers and as many open dishes.

Preparation. Wash the counting slide with water or soap and water only when it needs it; the less it is handled the better. Usually rinsing it in clean water and drying with a cloth is sufficient. Prepare small beakers of distilled water and the diluting solution. Clean the pipette as usual.

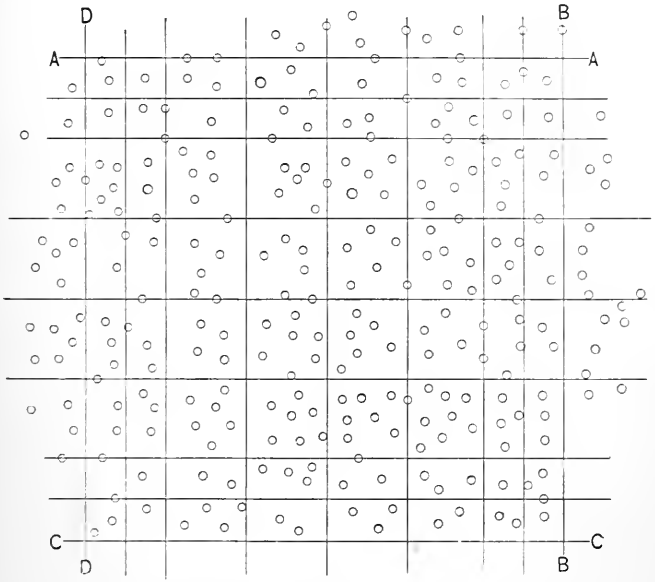
Technique. Having prepared the apparatus and solutions, make the puncture and fill the pipette by gently sucking a continuous column of blood up to the mark "0.5" or "1" on the pipette, which is near the bulb. Wipe the end of the pipette free from blood with a clean cloth, but do not allow any blood to be drawn out by the capillary attraction of the cloth. As soon as possible now insert the point of the pipette into the diluting solution and suck up a continuous stream of solution until the mark "101" above the bulb is reached. Roll the bulb between the thumb and finger as the blood enters the bulb.

If there is not blood enough to reach the mark "1," draw it only to mark "0.5" and proceed in the same manner. Now hold the pipette in the horizontal position with ends free and roll it back and forth for three minutes to thoroughly mix the blood and solution. When thoroughly mixed blow out the contents of the capillary below the bulb and then place a small drop on the marked plate of the counting slide, putting just enough of the mixture on it to fill the space between the marked plate and cover-glass, and being careful not to allow any of the mixture to get into the moat. Adjust the cover-glass over the drop quickly and carefully by placing one edge of cover-glass in contact with the slide and letting the opposite edge down gently with a needle.

Place the counting slide when properly filled under the microscope and find the upper left-hand corner of the marked area. Wait until the corpuscles come to rest upon the surface of the marked plate,

then begin the actual estimation by counting all the corpuscles in the first marked space, including those that are on the upper and left-hand lines of the space. Then count those in the space to the right, including the corpuscles on the upper and left-hand lines as before. Continue counting each space to the right until six spaces are counted; then drop down to the next space below and count

FIG. 68



Appearance of slide under about 500 diameters magnification. One counts all corpuscles which lie upon the upper and left boundaries of each square.

each space to the left until six spaces in the second row are counted. Then drop down to the next row of spaces and continue counting back and forth in the same manner until six rows of six spaces each are counted, as shown in Fig. 68. Place the results of counting on paper in the same relation to each other as the spaces illustrated, as follows:

5	6	5	4	7	5
9	6	6	6	6	7
4	5	5	5	8	7
7	5	8	6	4	7
6	6	7	7	9	7
6	6	6	6	5	7
37	34	37	34	39	40

$$37 + 34 + 37 + 34 + 39 + 40 = 221$$

$$221 \div 36 = 6\tfrac{5}{36}.$$

Having made the count, the slide and cover-glass should be cleaned as previously described. The pipette, which has been left in a hori-

zontal position in a safe place, should be rolled again for three minutes. Fill the counter and adjust the cover-glass carefully as before; count another group of thirty-six spaces and record the results obtained. If the averages of the two counts differ more than one, the same procedure must be carried out the third time, and the average of the two fields nearest alike taken and the estimate made.

To compute the number of corpuscles per cubic millimetre, find the average number of cells for each space and multiply this by 4000, as each space is $\frac{1}{20}$ mm. $\times$ $\frac{1}{20}$ mm. $\times$ $\frac{1}{10}$ mm. This will give the actual number of cells per cubic millimetre in the diluted blood. Then make the correction for the dilution of the blood by multiplying by 100 or 200, and the result will be the number of red cells per cubic millimetre in the specimen of blood taken. In the example given above it would work out as follows:

First 36 spaces, $6\frac{5}{36}$; second 36 spaces, $6\frac{13}{36}$. Average of the two groups, $6\frac{9}{36}$. $6\frac{1}{4} \times 4000 = 25{,}000 \times 200 = 5{,}000{,}000$, the number of red cells per cubic millimetre.

Precautions. The cement used on the counting slide is dissolved by alcohol or ether; so these liquids should not be used on the plate. Roll the filled pipette between the thumb and finger, and do not shake the pipette, as some of the solution is sure to be lost. A common source of error that can easily be detected, but which is often overlooked, is the unequal distribution of cells on the marked plate. As soon as the drop is placed on the marked plate the cells begin to settle, and, of course, most of them settle where the drop is thickest, that is, in the center. This can be avoided by getting the cover-glass in place quickly and making the whole drop of an even thickness. Each specimen, before being counted, should be tested to see that the corpuscles are evenly distributed over the whole drop. For the same reason the filled counting slide should be kept in a horizontal position.

Theoretically, counting the cells in one small space should be sufficient, and it would be if the measurement and dilution of the blood and distribution of the cells were all perfectly accurate. This is impossible, and the errors are mostly eliminated by the methods given. It is best for beginners always to make three counts of 36 spaces each from the same pipette and take the average.

Questions. 1. Why is alcohol used to dry and not to clean the pipette?

2. Why should the marked plate be dried without friction?

3. What does hydrogen peroxide do to clean the pipette?

4. Why rotate the needle while withdrawing it from the ear?

5. Why wipe away the first drop of blood?

6. Why wipe the end of the pipette before putting it into the diluting solution?

7. Why roll the pipette as the blood enters the bulb?

8. Why blow out a few drops before putting a drop on the slide?

9. Why draw air through the pipette after the ether is drawn out?
10. What kind of a solution should be used to dilute the blood; that is, what properties should it have?
11. Why are there 101 parts in the pipette instead of 100?
12. Is there any appreciable variation in the number of red cells in normal individuals?
13. If there is a variation, give some of the reasons.
14. Account for the variations observed in members of your section.

B. To Count the White Blood Corpuscles.

In counting the number of white cells per cubic millimetre in the blood, the principle of diluting and counting is exactly the same as in counting the red cells. There are some differences in the details, since we must first get rid of the red cells so that the white cells can be seen, and because of the small number we must dilute less and count larger areas.

Appliances. The instruments are the same, with two exceptions or modifications. The diluting pipette is just like the red-cell diluting pipette, except that it is larger in the capillary and smaller in the bulb, so it can make dilutions of ten to one hundred instead of one to one hundred. The counting plate should have the modified markings as shown in Fig. 68. One can use the lower power ($\frac{2}{3}$) objective of the microscope just as well and save time by it, but it is not necessary.

Reagents. The same as when counting the red cells except that a different diluting solution must be used. A $\frac{1}{2}$ per cent. solution of acetic acid in distilled water will destroy the red cells and render them invisible, while it will not destroy the white cells, but make them show more plainly.

White-cell Diluting Solution.

Acetic acid	0.5 c.c.
Distilled water . . . . . q. s. ad	100.0 c.c.

Technique. The technique of obtaining the blood and filling the pipette is the same as with the red cells, except that the capillary tube is so large that we must have more blood. For this reason for beginners we recommend that only a half-part of blood be taken at first. More accurate results can be obtained by using one part, but much time and practice is necessary to fill the tube easily. The capillary is so large that solutions will not stay in it, but run out quickly when the tube is out of the horizontal position. First, then, we must have more blood; usually two or three good-sized drops are sufficient. Second, the tube must be held horizontal or the blood and solution will run out. As the capillary tube is large it is very easily cleaned and dried.

Roll the pipette as before when filled and in a few moments the mixture will turn quite dark; when it no longer changes color it is ready to be counted. Allow a few drops to flow out of the tube as in the case of the red-cell pipette, then place a small drop from the end of the pipette on the ruled plate. It is not necessary to blow the fluid out; it will run out. Take the same precautions in filling the counter and adjusting the cover-glass as before, except that there is no need of haste in placing the cover-glass, because the white cells are lighter.

Here, because we have a clear field with little in it, and the cells are quite large, we can use a lower power of the microscope and see a whole square millimetre at once. Begin at the upper left-hand corner and count the cells in each space 1 mm. square, and observe the same method in keeping the record as when counting the red cells. Clean the counting slide, roll the pipette for a moment, and refill the marked plate and count the nine spaces again, keeping the records as before. Do this at least three times, so that the area counted will be 27 spaces, each 1 mm. square. The more cells counted the more accurate the results should be, but the three fields should be sufficient.

To estimate the number of cells per cubic millimetre in the blood specimen used, add together the number of cells and divide by the number of millimetre spaces counted. Each space is $\frac{1}{10}$ mm. $\times$ 1 mm. $\times$ 1 mm. or $\frac{1}{10}$ c.mm. Now multiply the average number of cells in each space by 10 to find the number of cells in the diluted blood, and then by 10 or 20 according as the blood was diluted, and that will give the number of white cells per cubic millimetre in the blood specimen, as follows:

33	45	56	47	39	57	51	43	49
48	57	39	55	45	61	37	61	53
61	53	59	43	51	41	57	39	40
142	155	154	145	135	159	145	143	142 = 1320

$1320 \div 27 = 48\frac{8}{9} \times 10 \times 20 = 9777\frac{7}{9}$ white cells per cubic millimetre of the blood examined.

Questions. 1. What is the number of white cells per cubic millimetre in the blood in the normal individual?

2. What is the normal variation?

3. What are some of the causes of the variations?

C. To Count both Red and White Cells at the Same Time.

In general the whole technique is followed out and the same instruments used as when counting the red cells alone. The method consists of using a diluting solution containing a stain that will stain the white cells only, and then counting the red and white cells separately.

COLORED DILUTING SOLUTION.

Methyl violet	0.025 gram.
Sodium chloride	1.000 gram.
Distilled water	100.000 c.c.

Count the red cells in a group of thirty-six spaces first, and keep the record as before. Next count the white cells in all of the nine square-millimetre spaces and keep the record as before. This should be repeated until at least two groups of red cells and three or four groups of white cells are counted from different specimens on the counter, and each record should be kept so that the average may be taken and the number per cubic millimetre be estimated in each case.

Estimate the number of cells by taking the average and estimating the number just as when counting the red cells alone. Estimate the number of white cells just the same as before by taking the average for each $\frac{1}{10}$ c.mm., but multiply that by 100 in this case instead of 10 or 20, as the blood in this specimen was diluted 100 times.

D. Centrifugalization of the Blood. To Determine the Relative Volume of Red Corpuscles and Plasma. To Estimate the Number of Red Corpuscles from Their Volume.

Appliances. Electric or hand hæmatocrit (Fig. 67); small rubber tubing to fit capillary tube; glover's needle; white paper; fine wire for cleaning tubes.

Reagents. Distilled water, hydrogen peroxide, alcohol, and ether.

Preparation. Adjust rubber to capillary tube. Put empty tube in one arm of cross-piece to preserve balance. Use fine wire to remove blood from the capillary tube, then clean and dry as other tubes.

Technique. Obtain blood from the lobe of the ear as heretofore described. Draw capillary tube full of blood. Remove the rubber tube by pushing it off and not by pulling. Remove any blood from the outside of the capillary, and make a record of the amount of blood in the capillary. Place the tube in the cross-piece of the instrument as quickly as possible and centrifugalize at least three minutes at the rate of 7000 to 10,000 rotations per minute. Take out the tube and lay on a piece of white paper to read the divisions. Each degree of the scale is estimated to contain about 100,000 cells; hence, a tube in which the red column stands at 50 would indicate about 5,000,000 red corpuscles per cubic millimetre. The use of this instrument is designed, however, chiefly to show *the volume of red corpuscles rather than the number.*

Precautions. Do not displace the rubber pads in the outer ends of the rotating arm, as the blood will be thrown out of the tube and

necessitate the repetition of the test. Before starting each test see that the pads are in place.

If the tube is not adjusted in the apparatus and set to rotating within a few seconds after the blood is drawn, coagulation will set in and hinder the complete separation of the corpuscles from the plasma. Should separation not be complete in three minutes the test should be repeated. The instrument should be started and stopped gradually, as the sudden starting and stopping injures it.

Questions. 1. Determine the volume percentage of red blood corpuscles in a number of normal individuals.

2. Do apparently normal individuals have the same or approximately the same volume percentage of red blood corpuscles? If not, seek for causes of the variations in different individuals.

3. Does the same individual have the same volume percentage of red blood corpuscles all the while?

(*a*) If there is a variation, is there any periodicity to be observed?

(*b*) Seek for causes of any variation in the same apparently normal individual.

4. The volume percentage as recorded by the hæmatocrit varies with the product of two factors: the average volume of the individual corpuscles by the number of corpuscles per unit volume. ($V = v \times n$).

(*a*) Is the average volume of the individual corpuscles (v) necessarily constant?

(*b*) If it is not constant, would one be justified in drawing conclusions regarding the number of corpuscle per unit volume (n) after observing the volume percentage (V) with the hæmatocrit?

5. What variation of the observation as above made would enable one to determine with reasonable accuracy the number of corpuscles per cubic millimetre?

6. If the tube were only partly filled at first, could one make an accurate test? If so, tell how to proceed.

II. THE ESTIMATION OF THE PERCENTAGE OF COLORING MATTER IN THE BLOOD.

The estimation of the coloring matter in the blood is based on the supposed fact that a normal individual under normal surroundings has a normal amount of coloring matter, and that is called 100 per cent.

The instruments that have been devised for making the estimation are numerous, and all, while theoretically correct, practically are liable to a greater or less error according to the experience and carefulness of the observer. They are, however, in a skilful and conscientious operator's hands, quite accurate, and are especially so when used to compare the tests of the same patient's blood, week by week.

The hæmoglobin contains practically all the coloring matter, and it constitutes 90 per cent. of the red cell. The hæmoglobin consists of 96 per cent. globulin and 4 per cent. hæmatin. In the hæmatin is the iron of the corpuscles; the coloring matter of the blood varies as does the amount of iron. Theoretically, the most accurate way to test the hæmoglobin would be to measure the amount of iron in a certain amount of blood. But the chemical extraction and weighing of so small an amount of iron is too difficult and tedious. Because of this, other tests have been devised, which depend upon the observer's eye to detect the likeness of shades of red as represented by the blood and colored glass, solutions or paper. Again, the specific gravity of the blood except in rare cases depends upon the amount of iron in the red cells, and varies as the iron does. Then we can estimate the percentage of hæmoglobin by finding the specific gravity of the blood.

The principal tests may be classified as follows:

1. Estimation of iron in the blood.
   Jolles' ferrometer.
2. Estimation of percentage of coloring matter by color tests.
   A. Fleischl's hæmometer.
   B. Gowers' hæmoglobinometer.
   C. Dare's hæmoglobinometer.
   D. Tallquist's hæmoglobinometer.
3. Obtaining the specific gravity of the blood by Hammerschlag's method.

A. Fleischl's Hæmometer.

Appliances. Fleischl's hæmometer; glover's needle; pasteboard tube two inches in diameter; artificial light; small beaker; a dark room or cupboard.

Fleischl's hæmometer consists of a sliding colored-glass wedge which moves in a standard underneath a cylindrical metallic cup, and a capillary tube. This cup is divided into two equal compartments and has a glass bottom and a detached glass top. The capillary tube is very small and is held by a small metallic band on a handle. The glass wedge and the capillary tube are the important parts of the instrument and are made to be used together. There is a number on the handle of the capillary tube, indicating its capacity, and this same number is stamped on the top of the standard; also a number is placed on the end of the sliding frame that holds the glass wedge, and the same number appears on the base of the standard of the instrument to which it belongs (Fig. 69).

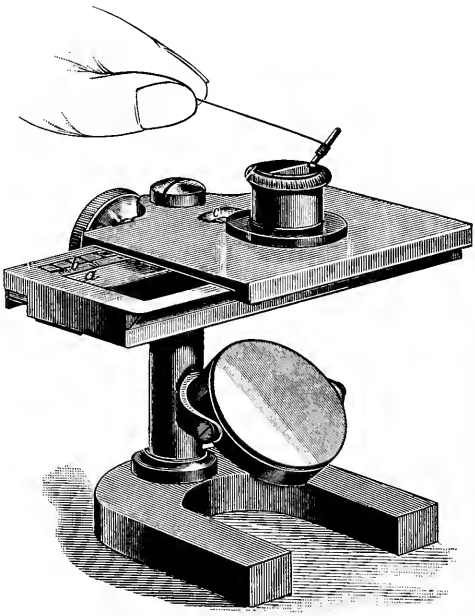
Reagents. Distilled water and hydrogen peroxide.

Preparation. Clean metallic cell or well with water and dry with a cloth only when it needs it. The capillary tube should be cleaned with water and hydrogen peroxide, and then with water again, by

waving it back and forth in the solutions for a moment or two. Then carefully dry the tube by blowing air through it, holding the tube about two inches from the mouth so as to avoid the moisture of the exhaled air. Fill each side of the metallic cup about three-fourths full of distilled water. Prepare the needle and the ear or finger as in other tests.

Technique. Obtain the blood in the usual manner. Hold the lobe of the ear with the thumb and finger. Use the second drop. Hold the capillary tube horizontally and carefully touch the drop of blood with the end of the tube only. If the tube is clean it will fill rapidly

FIG. 69



Fleischl's hæmometer.

by capillary attraction. If there is any blood on the outside of the tube or air-bubbles inside, it must be cleaned, dried, and refilled properly. If the capillary is overfull, remove the excess by touching the tip to a cloth or filter paper. Then quickly put the capillary tube into the water in one compartment of the metallic cell and wave it back and forth or up and down, and the blood, if fresh enough, will readily mix with the water; then allow a few drops of water from the medicine dropper to flow through the capillary into the same compartment to wash the blood that sticks to the tube. Now fill each compartment almost full with distilled water, taking care that the contents of either compartment does not flow into the other. Take

the handle of the capillary and stir the one that contains the blood so as to make the mixture complete. Now carefully slide the thick cover-glass over the compartments and gradually fill each cell with water as the cover-glass is put on until there is no air left in either cell. Exclude daylight by use of a dark room or a cupboard, and adjust the reflector so that the artificial yellow light is thrown up through the diluted blood and water from the side of the instrument, thus placing both cells in same relation to the reflector and the light. While making the test always shade the eyes from the light by placing some thick paper or a pasteboard tube, that reaches from the instrument to the forehead, before the eyes. It is better to use only one eye at a time, and look only for a few seconds at each time, giving the eye a rest and a chance to regain the ability to distinguish tints. Stand at one side of the instrument or turn the instrument so as to face the light and to bring the two cells into similar relations with the eye. Begin with a glass of a lighter color than the blood, and move the colored-glass slide by quick turns about one-fourth of an inch each time until the color or tint of the diluted blood appears to be the same as that of the colored slide; then make the reading. Next turn the colored glass on until it is darker than the diluted blood and do the same as before, except in the opposite direction, turning the slide until the color of the glass and blood are the same, and then make the reading. Usually the first reading will be too low and the second too high. The difference will usually be about 10 per cent. The correct result will be between these two readings, which can now be obtained by carefully moving the glass back and forth or by taking the middle point between the two readings. It is almost impossible to make the reading accurately and honestly unless great care is taken, and the writer has found the method given to produce the best results by far. This method should be practised again and again and done with care. A hasty reading is rarely correct. Repeat the whole test until you can obtain the same result each time with the same individual's blood.

Precautions. If the capillary tube is not perfectly clean it will not take blood by capillary attraction. While cleaning the tube always test it by touching a drop of water, when it should fill immediately. This will save time and ensure quick work. The amount of blood taken is so small and this is diluted so much that the least error is multiplied many times. We can expect accurate results only when every known chance of error is safely guarded. If the capillary has moisture or foreign matter in it, the tube will not hold the right amount of blood and the result will be too small. The blood must be obtained and mixed in the metallic cup with the water very quickly, or it will clot and stick in the capillary, or if it does leave it it may remain as a clotted thread of blood in the bottom of the cell. It takes a little practice to learn to wave

the capillary in the small space of the cell. Very gentle constant waving back and forth or into the water and out is the most effective in getting the blood out of the tube. Too vigorous movements are liable to break the glass tube. When completing the filling of the cells with water, fill the cell containing water only first, and then there is no danger of getting any of the diluted blood into the water compartment. If you neglect to stir the blood and water just before adjusting the glass cover the blood will remain in the lower part of the cup with the water on top, and it will have a darker color than it should because the blood really is not diluted as necessary. This will give a higher reading than is accurate. The glass cover should always be used; it not only makes the amount of dilution accurate, but it gives an even surface for the transmitted light rays. Without the glass the surface of the water is either concave or convex.

The metallic cup should not be taken apart unless it is very dirty. If the glass is clean, that is sufficient. As a laboratory precaution, where several instruments are in use, always compare the markings to be sure that you have the capillary tube that goes with the glass wedge that you have.

Questions. 1. Why use distilled water to dilute blood and not a saline solution similar to the plasma of the blood?

2. Name four common sources of error in the technique.

3. Can different individuals make approximately the same reading of the same test?

4. Can different individuals make approximately the same results from the same individual's blood?

5. Does every individual in ordinary health have the same percentage of hæmoglobin?

6. How would you explain the variation, if any?

7. Do individuals who have a low percentage of hæmoglobin have a correspondingly lessened number of red cells per cubic millimetre?

8. Is the reverse of the above true?

B. Gowers' Hæmoglobinometer.

Gowers' hæmoglobinometer consists of three pieces: a capillary measuring pipette, a graduated tube, and a sealed tube containing a standard colored solution. The standard colored solution represents the color of 1 per cent. solution of normal blood. The graduated tube is marked in 100 or more parts, and each part represents 20 c.mm. The capillary pipette holds 20 c.mm. up to the mark on the tube. If the blood is normal it will be necessary to add water to the hundredth mark in order to make the colors correspond. If the blood is not normal the percentage can be read off the graduated tube at the top of the diluted blood when the colors correspond. There are two kinds of instruments: one for use with daylight,

which has a white substance in the sealed end of the tube containing the colored solution; the other is for use with artificial light and has a black substance in the sealed end of the tube (Fig. 70).

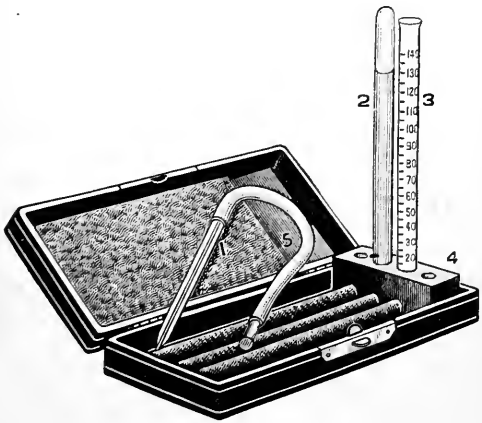
Appliances. Gowers' hæmoglobinometer and a glover's needle.

Reagents. Distilled water, hydrogen peroxide, alcohol, and ether.

Preparation. Clean the instruments in the usual manner. The capillary pipette should be cleaned with the same care and in the same way as the diluting pipette, being careful to first clean and then to dry the pipette. Fill the graduated tube to the mark 20 or 30 with distilled water; prepare the needle and finger or ear as usual.

Technique. Obtain the blood in the usual way except that a larger quantity of blood is needed than for the preceding instrument. Hold the ear in the same way and fill the pipette as when

FIG. 70



Gowers' hæmoglobinometer: 1, blood capillary; 2, solution of standard color; 3, tube in which blood is diluted.

obtaining blood in the pipette for counting corpuscles. Wipe away the first drop of blood. Touch the tip of the pipette to the drop of blood, resting the pipette on the end of the thumb, which is behind the ear, and slowly suck the blood up to the mark on the pipette, but do not allow the blood to go beyond that point. If the drop is not sufficient, quickly obtain the second drop by gentle pressure high above the wound in the ear. If there is any excess of blood on the point or sides of the pipette, quickly wipe it away. Insert the pipette into the tube almost to the water and slowly blow the blood, drop by drop, into the water. Now immediately shake the tube to mix the water and blood; this is to prevent the blood clotting or remaining as a thread in the bottom of the tube. Blood still remains in the capillary on its sides; so fill the pipette with distilled water and blow this into the tube, three or four times. Then thoroughly mix

the blood and water by shaking or rolling the tube gently. Do not place the thumb over the end and shake, as an appreciable amount of color will be lost and a foam is formed that delays the reading.

Now place the tube in the rubber block beside the tube containing the standard solution and add distilled water, drop by drop, to the diluted blood, always shaking the tube between the additions to keep the blood and water well mixed. Continue this until the color of the blood solution is not darker nor lighter than the standard solution. The comparison of the colors is made either by transmitted or reflected daylight. The eye will be assisted by placing the tube behind a piece of white paper and holding them toward the window light. The reading is made directly from the graduated tube, in percentage of hæmoglobin when the color of the diluted blood is the same as the standard solution. Repeat the test until the same result is obtained continually.

Precautions. If air bubbles are drawn up into the capillary, or if it is either over or underfilled, the tube must be recleaned and dried and the test repeated until done accurately. If the pipette contains moisture or foreign matter the measurement will not be accurate. Always remove any blood that may happen to get on the outside of the pipette, as it will increase the result. It is a good plan to have a large drop of blood ready before you begin to fill the pipette, rather than to take two or three small drops. Because of the time consumed to obtain the amount of blood needed there is liability of the blood clotting and sticking in the capillary. When only partially clotted the blood is blown into the graduated tube and remains as a clotted thread in the bottom. Gently striking the finger against the tube or shaking the tube sidewise is sufficient to mix the blood and water, and is far better than placing the thumb over the mouth of the tube and shaking it up and down. If this latter method is used it will make a difference of 5 to 10 per cent. in results. Always be sure to wash the capillary out a number of times and place the washings in the graduated tube, or the result obtained will be less than the test should show. If a tube has been partially or improperly filled, do not leave it so; always blow out the blood before it can clot, and much time will be saved. A reading should be made each time and recorded before more water is added, for if one should dilute the blood too much and had no record of the last reading the test is spoiled and the work lost.

C. Dare's Hæmoglobinometer.

Appliances. Dare's hæmoglobinometer, a candle, and a glover's needle.

Preparation. Dare's instrument estimates the percentage of hæmoglobin by comparing the color of a thin film of blood of a cer-

tain thickness with a revolving colored, wedge-shaped disk of glass. The only preparation necessary is to clean and polish the glass plates that hold the blood, and adjust them in their holder.

Technique. Obtain a good-sized drop of blood in the usual manner. Touch the edge of the plates to the drop of blood, and the space between them will be filled with blood by capillary attraction. Place the holder in its socket, adjust the telescopic tube and the lighted candle, and make the reading in the same manner as with the Fleischl instrument. A dark room is not necessary, but it is well to hold the instrument toward some dark object as a background.

D. Tallquist's Hæmoglobinometer.

Tallquist's hæmoglobinometer consists of a chart or a paper on which are twelve oblong, red-colored stripes, ranging from 10 to 120 per cent. in degree of color. The color of the stripe marked 100 is supposed to be the same color and shade as that of a piece of filter paper in which there is normal blood.

The other stripes vary from this as the numbers indicate.

Appliances. Tallquist's hæmoglobinometer chart; fine Swedish, tightly woven filter paper, and a glover's needle.

Preparations. Take a large piece of light yellow-colored paper and cut an oblong hole in its centre, not quite as large as one of the colored stripes on the chart. Take a piece of the filter paper, at least twice the size of the colored stripes, and cut a straight edge on one side of the paper. Prepare the needle and ear as usual.

Technique. Obtain the blood in the usual manner. Take the prepared piece of filter paper and allow drop after drop of blood to be absorbed into the paper until it is covered with blood over an area as large as one of the colored stripes of the chart. Put on just enough blood to saturate the paper, no less and no more. If there is too little blood on the filter it will be white still on the under side. If there is too much blood on the paper it will have a glistening surface, and later will clot upon the paper. This must be prepared quickly and very evenly and then compared with the colored stripes of the chart at once. It will be noticed, when the blood is first put upon the filter paper and is still fresh, that it has a glistening appearance, but that it soon loses this and appears dull red for a few moments, and then it takes on a darker red appearance of clotted blood. The time to take the reading is while it has the fresh, dull-red color, just after the glistening surface has disappeared and before the dry, darker red color comes. This gives only a few moments in which to make the reading. Place the perforated paper on the colored chart and place the filter paper with the blood right next to the oblong perforation. The examiner must control his inclination to manufacture results. This is best accomplished by using the same method

as with the Fleischl instrument. Do not allow the numbers to show. Begin the comparison with a colored stripe much lighter than the blood specimen and move up one stripe at a time until the colors appear the same; then make a reading. Next begin with a stripe of a darker color than the blood and compare colors in the opposite direction until they appear the same, and then make a second reading. The correct result will be between these two readings, and usually the two readings will be 10 or more per cent. apart. The test is made by reflected daylight. It is well to have a good, bright light, although direct sunlight is not good.

Precautions. The amount of blood that the filter paper will absorb is quite constant, and yet the amount that can be put on to make the paper red is variable. If you take long enough and the blood does not clot quickly, a very small amount of blood will saturate the paper. It should be saturated quickly before the glistening effect is gone, and then the amount is quite constant. This is one of the greatest errors to be avoided, and necessitates strict and accurate attention to details. The error made with reading is the same as with the other color tests, except as the specimen changes color. A spot of blood 1 cm. in diameter is not large enough to compare with the large red stripe of the chart. Colored stripes of paper of equally large size can be more accurately compared than when one is very small; the eye is overpowered by the larger amount of color. Many shades of color before the eye at one time are confusing; so it is important that all colored stripes should be covered except the one being used. The above common errors are partly responsible for the disrepute in which the method is held in the minds of some observers. The errors are of such a nature that the accuracy of the test depends almost entirely upon the operator. The technique is easily and quickly performed, but the beginner should repeat the test until he can obtain the same result a number of times with the same blood.

The filter paper containing blood is wet and will destroy the colored stripes if it touches them.

E. Estimation of Percentage of Hæmoglobin of the Blood by Finding the Specific Gravity.

The specific gravity of the blood can be obtained direct from a quantity of blood as with other solutions. This is not necessary, because when a drop of any fluid is put in another fluid of the same specific gravity that the drop does not mix with, it will go to the center of the latter fluid and remain there. If it is lighter it will come nearer the surface, and if it is heavier it will sink. There are a number of solutions that might be used. One of the most accurate is sodium sulphate in solution, placed in different cylinders in different strengths.

The specific gravity of the blood, except in some cases, as in

leukæmia and dropsy, varies as the amount of iron in the corpuscles. It must therefore be evident that the specific gravity of the blood varies as the percentage of hæmoglobin varies. By consulting the table of Hammerschlag, given below, the percentage of hæmoglobin can be read for the specific gravity of the blood at once.

The most practical solutions to use for finding the specific gravity of the blood are benzole and chloroform, because of the ease and speed with which they may be used.

TABLE OF HAMMERSCHLAG.

Specific gravity.		Hæmoglobin.	Specific gravity.		Hæmoglobin.
1.033-1.035	=	25-30 per cent.	1.048-1.050	=	55-65 per cent.
1.035-1.038	=	30-35 " "	1.050-1.053	=	65-70 " "
1.038-1.040	=	35-40 " "	1.053-1.055	=	70-75 " "
1.040-1.045	=	40-45 " "	1.055-1.057	=	75-85 " "
1.045-1.048	=	45-55 " "	1.057-1.060	=	85-90 " "

Appliances. Specific gravity bulb or hydrometer; a quadrilateral or cylindrical graduated glass tube about six inches high; a pipette or pointed glass rod; a stirring rod, and a glover's needle.

Reagents. Those for cleaning capillary pipette, graduated tube, and needle, also benzole and chloroform.

The hydrometer is a glass tube containing mercury and air, and graduated so that when placed in distilled water at room temperature it reads 1.000.

Preparation. Clean all the apparatus as usual, and make a mixture of benzole and chloroform in the glass tube of a specific gravity of about 1.060.

Technique. Secure the blood in the usual way. Suck at least three good-sized drops of blood into the pipette. Now before the blood clots insert the point of the pipette into the solution and blow out one or two drops of blood, but no air. If the drop of blood goes to the center of the mixture and remains there after the mixture is well stirred, then the specific gravity of the blood is the same as that of the mixture. If the drop comes to the top it is lighter than the mixture, and benzole must be added and stirred in. If the drop goes toward the bottom it is heavier than the mixture, and chloroform must be added. Add just a few drops of benzole or chloroform at a time and stir well and test before adding more. The quickness with which the test is performed depends upon the carefulness in adding the benzole or chloroform and in keeping the mixture stirred. Repeat the test until the same result is easily and quickly obtained.

Precautions. The blood will stick to whatever it comes in contact with, the sides of the graduated tube, the stirring rod, or the specific gravity bulb, if they are not clean and dry. There is danger, when the pipette is used to obtain the blood, of blowing small air-bubbles into the drop as it is put into the solution, which will cause it to float. If the mixture is lighter than the blood, the drop will go

straight to the bottom and adhere with the force of the fall. The difficulty with the pipette can be overcome easily by using a pointed glass rod. Secure the drop of blood on the point of the rod and shake it off into the solution. The benzole and chloroform evaporate very rapidly and change the specific gravity of the mixture. The two liquids do not stay mixed, but need stirring frequently. Do not attempt to work with the same drop of blood more than two minutes; take a fresh drop and continue. Make the specific gravity of the mixture as near that of the blood as possible before adding the second drop. One or two drops will always determine approximately what the specific gravity of the blood is; then take a third drop and prove it exactly. The solutions of benzole and chloroform can be put into a glass-stoppered bottle and used again; so there is little waste except from evaporation. This is one of the best tests for obtaining the percentage of hæmoglobin, as the personal equation is largely eliminated and the burden of accuracy is placed upon the instrument.

Questions. 1. Why make the mixture 1.060 to begin with?

2. What is the specific gravity of benzole? Of chloroform?

3. Why are they better than other solutions for a quick test?

III. EXAMINATION OF FRESH BLOOD.

A. Coagulation of Normal Blood.

The coagulation of normal blood is a phenomenon that takes place quite constantly in from three to five minutes. But in disease this time may be prolonged indefinitely. The coagulation may be approximately tested by taking a large drop of blood on a warmed slide, and, while holding it in the hand, draw through the drop a needle or a straw from an ordinary broom every quarter or half-minute, and note when a clot follows the straw out of the drop. Wright's instrument for testing coagulation is slightly more accurate.

Questions. 1. What is coagulation?

2. What becomes of the corpuscles?

3. Is there any variation in the time of coagulation among the individuals in your section?

B. Microscopic Examination of Blood.

The microscopic examination of fresh and stained blood is of great clinical importance. In quite a number of diseases it gives a specific diagnosis which could not otherwise be gained.

Appliances. Microscope, with one-eighth or one-twelfth oil-immersion objective; eye-piece micrometer; white ground-glass slides, seven-eighths inch square; No. 1 cover-glasses; glover's needle, and alcohol lamp or Bunsen flame.

The micrometer is a small piece of glass on which there is a scale marking off equal spaces. This is placed on the diaphragm of the eye-piece of the microscope and put in focus by pushing it up or down as needed. The scale is then compared with a stage micrometer marked in microns and the value of the eye-piece scale thus determined.

Preparation. Wash the cover-glasses and slides with soap and water and then thoroughly rinse in clean, warm water. Polish the glasses with a clean, soft towel. When handling the slides or cover-glasses hold them always by their edges, and never touch a flat surface. Success in preparing fresh-blood specimens depends largely upon the absolute cleanliness of the glasses used. Before using the glasses pass them through the Bunsen or alcohol flame six or eight times while holding them with the fingers, then they will not be broken. Put the glasses down in a clean, safe place, with the heated side up, as this is the side to be used.

Technique. Obtain the blood as before, using the second or third drop. Bring one of the previously heated cover-glasses underneath the drop of blood and allow it to just touch lightly the center of the glass; then quickly place the cover-glass, blood side down, upon a glass slide. If the glasses are clean, the blood fresh enough, and of the right amount, the blood will spread out into a thin layer, in which the corpuscles lie on the flat surface in a single layer. Around the margin the cells will be more or less grouped together. The specimen should then be placed under the microscope and studied at once. Mix a small drop of blood with a small drop of water on a slide and cover with a cover-glass and examine. Smear a drop of blood on a slide by drawing another slide over it, cover one with a glass, and make another and leave uncovered, and examine.

Precautions. It is very important that the slides and cover-glasses should be kept perfectly clean and dry. If alcohol is used an alcoholic residue is left upon the glass and often interferes with the examination. Touching a glass surface with a freshly cleaned finger will leave enough fat and dirt to prevent the blood spreading. The blood must be transferred to the glasses and covered quickly, or it will partially clot and prevent spreading. The drop must be large enough to give a thin clean field of at least one-half inch in diameter, but it must not be so large that the blood cannot spread out into a thin film between the glasses. The spreading must take place entirely by capillary attraction; pressure must never be used to continue or cause spreading. The glass must touch only the tip of the drop while obtaining the blood; if it touches the ear the blood will not spread.

Questions. Red Cell. 1. Describe a red cell.

2. What is the shape of a red cell?

3. How may the shape of a red cell be demonstrated?

4. Are the red cells nucleated?

5. What are the dimensions of the red cells?
6. Are there any variations in the size of the red cells?
7. What are the maximum and minimum dimensions?
8. What percentage are large, normal, or small?
9. How may the elasticity of the red cells be demonstrated?
10. What are the causes of the movements of the red cells?
11. How are the red cells arranged?
12. What causes crenation?
13. What causes vacuolation?
14. How large are blood platelets?
15. What happens to the red cells in the presence of water?
16. What happens to the red cells while drying in the air?
17. What happens to the red cells when spread by pressure?
18. Of what does a red cell consist?

White Cell. 1. Describe a white cell.
2. How can the shape of a white cell be demonstrated?
3. How can you demonstrate the elasticity of a white cell?
4. How can you demonstrate the nucleus of a white cell?
5. What are the dimensions of a white cell?
6. Are there any variations in the size of a white cell?
7. What are the percentages of the various sizes?
8. Do they float readily under the cover-glass?
9. What becomes of the white cell in the presence of water?
10. What becomes of the white cell while drying in the air?
11. Of what does a white cell consist?

C. Spreading Blood for Staining.

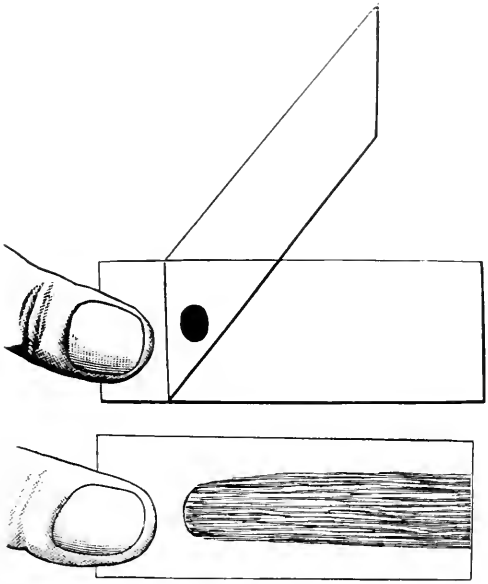
Glass Slide Method. Take one of the carefully prepared glass slides and allow the drop of blood to touch it near one end, as shown in Fig. 71. Place this on a table and hold the glass by placing a finger on the slide beyond the drop of blood, as shown in figure. Then take a ground-glass slide, hold by the edge between the thumb and fingers of the other hand, and place the edge of one end between the drop and the finger holding the slide, as shown in figure. Now, with a free-arm movement from the shoulder, quickly sweep the second slide across the remainder of the surface of the first slide, exerting a very slight but even pressure; the resulting smear will be as shown in lower slide of figure.

The speed can be made slowly by exerting more pressure, but it will not give a thin, even film of blood, and it will distort the corpuscles more. Make a number of smears for further use.

Cover-glass Method. Take a cover-glass between the thumb and first finger of each hand, with the heated surface up in the left and the heated surface down in the right hand, as shown in Fig. 72. Allow the center of the glass in the left hand to just touch the fresh

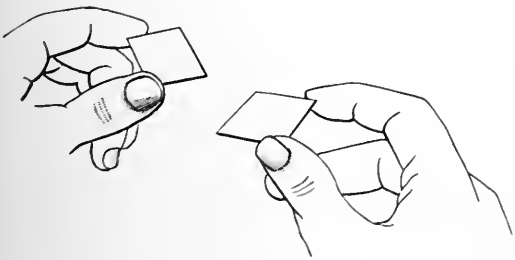
drop of blood, as shown in Fig. 73. Next, quickly drop the cover-glass in the right hand on the drop of blood, placing the glasses

FIG. 71



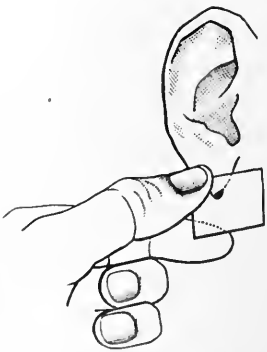
Showing method of spreading for examination of fresh blood.

FIG. 72



Showing the way to hold the cover-glasses.

FIG. 73



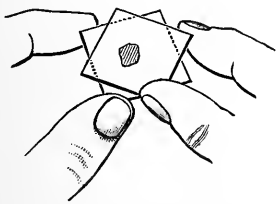
Touching the cover-glass to the blood drop.

together, so that each corner will be free, as shown in Fig. 74. This is easily done by bringing the thumbs closely together and holding the cover-glass in just the proper position before letting it drop.

The blood will then spread between the surfaces of the glasses by capillary attraction. As soon as the blood has entirely stopped spreading, take firm hold of the upper glass by the projecting corner with the thumb and first finger of the right hand, on the flat surface this time, as shown in Fig. 75. Now, with a quick, full swing of the whole arm, pull the upper glass away from the lower one as quickly as possible. Always pull the glass away in the same plane it was in, and do not tilt one glass upon the other, otherwise the spread will show ridges and will be of no use. Make a number of specimens for further study.

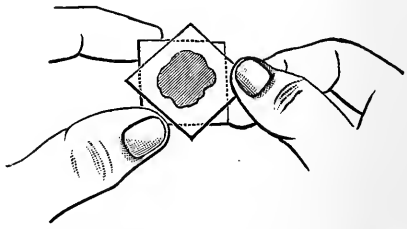
Fixing Blood Films. After the blood is spread the glasses are to be placed under a bell jar, with blood side upward, and allowed to air-dry until perfectly dry. The length of time for drying depends upon the thickness of the film; usually five to fifteen minutes are sufficient. The method of fixing blood spreads may be divided into two classes: chemical and thermal.

FIG. 74



Dropping cover-glass upon the drop of blood.

FIG. 75



Showing manner of holding the cover-glass to jerk them apart.

The principal chemicals are absolute alcohol and ether, absolute alcohol, ether, 95 per cent. alcohol, and 1 per cent. formaldehyde. The heating or baking method may be done crudely by passing the spread back and forth through the Bunsen flame for five minutes, while being held with the fingers. There are also copper ovens, copper plates, and copper receptacles, containing water, used to bake the blood. For staining with the ordinary histological or bacteriological stains the chemical method of fixing is sufficient, but when some of the finer differential staining is desired, as with the Ehrlich triacid stain, the baking method is necessary, and a copper oven controlled by a thermostat is the best. Fifteen to thirty minutes is the time required for baking, with the temperature about 100° C. The time depends upon the thickness of the film. Some observers employ higher temperatures with success. With the film on the slide, the blood-fixing jar is convenient for the chemical method. It is a quadrilateral jar, wide enough to allow four or five slides to stand in the jar, with a ridge of glass between

each slide. After removing them from the fixing solution, the specimens must be air-dried under a bell jar until all the solution is completely evaporated.

D. Staining Blood Films.

There are many stains that may be used to stain the blood corpuscles. The choice of stain depends largely upon the purpose of the staining. For ordinary histological purposes eosin and hæmatoxylin are the best.

Technique (1). Fix the blood film in alcohol fifteen minutes, dry, and stain with 1 per cent. aqueous eosin in 50 per cent. alcohol for two minutes, and then counterstain with Delafield's hæmatoxylin for a half-minute. Wash in water, allow to air-dry, and then mount in balsam. But for finer work, when parasites are suspected, as the plasmodium malariæ, or when a differential count of the white cells is desired, eosin and methylene blue are necessary.

Technique (2). Just the same as the above, except that a 10 per cent. methylene blue in 50 per cent. alcohol is used instead of the hæmatoxylin.

Technique (3). The other stain mentioned, Ehrlich's triacid, contains both acid and basic stains, and stains all structures, differentiating each. In special diseases of the blood this is the best stain, for among its other advantages it differentiates the nucleus of a red cell from that of a white cell.

All blood films must be fixed carefully by heat, preferably in the oven. Allow the stain to remain on the glass for four or five minutes; then wash, air-dry, and mount in balsam as usual. The stain must be of the best quality and accurately prepared. Then the staining depends largely upon the baking. An underheated or overheated specimen will not stain well; the first will appear too red from the acid fuchsin, and the latter will be a pale-lemon color from the orange G. stain.

E. Differential Counting of the Cells.

Appliances. Blood films stained with eosin and methylene blue or triacid stain; microscope, with one-eighth or one-twelfth oil-immersion objective and a mechanical stage.

Technique. The stained blood film should have a space at least one-half inch square in its center in which the corpuscles do not overlap each other. Place the film in focus with the microscope and begin at the upper left-hand corner of the specimen. Count all the various kinds of white cells, and keep a record of the number of each kind counted. Next count all the red cells in the field, and keep a record of the number and also of any peculiar forms and their

number. By the scale on the mechanical stage, measure the width of the microscopic field and then move the specimen to the next field to the right. Count and keep a record of all the various corpuscles in this field. Continue counting each field to the right until across the specimen; then drop down to the next row of microscopic fields and count to the left, and so on until the whole specimen has been counted and a record of the various corpuscles on the specimen has been obtained. Usually there are not enough white cells on one specimen to take an average from; in that case continue counting specimens until at least 100 white cells in all have been counted. They normally occur in the percentages as given in the table below. If there is difficulty in keeping the microscopic field because it is round, take a piece of stiff paper and cut a square hole in it just the size to make the field square, and place the paper on the diaphragm in the eye-piece of the microscope.

Corpuscles of Normal Blood.

1. **Red,** 5,000,000 per cubic millimetre. A biconcave disk 7.7 microns in diameter.
2. **White**, 8000 per cubic millimetre.

Classification of Leukocytes.

I. **Small Mononuclear.** Irregularly spherical, 8 to 10 microns in diameter; 20 to 30 per cent. of white cells. The nucleus nearly fills the cell and may or may not stain deeply blue according to technique, though it usually stains well. The protoplasm forms a thin rim around the nucleus, stained faintly blue.

II. **Large Mononuclear.** Irregularly spherical, 12 to 13 microns in diameter, 4 to 8 per cent. of white cells. The nucleus, about half the size of the cell, lies eccentrically, takes the blue stain lightly, and is surrounded by protoplasm very faintly blue, with the layer next the nucleus being almost unstained.

Transitional Forms. Same as above, except that the nucleus is indented or horseshoe-shaped.

III. **Polynuclear.** (*a*) **Neutrophile.** Irregularly spherical, 12 to 14 microns in diameter; 60 to 70 per cent. of white cells. Two or more nuclei in an irregular group. Nuclei are stained clearly. Protoplasm is partially filled with fine granules that stain red or pink, with a bluish or pinkish background.

(*b*) **Eosinophile.** Irregularly spherical, 12 to 14 microns in diameter; $\frac{1}{2}$ to 4 per cent. of white cells. Two or more nuclei, faintly stained. Protoplasm is filled with coarse granules stained a bright red with pinkish or unstained background.

Fig. I

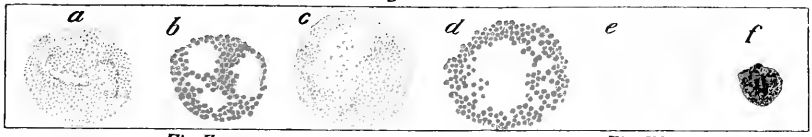


Fig II.

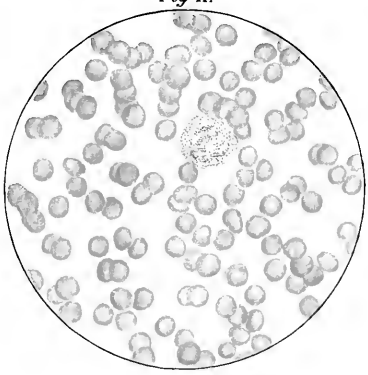


Fig III

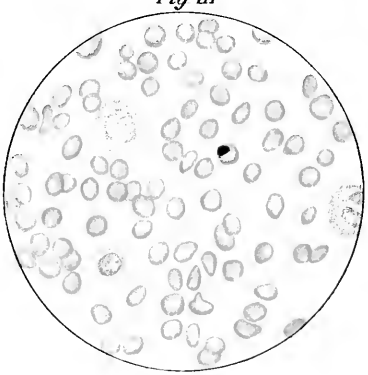


Fig IV.

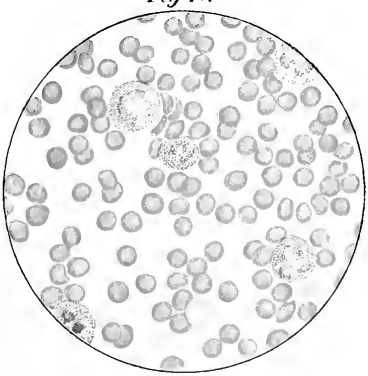


Fig. V.

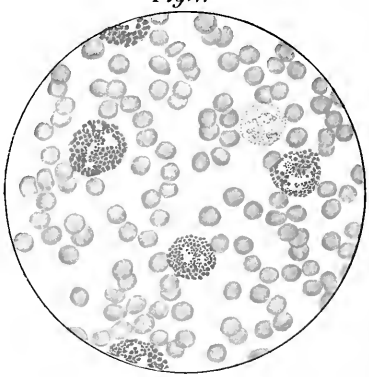


Fig. VI.

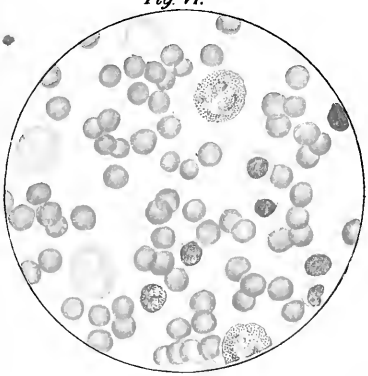


Fig. VII.

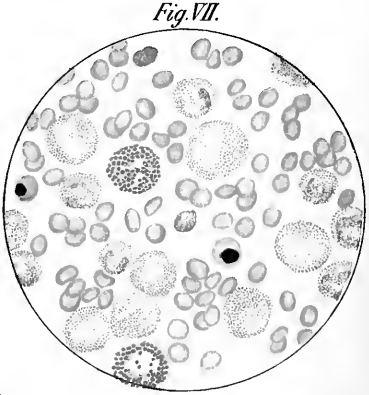
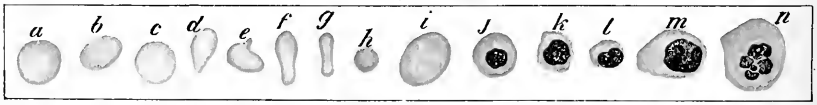


Fig. VIII.



DRAWN BY J. N. Z. CHASE

BLOOD.

(Ehrlich triple stain.)

(Prepared by Dr. I. P. Lyon.)

Fig. I. TYPES OF LEUCOCYTES.

a. Polymorphonuclear Neutrophile. *b.* Polymorphonuclear Eosinophile. *c.* Myelocyte (Neutrophilic). *d.* Eosinophilic Myelocyte. *e.* Large Lymphocyte (large Mononuclear). *f.* Small Lymphocyte (small Mononuclear).

Fig. II. NORMAL BLOOD.

Field contains one neutrophile. Reds are normal.

Fig. III. ANÆMIA, POST-OPERATIVE (secondary).

The reds are fewer than normal, and are deficient in hæmoglobin and somewhat irregular in form. One normoblast is seen in the field, and two neutrophiles and one small lymphocyte, showing a marked post-hæmorrhagic anæmia, with leucocytosis.

Fig. IV. LEUCOCYTOSIS, INFLAMMATORY.

The reds are normal. A marked leucocytosis is shown, with five neutrophiles and one small lymphocyte. This illustration may also serve the purpose of showing the leucocytosis of malignant tumor.

Fig. V. TRICHINOSIS.

A marked leucocytosis is shown, consisting of an eosinophilia.

Fig. VI. LYMPHATIC LEUKÆMIA.

Slight anæmia. A large relative and absolute increase of the lymphocytes chiefly the small lymphocytes) is shown.

Fig. VII. SPLENO-MYELOGENOUS LEUKÆMIA.

The reds show a secondary anæmia. Two normoblasts are shown. The leucocytosis is massive. Twenty leucocytes are shown, consisting of nine neutrophiles, seven myelocytes, two small lymphocytes, one eosinophile (polymorphonuclear) and one eosinophilic myelocyte. Note the polymorphous condition of the leucocytes, *i.e.*, their variations from the typical in size and form.

Fig. VIII. VARIETIES OF RED CORPUSCLES.

a. Normal Red Corpuscle (normocyte). *b, c.* Anæmic Red Corpuscles. *d-g.* Poikilocytes. *h.* Microcyte. *i.* Megalocyte. *j-n.* Nucleated Red Corpuscles. *j, k.* Normoblasts. *l.* Microblast. *m, n.* Megaloblasts.

IV. STAINING BONE-MARROW.

Appliances. A strong vice; saw; microscope, with one-fifth to one-eighth-inch objective; fresh bone containing red marrow; slides, cover-glasses, with usual equipment necessary for fixing and staining films.

Technique. Place the bone in the vice and fasten it just sufficiently to hold it for the saw. Saw off an end and then close the vice on the bone and crush it enough to make the marrow leave the bone. As soon as the drop of bone-marrow forms, touch a clean glass slide to it and make a spread by the slide method. Make two or three spreads so as to be sure to have a good one. Then fix and stain as with the blood films, using either eosin and methylene or the triacid stain.

Precautions. The bone should be as fresh as possible. The piece should be sawed just before using so as to have a freshly cut surface from which to take the marrow. The spread is apt to be too thick. Use just as much care in cleaning the slides and making the spreads as when making the blood films.

Questions. 1. Name and describe the different cells found in the red bone-marrow.

2. Are there cells found here that are not found in the normal blood?

3. What is the difference between a myelocyte and a leukocyte of the same size?

Corpuscles of Red Bone-marrow. **Red Cells.** *Normocyte,* non-nucleated, 6 to 8 microns in diameter; *normoblast,* nucleated, 6 to 8 microns in diameter; *microcyte,* non-nucleated, 4 to 6 microns in diameter; *microblast,* nucleated, 4 to 6 microns in diameter; *megalocyte,* non-nucleated, 8 to 10 microns in diameter; *megaloblast,* nucleated, 8 to 10 microns in diameter; *poikilocyte,* non-nucleated, distorted.

White Cells. Same as those in normal blood.

Myelocytes. (*a*) *Neutrophile.* Irregularly spherical, 10 to 20 microns in diameter. It has a single or partially divided nucleus, nearly filling the cell, and stained a pale blue. The protoplasm is filled with neutrophile (fine) granules stained a bluish red.

(*b*) *Eosinophile.* Same as above, except the nucleus is less distinct and the protoplasm is filled with coarse granules stained a bright red.

CHAPTER VI.

DIGESTION AND ABSORPTION.

DIGESTION.

As stated in the Introduction, it is taken for granted that by the time a medical school has found the conditions propitious for the establishment of a laboratory of experimental physiology, the whole province of chemical physiology will have been occupied by the department of chemistry as a legitimate growth of that department.

The American laboratory of experimental physiology will present almost exclusively the physical problems of physiology. But even where such are the conditions, it may seem advisable to introduce into a course of lectures or recitations on the physiology of digestion a series of demonstrations.

The following exercises in the chemistry of digestion and the physics of absorption may be given either as demonstrations or as laboratory exercises.

This chapter is not intended as a substitute for any of the excellent treatises now used in medical schools, but rather as a supplement to them.

It will be taken for granted that the student has had at least one year of chemistry before he enters upon this course.

To give the course which is outlined, one will need the following apparatus and reagents:

1. **Appliances.** (*a*) **Glassware Utensils, etc.** 10 evaporating dishes, assorted sizes; 10 filters, assorted sizes, 5 cm. to 20 cm.; 100 test-tubes, 15 cm.; 10 beakers, 30 c.c.; 10 beakers, assorted, 50 c.c. to 2 l.; 10 50-c.c. graduated cylinders; 4 graduated cylinders, 100 c.c., 200 c.c., 500 c.c., 1000 c.c.; 3 Wedgewood mortars, $2\frac{3}{4}$, 4, and 7 inches in diameter; filter papers; labels; pig-bladders; thread; rubber tubing, and glass stirring rods.

(*b*) **Apparatus.** Bunsen burners, with rubber tubing; filter stand; 2 supports, with rings and gauze; dialyzers; 1 incubator; drying oven; meat hasher; desiccator; water-baths; platinum dishes, 15 c.c. to 100 c.c.

2. **Reagents.** Diluted iodine, Fehling's solution, sodium hydrate and potassium hydrate, copper sulphate, distilled water, 6 thistle tubes, neutral litmus, concentrated nitric acid, strong ammonia, acetic acid, osmic acid (1 per cent.), pure standard pepsin, muriatic acid (c. p., sp. gr. 1.16=31.9 per cent. abs. HCl), absolute alcohol,

ether, chloroform, calcium chloride, 25 per cent. solution NaOH, 25 per cent. solution KOH, and one-half saturated solution Na_2CO_3. Non-medicated absorbent cotton for rapid filtering of mucilaginous or albuminous liquids.

I. THE CARBOHYDRATES.

1. **Materials.** Potato starch, dextrin, dextrose, maltose, lactose, saccharose, and cellulose, represented by absorbent cotton and ashless filter paper.

2. **Preparation.** (1) **To Prepare Fehling's Solution.** (*a*) Into a half-litre, glass-stoppered bottle put 34.64 grams $CuSO_4$, c. p., and enough H_2O dist. to make 500 c.c. Label the solution: *Fehling's Solution* (*a*).

(*b*) Into a similar receptacle put 173 grams of potassic-sodic tartrate, $KNaC_4H_4O+4H_2O$ (Rochelle salt), and 50 grams of NaHO, weighed in sticks; add enough water to make 500 c.c. Label: *Fehling's Solution* (*b*). For use, mix these solutions in equal parts. A convenient quantity for the following experiments is 100 c.c. of each solution.

(2) Prepare a starch paste by rubbing 1 gram of starch to a creamy consistence with water, add 100 c.c. of distilled water, and boil.

(3) Prepare a dilute solution of iodine by direct solution in water or by diluting an alcoholic solution.

3. **Experiments and Observations.** (1) Put a little dry starch into an evaporating dish; add some dilute iodine. The starch turns blue. Pour a few drops of starch paste into a test-tube; add a few drops of iodine. Iodine may be used to detect the presence of raw or of cooked starch.

(2) Put some raw starch into a test-tube or beaker; add water and stir. The starch does not seem to be at all soluble in water. Stir or shake the mixture to bring the starch into suspension in the water; pour upon a filter. A clear filtrate passes readily through. Test the filtrate for starch; result, negative; pour a few drops of iodine upon the filter, starch present. Conclusions:

(*a*) Potato starch is insoluble in cold water.

(*b*) The granules of potato starch will not pass through common filter paper.

(3) Dilute a few centimetres of starch paste; pour it upon a filter; to the filtrate add iodine. The blue color indicates that in the cooking of starch the grains are broken up into particles sufficiently small to pass readily through the meshes of common filter paper.

(4) In order to determine whether dilute starch paste will, in response to the laws of osmosis, pass through an animal membrane, fill a dialyzer with dilute starch paste. Set aside to be tested one or two days later.

(5) Put a bit of absorbent cotton into a beaker or test-tube; add water; boil; add iodine. Cellulose as represented by cotton fibres does not respond to the iodine test.

(6) Put a few bits of ash-free filter paper into a test-tube; add water; boil; add iodine. Cellulose as represented by the fibres of ash-free filter paper is insoluble in water, and responds to the iodine test. One must remember in this connection that in the preparation of ash-free filter paper mineral acids are used to dissolve out the salts; and mineral acids, especially sulphuric acid, so modify cellulose that it responds to the iodine test with a blue color.

(7) Add water to dextrine in a beaker; stir with a rod. Dextrine is readily soluble in cold water. To a small portion add iodine. The solution will probably assume a wine color; the typical reaction of erythrodextrine.

(8) Fill a dialyzer with diluted dextrine solution and leave for subsequent examination.

(9) Add water to dextrose; it is readily soluble. Add iodine to a portion of the solution; result negative.

(10) **Fehling's Test for a Reducing Sugar.** To a few drops of the solution add several cubic centimetres of Fehling's solution and boil. A yellowish precipitate of cuprous oxide (CuO) appears. If the boiling is continued, the color changes to a brick-dust red.

(11) To a solution of maltose add Fehling's solution and boil; the copper solution is reduced and CuO is precipitated.

(12) To a solution of lactose add Fehling's solution and boil; reduction takes place.

(13) Subject a solution of saccharose to the Fehling test. No reduction occurs. Vary the test by boiling the solution with a few drops of dilute HCl before adding the Fehling solution. The acid splits the disaccharid cane-sugar into its monosaccharid components, one of which reduces the Fehling solution.

(14) **Trommer's Test for a Reducing Sugar.** To any liquid suspected of containing a reducing sugar, add a few drops of dilute $CuSO_4$ solution; to this mixture add an excess of NaOH (or KOH); boil; if the suspected liquid contain a reducing sugar the $CuSO_4$ will be reduced with precipitation of CuO. Subject all of the solutions of sugar in turn to the Trommer test. Note that the appearance is practically the same as with the Fehling test. Any differences are due only to a difference in the proportions of the two reagents. The Fehling test is the more satisfactory one.

(15) Fill a dialyzer with a dilute solution of dextrose for subsequent examination.

(16) Fill a dialyzer with a dilute solution of maltose or lactose for subsequent examination.

(17) Fill a dialyzer with a dilute solution of saccharose for subsequent examination.

QUESTIONS AND PROBLEMS.

- Carbohydrates
  - I. Monosaccharids . . . .
    - Dextrose.
    - Levulose.
    - Galactose.
  - II. Disaccharids . . . .
    - Saccharose.
    - Lactose.
    - Maltose
  - III. Polysaccharids
    - Dextrines
      - Erythrodextrine.
      - Achroödextrine.
    - Gums . Gum arabic.
    - Starches
      - Vegetable.
      - Animal: glycogen.
    - Cellulose.

(*a*) How may carbohydrates be classified? (Make three classes.)
(*b*) Which class has the lowest grade of hydration?
(*c*) How many of this class are soluble in cold water?
(*d*) How many are diffusible?
(*e*) Which class has the highest grade of hydration?
(*f*) Are all of those which belong to Classes I. and II. soluble in water?
(*g*) Which are diffusible?
(*h*) How many of the carbohydrates reduce $CuSO_4$ in the presence of an excess of NaOH or KOH?
(*i*) How many of the carbohydrates are diffusible?
(*j*) How may one determine whether or not cane-sugar passed through the animal membrane?

II. SALIVARY DIGESTION.

1. **Materials.** Bread; fibrin; pig-fat; olive oil; starch paste; cane-sugar.

2. **Preparation.** (*a*) Remove the parotid and submaxillary glands of several rabbits or rats, hash them, rinse quickly with water to remove blood, and cover with water. After a few hours (twelve to twenty-four) filter or strain off the opalescent, aqueous extract. It should contain an aqueous solution of ptyalin. Label: *Salivary Extract.*

(*b*) Chew a piece of rubber or paraffin. The flow of saliva is stimulated; catch the secretion in a beaker; dilute and filter. Label: *Salivary Secretion.*

(*c*) Fibrin for use in experiments on digestion may be procured in any quantity at a slaughter-house. Rid it of all red coloring matter and of accidental contamination by repeatedly soaking and washing in water. The white, elastic threads of fibrin may be kept

indefinitely in pure glycerin. For use one needs only to wash out the glycerin thoroughly.

3. **Experiments and Observations.** (1) Subject saliva (*a*) and (*b*) to the Fehling test. It will be found that neither the extract nor the secretion will reduce the $CuSO_4$.

(2) Subject starch paste to the same test. The result is negative.

(3) Mix equal volumes of starch paste and salivary extract in a beaker. Place the mixture in the incubator, which is kept at a temperature of 35° to 40° C. After ten or fifteen minutes subject the mixture to a test with Fehling's solution. If the conditions are normal a copious precipitate of CuO indicates that a change has been wrought in the mixture. The starch has been changed to a reducing sugar by the ptyalin of the salivary extract.

(4) Mix equal volumes of starch paste and salivary secretion in a beaker; place the mixture in the incubator for ten or fifteen minutes; test with Fehling's solution. The presence of a reducing sugar shows that the secretion of the human salivary glands has the power to change starch to sugar; to change an insoluble diffusible foodstuff to a soluble diffusible one.

(5) Put a few crumbs of bread in a test-tube; add dilute iodine. Starch is an important constituent of bread.

(6) Put a few crumbs of bread in a beaker; add salivary extract; place in the incubator twenty minutes. Disintegration of the pieces and a marked increase of the amount of reducing sugar indicates the digestive action of saliva upon bread.

(7) Put a bit of fibrin into salivary extract; place in the incubator. An hour or a day will show no apparent change in the fibrin. Had one used any other proteid the result would have been the same. We are justified in the conclusion that saliva contains no ferment capable of changing proteids.

(8) Put a bit of fat or a drop of oil into a few cubic centimetres of salivary extract, shake vigorously; place in incubator. After an hour or a day one can see no change in the fat or oil, and is justified in the conclusion that saliva contains no ferment which acts upon fats.

(9) To a small amount of *raw* starch add salivary extract; place the mixture in the incubator; shake frequently; after fifteen minutes test for reducing sugar. There will probably be a relatively small amount of reducing sugar. If one watches the progress of digestion for several hours he will be convinced that the cooking of starch very greatly facilitates its digestion by saliva.

(10) Boil a few cubic centimetres of saliva; add starch paste; place in the incubator for ten minutes; test for reducing sugar. What is the verdict?

(11) Test the salivary secretion with neutral litmus. Determine whether its faint alkaline reaction is essential to its action as a digestive fluid.

(*a*) To a portion of saliva add an equal volume of 0.3 per cent. hydrochloric acid and the same amount of starch paste. The mixture represents 0.1 per cent. hydrochloric acid. Place the mixture in the incubator for fifteen minutes; test with Fehling's solution. Verdict?

(*b*) Repeat the experiment, substituting for the hydrochloric acid lactic acid of the same strength; place in the incubator for fifteen minutes; test with Fehling's solution.

What is the conclusion?

(12) *To Determine the Course of Salivary Digestion.* Mix 50 c.c. of salivary extract with an equal amount of starch paste. Test a portion with iodine at once. Test another portion at once with Fehling's solution. Keep the beaker in a water-bath at blood temperature. Test a portion of the mixture every minute with iodine and another portion every minute with Fehling's solution.

(*a*) What is the first change noted in the digestion of the starch?

(*b*) How many steps may be made out with the means used and under the conditions existing in the experiment?

(*c*) In what order do the changes occur?

(13) Place some starch paste in a beaker which may be floated in ice-water; similarly float a beaker with saliva. After both liquids have been cooled down to near the temperature of the surrounding water, mix them in one of the beakers, keep the mixture at the low temperature while subjecting portions of it every two minutes to the tests suggested above.

(*a*) May the same changes be made out in this experiment as in the previous one?

(*b*) Are the changes in the same order?

(*c*) State any differences in salivary digestion at blood temperature and at low temperature (0° C.) used in this experiment.

(14) (*a*) Sum up the day's work in a series of conclusions.

(*b*) What is the chemical formula of starch? Of erythrodextrine? Of maltose? Of dextrose?

(*c*) Write a chemical reaction or a series of reactions which will be in harmony with the observations and show as nearly as possible the course of salivary digestion.

(*d*) What change has the ferment wrought in the starch molecule to render the resulting carbohydrate capable of diffusion through animal membrane?

III. THE PROTEIDS.

1. **Materials.** An egg; fibrin; gelatin; myosin; syntonin; acid albumin; commercial peptone (mixed albumoses, proteoses, and peptones); Grübler's pure peptone.

2. **Preparation.** (*a*) **To Prepare Myosin.** (1) Take one pound of lean meat, grind it in the meat hasher; soak and wash repeatedly until the tissue is nearly white and quite free from hæmoglobin.

(2) Put the washed muscle tissue into a flask with an equal bulk of a 20 per cent. solution of ammonium chloride; shake from time to time for twenty-four hours.

(3) Strain off the liquor and add it to twenty volumes of distilled water. Myosin is precipitated. Wash the precipitate, redissolve one-fourth of the precipitate in 10 per cent. NaCl and label: *Saline Solution of Myosin.*

(*b*) **To Prepare Syntonin.** To the remaining three-fourths of the washed myosin add several volumes of 0.1 per cent. hydrochloric acid. In a very short time the myosin will be dissolved and changed to *syntonin.*

(*c*) **To Prepare Dilute Egg Albumin.** Make an opening in end of the shell of an egg; drain off the white of the egg, catching it upon a coarse linen cloth—a towel serves the purpose well; press the albumin through the meshes of the linen into a beaker; add 400 or 500 c.c. of distilled water; transfer the mixture to a 1-litre cylinder, and shake vigorously; after a short time filter through pure absorbent cotton or strain through fine linen.

(*d*) **To Prepare Acid Albumin.** To 100 c.c. of dilute egg albumin add an equal quantity of 0.2 per cent. hydrochloric acid; place the mixture in the incubator for two or three hours. Though the change begins at once, it will probably not be complete before the time suggested. If one wishes to isolate the acid albumin from the mixture, he has only to carefully neutralize with sodic hydroxide, precipitating the acid albumin, and to wash the precipitate with distilled water. For the purposes for which it is to be used in the following demonstration, it may be left in the acid solution, which represents 0.1 per cent. HCl. Label: *Acid Albumin Solution in* 0.1 *per cent. HCl.*

(*e*) Make an aqueous solution of the commercial "peptone," and, though the peptone is present in small proportions, label it *Proteoses.*

(*f*) Make an aqueous solution of a few grams of Grübler's pure peptone and label: *Peptone.*

(*g*) Dissolve a few grams of gelatin in distilled water.

(*h*) **To Prepare Millon's Reagent.** 1. To 100 grams of pure mercury add an equal weight of concentrated nitric acid, c. p. The reaction proceeds at room temperature, though gentle heat may be applied to complete the solution of the mercury. 2. Cool the mixture; add two volumes of water; after twelve hours decant the supernatant liquid—*Millon's Reagent.*

3. **Experiments and Observations.** (1) **The Heat Test.** Pour into test-tubes a few cubic centimetres of each of the following proteid solutions and subject each in turn to a temperature of 63° C., and,

finally to a temperature of 100° C., by dipping the tubes into water-baths of the temperatures named:

(*a*) Dilute egg albumin.
(*b*) Saline solution of myosin.
(*c*) Syntonin in acid solution.
(*d*) Acid albumin in acid solution.
(*e*) Gelatin in aqueous solution.
(*f*) Proteoses.
(*g*) Peptone.

Record results in a table and formulate conclusions.

(2) **The Cold Nitric Acid Test.** Subject the same series of proteids to the cold nitric acid test by first pouring 1 c.c. or 2 c.c. of strong nitric acid into a test-tube; then, with pipette, carefully floating the proteid liquid upon it. In the case of the dilute egg albumin, a characteristic white ring forms between the acid and the albumin. Note in each case whether or not a typical ring is formed.

(*a*) Dilute egg albumin.
(*b*) Saline solution of myosin.
(*c*) Syntonin.
(*d*) Acid albumin.
(*e*) Gelatin.
(*f*) Proteoses.
(*g*) Peptone.

Tabulate results and formulate conclusions in a concise statement.

(3) **The Xanthoproteic Test.** Use the tubes and materials already prepared in the cold nitric acid test. Shake the tubes to mix the acid with the proteid. In some cases a coagulum will be formed, and this coagulum turns yellow on boiling if the tube is held in a Bunsen flame. After the coagulum has been boiled in the acid, cool under the hydrant or in a pail of ice-water and add strong ammonia to alkaline reaction. The light-yellow coagulum which forms in the case of the egg albumin turns to an orange color. This test is usually given as a universal proteid test. Tabulate results on the above suggested series (*a*) to (*g*), noting any variations of the reaction with different proteids. Note variations in the reaction with different strengths of solution of the same proteid.

(4) **Millon's Test.** A general test for proteids is to heat a proteid-containing liquid with half its volume of *Millon's reagent*. A precipitate appears, which is yellowish at first, but turns red under the influence of heat. Test each of the above list of proteids (*a*) to (*g*) with Millon's reagent. Record results.

(5) **The Biuret Test.** To a suspected liquid add an excess of sodic hydrate; shake well, and to the mixture add one or two drops of a very dilute solution of cupric sulphate. A violet color appears, which, on heating, becomes deeper in shade.

A most convenient reagent for this reaction is a mixture of the

solutions (*a*) and (*b*) of the Fehling solution, but in the proportion of nine parts of the sodic hydroxide solution (*b*) to one part of the cupric sulphate solution (*a*), and add an equal volume of the distilled water to the mixture.

Tabulate results on the proteid series (*a*) to (*g*).

(6) Subject each of the series of proteids (*a*) to (*g*) to each of the following reagents, tabulating results:

(I) Picric acid, saturated solution.
(II) Absolute alcohol.
(III) Mercuric chloride, saturated solution.
(IV) Tannic acid, saturated solution.
(V) Silver nitrate, 10 per cent. solution.
(VI) Ammonium sulphate, saturated solution.

On which of the proteid solutions would one get a precipitate with silver nitrate independent of the presence of proteid?

(7) **To Separate Peptone from Other Proteids.** It will have been noted that ammonium sulphate precipitates all proteids except pure peptone. If one has peptone mixed with proteoses and unchanged proteids, one may demonstrate its presence by precipitating out the other proteids and then demonstrating by such a test as the Biuret test the presence of a proteid in the clear filtrate; that could be nothing else than peptone.

Test commercial peptone in this way and determine whether any appreciable proportion of it is peptone.

(8) **The Diffusibility of Proteids.** Fill seven dialyzers with proteids above studied. On the following day test the diffusates for proteids.

IV. (a) DIFFUSIBILITY OF PROTEIDS. (b) MILK.

(a) Diffusibility of Proteids.

1. **Materials.** The seven dialyzers filled at the end of the previous demonstration.

2. **Experiments and Observations.** (1) What reagent may best be used to determine whether or not any of the egg albumin has diffused through the animal membrane?

(2) How may one determine whether or not any of the salts of the egg albumin have diffused through the membrane?

(3) In the case of the saline solution of myosin (*b*), of syntonin (*c*), and of acid albumin (*d*), is there any contraindication against silver nitrate as a reagent to determine whether proteid has diffused?

(4) What tests would be most reliable in these cases to detect the presence of proteid in the diffusate?

(5) Would a trace of proteid in the diffusate necessarily demonstrate the diffusibility of these proteids through the walls of the alimentary tract? If not, why not?

(6) What tests may be used to determine the presence of gelatin in the diffusate? Is gelatin diffusible?

(7) The term proteoses is a general one and is used to designate the mid-products of proteid digestion. The mid-product of albumin digestion is albumose; of globulin digestion, globulose; of myosin, myosinose; of vitellin, vitellinose; of casein, caseinose; or, in general, of a proteid, proteose.

Dialyzer (*f*) contains products of peptic digestion of proteids, principally albumin. The progress of digestion was suspended at a stage where there were present not only peptone, but mid-products—albumoses; or, to use the general term, proteoses.

The problem which confronts us is to *determine whether or not proteoses are diffusible.*

(*a*) If peptone is diffusible, the diffusate will certainly contain peptone. Do peptone and proteoses respond alike to all the general tests for proteoses?

(*b*) How may peptone be separated from the proteoses? What single reagent is indicated in the case?

(8) Demonstrate the diffusibility of peptone.

(b) Milk.

1. **Materials.** One litre of fresh whole milk; one litre of milk for the preparatory steps of the demonstration.

2. **Preparation.** (1) On the day before the demonstration fill a 500-c.c. open-mouthed cylinder with milk and put it in a cool place.

(2) Two days before the demonstration weigh out 10 grams to 50 grams of whole milk in a platinum dish or in a thin porcelain dish. Place it in a drying oven at 90° to 95° C., and dry to constant weight. Record the dry weight.

(3) Before the hour of demonstration burn the residue by bringing the dish which contains the dry solids to a red glow in a Bunsen flame, allowing ample access of oxygen. After the dish and the white ashes have cooled in a desiccator, take the weight. All of these weights should, of course, be taken upon an analytical balance.

(4) Fill a dialyzer with diluted milk one day before the demonstration.

3. **Experiments and Observations.** (1) What proportion of milk evaporates at the temperature above suggested? It may be taken for granted that this proportion represents practically the water of the milk.

(2) Of the solids of milk, what proportion is organic and what proportion is inorganic?

(3) What bases predominate in the ashes?

(4) What is the character of the organic constituents of milk?

(*a*) Note that the milk that has been standing has separated into

two layers, an upper yellowish layer and a lower bluish-white layer.

(*b*) Draw off with pipette a few cubic centimetres of the cream and in a test-tube add an equal volume of osmic acid. To a few drops of olive oil in another tube add osmic acid. Shake both tubes vigorously. Osmic acid has the same effect upon cream as upon olive oil. The cream is, in fact, fat in physiological emulsion. Quantitative examination shows that about 4 per cent. of milk, or $\frac{4}{13}$ of the solids of milk, consists of fats in which olein predominates.

(5) Fill a siphon with water and introduce it through the cream to the bottom of the 500-c.c. cylinder; draw off 300 c.c. of the milk; add to it four volumes of water; slowly add 1 per cent. acetic acid, while stirring with a rod, until the casein separates as a copious flocculent precipitate. After the casein has partially settled, decant off a few cubic centimetres of the supernatant liquid and subject it to the Fehling test. The abundant precipitate indicated the presence of a reducing sugar. It is milk-sugar—lactose.

(6) Wash the casein by the repeated addition of water, followed by decantation; pour it into a linen sack or a towel and press out the water; further extract the water with absolute alcohol; extract the remnant of fat with ether; dry in the air. The white granular material that remains is nearly pure *casein*, the most important proteid of milk, and represents nearly 4 per cent. of milk.

(7) Heat 100 c.c. of the fresh milk in a beaker. Before the boiling point is reached a membrane gathers upon the surface of the milk. This membrane represents the *lactalbumin* of the milk, which has been coagulated by the heat and has collected in the membranous coagulum at the surface. The lactalbumin represents only a small proportion of the milk proteid. Subject the membrane to the xanthoproteic test or the Millon test to demonstrate that it is a proteid.

(8) To 30 c.c. of fresh milk in a beaker add common salt to saturation. Record results.

(9) To 30 c.c. of fresh milk in a beaker add magnesium sulphate to saturation. Record results.

(10) Dilute fresh milk to one-fifth normal and subject it to the following tests, recording results:

(*a*) Trommer's test.

(*b*) The xanthoproteic test.

(*c*) The biuret test.

(*d*) The osmic acid test.

(11) Fill a dialyzer with the diluted milk. One day later examine the diffusate:

(*a*) For any of the inorganic constituents of milk.

(*b*) For the carbohydrate constituents of milk.

(*c*) For the proteid constituents of milk.

(*d*) For the fatty constituents of milk.

(12) Formulate in a series of concise statements the facts demonstrated regarding milk:

(*a*) Its chemical constituents.

(*b*) Its physical properties.

V. GASTRIC DIGESTION.

1. **Materials.** Two fresh pig-stomachs; ½ kilo clean sea-sand; four eggs; fibrin; bread; milk; jellied gelatin; casein, and rennin.

2. **Preparation.** (1) **To Prepare Artificial Gastric Juice.** (*a*) Stretch a fresh stomach of a pig upon a board, with mucous surface up; fasten in place with nails.

(*b*) Rinse off the mucous membrane gently with cold water.

(*c*) Scrape thoroughly with a dull-edged table knife or an equivalent; collect the scrapings in a large mortar.

(*d*) Grind the scrapings in clean, fine sand.

(*e*) Add an equal volume of 0.2 per cent. HCl and leave for twenty-four to forty-eight hours, stirring occasionally.

(*f*) Strain through linen; filter, and preserve in a glass-stoppered bottle. Label: *Acidulated Aqueous Extract of Pepsin.*

(*g*) For use dilute this extract with three or four volumes of 0.1 per cent. HCl. Label: *Artificial Gastric Juice* (1).

(2) **To Prepare a Glycerin Extract of Pepsin.** (*a*) Rinse off the mucous membrane of a fresh pig stomach with cold water and remove the mucous membrane from the muscular walls of the stomach.

(*b*) Grind the mucous membrane in the meat hasher.

(*c*) Put the hashed tissue into a beaker and cover with two volumes of pure glycerin. Stir the mixture occasionally for several days. The glycerin extracts the pepsin ferment.

(*d*) Strain the *glycerin extract* through fine linen; preserve in a glass-stoppered bottle for future use. It will keep indefinitely.

(*e*) For use add to one volume of the extract thirty to fifty volumes of 0.2 per cent. HCl. Label: *Artificial Gastric Juice* (2).

3. **Experiments and Observations.** (1) To a bit of starch paste of the consistency of jelly add artificial gastric juice (1); place in the incubator; in ten minutes or one day note results.

(2) To a few drops of olive oil or to a bit of pure tallow add several cubic centimetres of gastric juice and keep at incubator temperature for one day. What effect has gastric digestion upon fat or oil?

(3) To a bit of pig-fat add gastric juice and keep at incubator temperature for several hours. What effect has gastric digestion upon adipose tissue?

(4) To a bit of fibrin in a test-tube add gastric juice. The warmth of the hand will be sufficient. If the preparation of artificial gastric

juice has been successful the fibrin will dissolve in one or two minutes. One may be certain that digestion is progressing rapidly, though complete solution of the fibrin does not necessarily indicate complete digestion of it; for complete digestion of a proteid implies that the foodstuff is both *dissolved and diffusible.* The fibrin is dissolved; it may or may not be diffusible. But this will be determined later.

(5) **To Determine the Active Factors of Gastric Digestion.** (*a*) To a few shreds of fibrin in a test-tube add a few cubic centimetres of 0.2 per cent. HCl. Carefully note results. Will dilute HCl dissolve fibrin? Is it possible to digest a proteid without dissolving it?

(*b*) To fibrin add dilute neutral glycerin extract of pepsin. Is solution effected?

(*c*) To tube (*a*) add a few drops of the glycerin extract of pepsin.

(*d*) To tube (*b*) add two volumes of 0.2 per cent. HCl. Note results. Formulate conclusions.

(6) **To Determine whether the Acid Factor of Gastric Digestion Need Necessarily Be Hydrochloric Acid.** Prepare a 0.4 per cent. solution of each of the following acids:

(I) Lactic acid.
(II) Sulphuric acid.
(III) Nitric acid.
(IV) Phosphoric acid.
(V) Citric acid.
(VI) Acetic acid.
(VII) Malic acid.

For each acid prepare four test-tubes as follows:

(*a*) Fibrin+1 c.c. glyc. ext. of pepsin+10 c.c. 0.4 per cent. acid.
(*b*) Fibrin+1 c.c. pepsin ext.+10 c.c. 0.2 per cent. acid.
(*c*) Fibrin+1 c.c. pepsin ext.+10 c.c. 0.1 per cent. acid.
(*d*) Fibrin+1 c.c. pepsin ext.+10 c.c. 0.05 per cent.

Proceed in a similar manner with each acid.

Tabulate results. May other acid or acids take the place of HCl as a factor in digestion? If so, in what minimum strength? Which one of the above acids is normally present in the stomach? May any of the above acids serve as digestives and as foods?

As digestives and as tonics?

As digestives, foods, and tonics?

Cite authorities.

(7) **To Determine the Optimum Strength of the Hydrochloric Acid.** Prepare with care the following three dilutions of hydrochloric acid: 10 per cent., 1 per cent., 0.1 per cent.

Into twelve test-tubes put as many small masses of fibrin; into each tube put 1 c.c. of neutral 10 per cent. dilution of glycerin extract of pepsin. Label and fill the tubes as follows:

Tube (*a*) 5 per cent.: Add to the fibrin 5 c.c. of 10 per cent. HCl and of distilled water a quantity sufficient to make 10 c.c.

Tube (*b*) 2 per cent.: Add 2 c.c. of 10 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Tube (*c*) 1 per cent.: Add 1 c.c. of 10 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Tube (*d*) 0.5 per cent.: Add 5 c.c. of 1 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Tube (*e*) 0.4 per cent.: Add 4 c.c. of 1 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Tube (*f*) 0.3 per cent.: Add 3 c.c. of 1 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Tube (*g*) 0.2 per cent.: Add 2 c.c. of 1 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Tube (*h*) 0.1 per cent.: Add 1 c.c. of 1 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Tube (*j*) 0.05 per cent.: Add 5 c.c. of 0.1 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Tube (*k*) 0.025 per cent.: Add 2.5 c.c. of 0.1 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Tube (*l*) 0.01 per cent.: Add 1 c.c. of 0.1 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Tube (*m*) 0.005 per cent.: Add 1.2 c.c. of 0.1 per cent. HCl and aqua dest. q. s. ad 10 c.c.

Place these twelve tubes in the incubator and note conditions every ten minutes for the first hour, every hour for the first six hours, and then at the end of one or two days make the final observations.

Tabulate results. Formulate conclusions. What range of strength may, from the experiments with the artificial gastric juice under artificial conditions, be considered the optimum strength for the acid? Is there any reason to doubt that the optimum strength as determined above is essentially different from the optimum strength in normal digestion?

(8) **To Determine How Dilute the Pepsin May Be and Still Be Efficient in Digestion.** This experiment requires a standard solution of pepsin to use as a basis. The *U. S. Pharmacopœia* (p. 295 of the 7th Decennial Revision) gives the following formula for a standard solution of pepsin:

Hydrochloric acid (absolute), 0.21 grm.

Pepsin (pure), 0.00335 grm.

Water (distilled), q. s. ad 100 c.c.

The following suggestions are made as to method of preparation: To 294 c.c. of water add 6 c.c. of dilute hydrochloric acid—sol. A. In 100 c.c. of sol. A dissolve 0.067 grm. of standard pepsin—sol. B. To 295 c.c. of sol. A at 40° C. add 5 c.c. sol. B. The resulting mixture is a *standard artificial gastric juice* of the formula given above, and has the power of completely digesting at 38° to 40° C. one-fifth its weight of coagulated egg albumin in six hours.

From a standard gastric juice prepare the following dilutions, using 0.1 per cent. HCl as a diluent. It is scarcely necessary to say that the greatest care should be taken (1) to make all measurements with precision, and (2) to thoroughly shake each dilution before drawing off material for the next lower dilution.

(*a*) Standard artificial gastric juice 10 c.c.+1 c.c. moist fibrin.

(*b*) 1:10 standard artificial gastric juice 10 c.c.+1 c.c. moist fibrin.

(*c*) 1:100 standard artificial gastric juice 10 c.c.+1 c.c. moist fibrin.

(*d*) 1:1000 standard artificial gastric juice 10 c.c.+1 c.c. moist fibrin.

(*e*) 1:10,000 standard artificial gastric juice 10 c.c.+1 c.c. moist fibrin.

(*f*) 1:100,000 standard artificial gastric juice 10 c.c.+1 c.c. moist fibrin.

(*g*) 1:1,000,000 standard artificial gastric juice 10 c.c.+1 c.c. moist fibrin.

Keep tubes in incubator or water-bath at 38° to 40° C. Note (1) time required to dissolve fibrin completely; (2) time required to change all acid albumin to proteose or peptone. Will one-millionth standard gastric juice digest fibrin at all? Will a lower dilution (one-ten-millionth) digest it? If so, how dilute, and how long a time required?

VI. GASTRIC DIGESTION (Continued).

Experiments and Observations (*Continued*). (9) **To Determine the Influence of the Hydrochloric Acid of the Gastric Juice upon Putrefaction in the Stomach.** It has been determined that the hydrochloric acid in the stomach destroys, under favorable conditions, at least the non-pathogenic forms of bacteria. Let us determine the strength of acid necessary to destroy the common bacteria of putrefaction. To each tube used in experiment (7) add a minute drop of any putrefying fluid. If the contents of a tube serve as a good culture field, any drop of the fluid may be found to be swarming with bacteria within a few hours. Within a few hours after infecting the tubes examine under high power—700 to 1000 diameters—a drop of the contents of each tube. While making the observations take care not to contaminate one tube with the contents of another. That the tubes containing 5 per cent. or 2 per cent. or 1 per cent. hydrochloric acid will be found to be free from bacteria goes without saying. Just how weak may the acid be and destroy the bacteria? How weak may the acid be and retard their development? Could one readily drink enough liquid at a meal to change the stomach from a sterilizing field to a culture field for the bacteria of putrefaction?

(10) **The Influence of Division upon the Time Required to Digest Proteids.** Boil an egg five to ten minutes; cool quickly; separate hard, coagulated white from yolk and envelopes.

(*a*) Cut out one-centimetre cube and put it into a beaker with 40 c.c. artificial gastric juice.

(*b*) Put into a second beaker of 40 c.c. gastric juice a centimetre cube which has been divided into eight half-centimetre cubes.

(*c*) Prepare another beaker in which are sixteen quarter-centimetre cubes in 10 c.c. of artificial gastric juice.

(*d*) Into another beaker with 10 c.c. of artificial gastric juice put one-quarter of a cubic centimetre of the egg albumin which has been finely divided by pressing through a fine sieve.

Note time required in each case to completely digest the albumin.

Has this any hygienic bearing?

(11) **The Influence of Temperature upon the Time Required to Digest Proteids.** Prepare five tubes by first providing each with 5 c.c. of artificial gastric juice; treat the several tubes as follows:

(*a*) Bring to 40° C. in water-bath; add fibrin; note time.

(*b*) Bring to 30° C. in water-bath; add fibrin; note time.

(*c*) Bring to 20° C. in incubator; add fibrin; note time.

(*d*) Leave at room temperature (10° C.); note time.

(*e*) Bring to 0° C. in ice-water; add fibrin; note time.

What is the optimum temperature?

Is the progress of digestion materially retarded by a reduction of the temperature?

Would the temperature of the stomach contents be essentially lowered by the occasional sipping of an iced beverage during a meal?

What is the hygienic significance of the experiment?

VII. GASTRIC DIGESTION (Continued).

Experiments and Observations (*Continued*). (12) **The Steps of Gastric Digestion.** Boil an egg five to ten minutes; cool quickly; separate out the white; press it through a fine sieve; put into a beaker with 100 c.c. artificial gastric juice, and place the beaker in a water-bath at 40° C. At intervals of two minutes for the first ten minutes, then at intervals of five minutes for the next twenty minutes, then at intervals of ten minutes for the second half-hour, afterward at intervals of one hour, subject the liquid to tests for egg albumin, for acid albumin, for albumose, for peptone. In what order and after what length of time do the several products appear? Is the one that is first to appear also first to disappear?

(13) **The Artificial Digestion of Various Proteids.** (*a*) To a small mass of jellied gelatin add ten to fifteen volumes of artificial gastric juice and note effect.

(*b*) Subject bread to the xanthoproteic test. The presence of proteid material is demonstrated. Put a small piece of dry bread into a beaker with gastric juice and note effect.

(*c*) Note the course of casein digestion.

(*d*) Triturate in a mortar well-cooked lean meat; digest with gastric juice.

(*e*) Try the xanthoproteic test upon cooked beans or peas; proteid is present. Triturate in a mortar; digest.

(*f*) In each case demonstrate the ultimate appearance of peptone.

(14) **The Artificial Digestion of Milk.** Of fresh milk take three portions of 5 c.c. each.

(*a*) To one portion add ten volumes of artificial gastric juice and place it in the incubator at 38° to 40° C.

(*b*) Prepare another beaker in the same way, but place it in a water-bath at 38° to 40° C. and keep the mixture well stirred, dividing the casein coagulum as fine as possible.

(*c*) Place the third portion of milk in the water bath. When it has become warm add a few centimetres of *rennin*. Fifteen minutes later add artificial gastric juice. Stir as in (*b*). In which of the first two does digestion seem to progress the more rapidly? Does the progress or process of the digestion seem to be materially different in the last two experiments (*b*) and (*c*)? Have any of the observations made on milk digestion any hygienic significance?

(15) **The Diffusibility of the Products of the Artificial Digestion of Proteids.** From the products of digestion in experiments (14, *b*) digested milk, (13, *a*) digested gelatin, (13, *b*) digested bread, fill three dialyzers—first neutralizing the acid with sodic carbonate. After twelve to twenty-four hours, test the diffusate for peptone. Why neutralize the liquid before filling the dialyzer?

Have all of these indiffusible proteids been wholly or in part changed to diffusible peptones by the action of the artificial gastric juice?

VIII. THE PROPERTIES OF FATS.

1. **Materials.** Olive oil; cream; butter; beef-tallow; lard; adipose tissue, and cotton-seed oil.

2. **Experiments and Observations.** (1) **The Osmic Acid Test.** Place in test-tubes a small amount of each of the above foodstuffs; add to each a few cubic centimetres of osmic acid. A characteristic reaction takes place, the result of which is a deep-brown coloration of the fat. If the conditions are favorable the stain deepens into a sepia black. The cream and the adipose tissue have proteid admixtures; note the variation of the reaction.

(2) **The Solubility of Fats and Oils.** Prepare three tubes each of olive oil and of tallow; treat each material with absolute alcohol, with ether, and with chloroform. It will be found that all of these

reagents are solvents of fats and oils. The alcohol, however, dissolves very much more of the fat or oil when warm than when cold, as may be demonstrated by making the alcoholic solution with the tube immersed in boiling water; after the alcohol seems to have reached the limit of solution at that temperature immerse the tube in cold water. A large part of the dissolved oil instantly separates out, but will readily redissolve on again immersing the tube in the boiling water.

(3) **The Saponification of Fats and Oils.** (*a*) To about 2 c.c. of olive oil in a test-tube add one to two volumes of a 25 per cent. solution of sodic hydrate. Shake the mixture vigorously; it is evident that a chemical reaction is in progress. The fat is undergoing the process of *saponification*. A complete and typical saponification requires a more careful apportionment of the amount of oil and of alkali used and an application of heat.

(*b*) Repeat the experiment, substituting a 25 per cent. solution of potassic hydrate. The result is similar.

(*c*) What is the chemical formula of palmitin? Of stearin? Of olein?

(3) Write the reaction which takes place in saponification of palmitin; of olein. Note the ready solubility of the products of this reaction in water.

(4) To a solution of soap add any aqueous solution of a calcium salt soluble in water—*e. g.*, calcium chloride; a curdy, white precipitate separates out. Write the formula of the reaction.

May the reaction have any relation to hygiene or therapeutics?

(5) **The Emulsification of Oils.** Gould defines an emulsion as "water or other liquid in which oil in minute subdivision of its particles is suspended." One may add: more or less permanently suspended. For if one shake together vigorously 2 c.c. of oil with an equal amount of water in a test-tube he is able to bring about a minute subdivision and temporary suspension of the oil in the water. While the oil is in this temporary physical condition it has the white color typical of emulsions in general. In a few minutes, however, the particles as they rise to the top of the liquid coalesce into minute globules; then into larger and larger globules, and finally into a homogeneous, supernatant oil-layer.

(*a*) Add to the mixture above described 2 or 3 c.c. of strained egg albumin; shake vigorously. One observes the same minute subdivision of the particles, but they show no tendency to coalesce on standing; the suspension is *more or less permanent.*

Why do not the particles coalesce? In what respects is this emulsion unlike milk?

(*b*) To 2 c.c. of olive oil add 2 c.c. of sirupy solution of any gum —*e. g.*, gum acacia; shake the mixture thoroughly. An emulsion will be formed. What characteristics has this emulsion in common with emulsion (*a*)?

(*c*) To 5 c.c. of cotton-seed oil containing a little free fatty acid add ten drops of strong sodium carbonate solution and shake. A good stable emulsion is made in this way.

In what way is this emulsion different from those which precede? Which one of the emulsions given above is most like the emulsions formed in the small intestine?

(*d*) What materials present in the small intestine tend to promote emulsification of fats?

(6) **The Diffusibility of Fats or Their Derivatives or Modifications.** Fill five dialyzers as follows:

(*a*) Milk.

(*b*) Solution of soap.

(*c*) 10 per cent. glycerin.

(*d*) Emulsion (5, *a*).

(*e*) Emulsion (5, *c*).

Complete the observations on the following day, determining what derivations or modifications of fat or oil are diffusible. How may the presence of soap in the diffusate be determined?

IX. INTESTINAL DIGESTION.

1. **Materials.** Two pig pancreases; 200 c.c. of pig or ox bile.

2. **Preparation.** (1) **Aqueous Pancreatic Extract.** (*a*) Free a pig pancreas of fat.

(*b*) Grind it in a meat hasher.

(*c*) Extract with water kept at a temperature of 25° to 28° C.

(*d*) After two hours strain through linen and filter through absorbent cotton.

(2) **Glycerin Extract of the Pancreatic Ferments.** (*a*) After freeing the gland of fat grind it.

(*b*) Place it in two volumes of absolute alcohol for two days.

(*c*) Drain off the alcohol and transfer to two volumes of pure glycerin.

(*d*) After one week press out the glycerin, which has extracted the ferments.

This glycerin extract will keep indefinitely. To make *artificial pancreatic juice* proceed as follows:

(*e*) To one volume of the glycerin extract add five or six volumes of water and sufficient sodium carbonate solution to give the mixture a distinctly alkaline reaction.

(3) **Preliminary Experiments on Bile.** This secretion may easily be procured from the slaughter-house at almost any time of the year.

(*a*) To diluted bile add dilute acetic acid. The copious yellow precipitate is mucin.

(*b*) To diluted bile add absolute alcohol; mucin is precipitated;

filter. To one portion (I) of filtrate add HCl. The yellow precipitate is glycocholic acid.

"To the other portion (II) of the filtrate add lead acetate, which throws down lead glycocholate. Remove this by filtration, and to the filtrate add solution of basic lead acetate, which gives a further precipitation of lead taurocholate."—*Chemical Physiology*, Long, p. 119.

(*c*) **Gmelin's Test for Bile Pigments.** To a few cubic centimetres of strong nitric acid containing nitrous acid carefully add dilute bile. At the junction of the liquids a play of colors, green, blue, violet, red, and yellow, will be noted; the green being next to the bile and the yellow next to the acid. This delicate and most reliable test may be applied to any liquid suspected of containing bile.

(*d*) The reaction of bile is found to be distinctly alkaline.

3. **Experiments and Observations.** (*a*) **The Action of Pancreatic Juice upon Foods.** (1) To raw or cooked starch add in one beaker aqueous extract of pancreas (*a*); in another add artificial pancreatic juice (*b*); place the mixtures in the incubator; after a short time test for reducing sugar. *Pancreatic juice contains an amylolytic ferment.*

(2) Subject fibrin to the action of both of the pancreatic preparations. *Pancreatic juice contains a proteolytic ferment.*

(3) Boil fresh milk and mix it with an equal bulk of the aqueous extract of pancreas and put the mixture into the incubator. Put also into the incubator boiled milk diluted with an equal volume of distilled water. The milk which is mixed with the pancreatic juice will curdle much sooner than the other. *Pancreatic juice contains a milk-curdling ferment.*

(4) Mix 5 c.c. or 6 c.c. of neutral olive oil with an equal volume of aqueous extract of pancreas; shake the mixture vigorously. No emulsion is formed. Place one-half of the mixture in the incubator. After a few hours any undigested oil may be emulsionized on shaking, or fresh oil may be emulsified. Explain.

(5) To the second part of the mixture add 3 c.c. bile; shake the mixture vigorously. A good emulsion is formed. How is this emulsion formed? What factor of an emulsion does the bile add? What is the relation of experiment (5) to experiment (4)? *Pancreatic juice contains a fat-splitting ferment whose action liberates fatty acids.*

(*b*) **The Action of Bile upon Foods.** (6) To starch paste add several volumes of dilute bile. Result?

(7) To fibrin add dilute bile. Result?

(8) To oil which contains free fatty acid add bile; shake the mixture vigorously. Result?

(9) To neutral oil add bile; shake the mixture vigorously. What is the result? Allow the mixture to stand in the incubator. After several hours shake the mixture. Is an emulsion formed?

(10) Summarize the results of the foregoing experiments, formulating a series of conclusions regarding the action of pancreatic juice; the action of bile and their combined action on each class of food.

ABSORPTION.

Physiologists have entertained the hope that all the phenomena of absorption of diffusible substances could be eventually explained by the laws of physics. That hope has practically given place to the conviction that however important it may be to the animal economy to produce, in its digestive processes, diffusible products, these products do not pass through the epithelial lining of the alimentary tract at the rate or in the proportions that would be observed in the dialyzer. This need occasion no surprise; in one case we have to deal with living, active cells; in the other with dead tissue.

Living cells of muscle tissue or of gland tissue have the power of *selecting* from the tissue plasma such materials as are needed for the replenishment of their substance. Not only does the animal select what shall be taken into the alimentary tract, but the epithelial lining of that tract seems to select what shall be absorbed and to absorb it according to laws which conform not at all to the laws of osmosis.

In order, however, to understand the current literature on the subject of absorption, it is necessary to be familiar with the terminology and the laws of osmosis and dialysis. To that end the student may profitably perform for himself a few simple experiments preliminary to more complex ones which the demonstrator may suggest or may perform for the class.

1. **Appliances and Materials.** Six dialyzers complete, including outer receptacles and supports, two or three 100 c.c. evaporating dishes, distilled water, sodium chloride, alcohol, egg, and mercury manometer.

2. **Preparation.** (1) Fit four of the dialyzers with membrane of pig bladder. The bladders should be carefully selected as to uniformity in thickness, and should be soaked for an hour or more in water before being stretched upon the dialyzers. The membrane should be stretched as nearly uniform as possible upon four dialyzers. Fit one dialyzer with parchment paper, such as is frequently used for this purpose. Furnish one dialyzer with some other animal membrane—*e. g.*, a cow's bladder or a rabbit's cæcum.

(2) Prepare dilute egg albumin by adding to strained undiluted albumin about nine volumes of distilled water.

3. **Experiments and Observations.** (1) Salt, in saturated aqueous solution, may be put into a dialyzer. So adjust the apparatus that the water in the outer receptacle shall be on a level with the solution in the vertical tube of the dialyzer. How much does the water rise in the tube? What degree of positive pressure within the dialyzer does that represent? How much pressure per unit area, measured with a mercury manometer will it be necessary to produce

within the dialyzer to stop the increase of the volume of its contents? (*Endosmotic pressure.*) Will that amount of pressure prohibit diffusion between the liquids?

(2) After osmosis has been allowed to take its unimpeded course for, say, one hour, starting with a 20 per cent. solution of NaCl within and distilled water without the dialyzer, note the height of the water in the tube and compute the number of grams of water which have entered the dialyzer. Determine how much NaCl has passed out of the dialyzer. An easy and sufficiently accurate method is to evaporate to dryness all or a known proportion of the liquid in the outer receptacle, and weigh the dry salt remaining. How many grams of water enter the dialyzer for each gram of salt that leaves? (*Endosmotic equivalent.*)

(3) Is the endosmotic equivalent constant for salt and water?

(*a*) Is it the same for different strengths of the salt solution—*i. e.*, for 10 per cent. or 1 per cent. as for 20 per cent.? (*b*) Is it the same for two hours or four hours as for one hour?

(4) Fill with 10 per cent. glucose three dialyzers provided with three different kinds of membrane. Does osmosis take place at the same rate in all three dialyzers? What is the endosmotic equivalent for glucose?

(5) What is the endosmotic equivalent for dilute egg albumin? When albumin is injected into the colon it is readily absorbed as albumin, there being no digestive changes in it.

(6) Fill a dialyzer with equal parts of 10 per cent. glucose and 10 per cent. NaCl. At the end of a convenient period, two to six hours, determine whether these substances have diffused according to their own endosmotic equivalents—*i. e.*, independent of each other, or have they been influenced, the one by the other?

CHAPTER VII.

VISION.

I. DISSECTION OF THE APPENDAGES OF THE EYE.

Appliances. Fresh ox-eyes, including as much of the appendages as possible; physiological operating case; dissecting board and pins, such as used for frogs; dog, cat, or rabbit; bone forceps; injection mass; syringe.

Dissection. Follow Cunningham or Quain, vol. iii., part iii.

(1) Before fixing the eye to the board make a careful examination of the organ.

(*a*) Trace the *conjunctiva*, describing its *ocular* and its *palpebral* portions. Describe the *plica semilunaris* and the *caruncula*. Do these two tissues have the same relative size in man and the ox? Find and describe the *puncta lacrymalia*. Find and describe the openings of the *lacrymal ducts*. How many are there? Enumerate the conjunctival landmarks which determine the inner from the outer side of the eye. Enumerate the conjunctival landmarks which determine the superior aspect of the eye. Is the eye which you have a right eye or is it a left one?

(*b*) Observe the appendages of the eye. Do you find a remnant of the *levator palpebræ* muscle? Find the *tarsal cartilages* and the remnant of the *orbicularis palpebrarum* muscle. Find openings of the *meibomian* and of *sebaceous glands*. Find and describe the *lacrymal* gland as to *location* and *size*.

Find and cut off ends of the *recti* and *oblique muscles* of the eye.

Describe location of the *optic nerve* with respect to the cornea.

What traces have you found of the *capsule of Tenon?*

Enumerate the new landmarks which determine the superior aspect of the eye; the internal aspect. Are these extra landmarks sufficient to determine whether the eye which you have is a right or a left one?

(2) Fix the eye to the board with the corneal surface down, pinning down flaps of the conjunctiva for support.

(*a*) Dissect out the four *recti* and the two *oblique* muscles. One will find in the ox a rather heavy retractor muscle in close relation to the optic nerve. This should be left undissected until the other muscles are demonstrated.

(*b*) Trace the intricate loculi of the *capsule of Tenon.*

(*c*) Carefully separate from the eyeball all connective and adipose tissue.

(3) Remove the retractor muscle of the ox-eye in process of dissection, taking care not to sever any important bloodvessels or nerves.

(*a*) Locate and describe the *venæ vorticosæ*. How many are there?

(*b*) Find the *anterior ciliary arteries*. How many can be found? Describe their relation to the tendons of insertion of the recti muscles. What tissues do they supply?

(*c*) Find the two *long ciliary arteries*.

(*d*) Locate and enumerate the *short posterior ciliary arteries*.

(*e*) Dissect out the *ciliary nerves*. What tissue do they supply?

(4) Let one member of the division dissect, for demonstration, the *orbital muscles* of a dog, cat, or rabbit. To facilitate the dissection fix the animal with dorsum up, and remove with bone forceps the upper and outer walls of the orbit.

(5) Let one member of the division inject, with carmine or vermilion mass, the internal carotid of a dog, cat, or rabbit, and dissect out for demonstration the *ocular branches* of the ophthalmic artery.

II. DISSECTION OF THE EYEBALL.

Appliances. The eyes, already partly dissected, which have been kept in an ice-chest. Let one man make an anterior and another a posterior dissection.

Dissection. 1. **Anterior Dissection.** Fix the eye to the board, cornea upward, pinning out the dissected muscles as guys.

(1) Describe the *cornea* as seen from the front. Does the radius of curvature of the lateral meridian seem to be the same as that of the vertical meridian? With heavy scissors remove the cornea, leaving a margin of one-sixteenth inch anterior to its junction with the iris.

Examine the cut surface of the cornea with a lens.

(2) Through the elliptical opening thus made examine the *iris* as to texture, etc.

(3) Holding the margin of the cornea with strong forceps, carefully dissect the *sclerotic coat* from the choroid for about one-eighth of an inch posterior to the angle of the anterior chamber. Locate four points in the margin from which the incisions may be made anteroposteriorly between the insertions of the recti muscles. From the points located make the incisions posteriorly as far as the equator of the eyeball. Dissect each flap from the underlying choroid; remove the pins which fix the recti muscles, and through traction draw the flaps back; fix.

(*a*) Make a drawing of the *choroid* with its *iridal* and *ciliary* portions thus exposed.

(*b*) Locate, if possible, the course and distribution of nerves and bloodvessels.

(4) With fine forceps grasp the margin of the iris and with fine scissors cut out a sector limited posteriorly by the ciliary body.

(*a*) Study the boundaries of the *posterior chamber.*

(*b*) Find fibres of the *suspensory ligament.*

(*c*) Describe the anterior surface of the *ciliary processes.*

(5) Make a circular incision with small scissors severing the choroid and retina at about the line of the *ora serrata.* Lift off from the dense vitreous humor the whole ciliary apparatus and lens; place them, anterior surface downward, upon a plate.

(*a*) Describe the posterior aspect of the ciliary processes.

(*b*) Describe the *lens* minutely, as viewed externally.

(*c*) Make a section of the lens; describe its appearance. Is the capsule discernible?

(6) Describe the *retina* as seen through the vitreous humor.

(*a*) Locate the entrance of the *optic nerve.*

(*b*) Can the *fovea centralis* be located?

2. **Posterior Dissection.**

(7) Let one member of the division remove the posterior half of the sclerotic coat, after fixing the eye with cornea downward, using the recti muscles, in this case also, for guys.

(*a*) Note the *venæ vorticosæ.*

(*b*) Follow the *ciliary nerves* from their entrance into the eyeball, along their course between the sclerotic and choroid coats.

(*c*) Do you find the long ciliary arteries, or the posterior ciliary arteries?

(8) Remove the choroid carefully.

(*a*) Note the character of its tissue, its vascularity, and its rich pigmentation.

(*b*) Describe the retina as seen from this direction. Its pigmented layer has probably come away with the choroid.

(9) Remove the posterior half of the vitreous body together with the retina.

Make a drawing of the posterior surface of the lens, suspensory ligaments, and ciliary processes as shown posteriorly.

(10) Remove the remnant of the vitreous body; sever the fibres of the suspensory ligament; lift out the lens.

Describe the ciliary body and the iris thus held in their normal relations by the supporting sclera.

III. PHYSIOLOGICAL OPTICS.

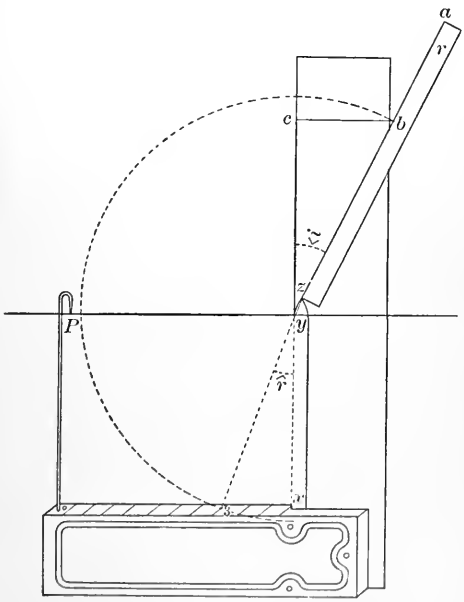
Determination of the Indices of Refraction of Water and of Glass.

1. **Appliances.** Apparatus for determining the index of refraction; a deep, flat-bottomed water-pan; a cube of glass 4 to 6 cm. in linear dimensions and polished on at least two opposite sides (the two polished sides must be absolutely parallel), whether the other sides are parallel or polished makes no difference; centimetre rule and dividers.

2. **Preparation.** A very convenient and sufficiently exact apparatus for making the required determination may be readily made as follows:

(1) Take a carpenter's tri-square, constructed wholly of iron; from the angle x (Fig. 76), where the graduated limb joins the body, measure off centimetres upon the inner surface of the body and cut them in with a file.

FIG. 76



Apparatus for determining index of refraction.

(2) Locate on the inner edge of the graduated limb any point, as y, 6 to 9 cm. from the point x. With file remove about $\frac{1}{2}$ cm. of the edge as indicated in figure, cutting deeply at z, so as to leave a slender point at y as indicated.

(3) Drill a hole in the inner surface of the body at *o;* fit and drive a heavy brass or iron wire into this; sharpen the upper end of the wire. The length of the wire above the body must be 2 or 3 cm. greater than the distance *x y*. Bend the point over so that distance *o p* shall equal *x y*.

3. **Experiments and Observations.** Place the instrument in the water-pan; fill the pan, so adjusting it that both points *p* and *y* will just touch the water, or rather almost touch the water, for the surface of the water at *y* must be absolutely plane. If the point touch it the surface will not be plane.

(1) (*a*) Bring a small rule (*R*) into position and clamp it to the limb of the instrument by means of heavy serre-fine forceps. So adjust the rule that as one sights along its upper edge the points *a, y,* and 3 seem to lie in one and the same straight line. Lift the apparatus out of the water and lay it on the table, taking care not to disturb the adjustment.

(*b*) With dividers measure the distance from the point *y* to line 3. This is the radius. Determine the point where the circumference would cut the upper surface of the rule—say, point *b*.

(*c*) From this point determine the perpendicular distance to the edge of the limb at *c*.

(*d*) The line *c y x* is a normal to the surface of the water at the point *y*. The angle *i* is the angle of incidence; the angle *r* is the angle of refraction. Imagine a circle whose centre is at *y* and whose circumference passes through *b* and 3. The line *b c* is the sine of the angle of incidence. The line *x* 3 is the sine of the angle of refraction.

(*e*) What is the ratio of sine *i* to sine *r*, or

$$\frac{\text{sine } i'}{\text{sine } r'} = ?$$

(2) In the same manner determine the ratio of the sines of these angles when the rule is so adjusted as to bring *a′ y* 4 in apparently one straight line. What is the ratio of sine *i′* to sine *r′*, or

$$\frac{\text{sine } i'}{\text{sine } r'} = ?$$

(3) If the instrument has been carefully constructed, and if the determination has been made with sufficient care, the ratios will be found to be practically equal—*i. e.*,

$$\frac{\text{sine } i}{\text{sine } r} = \frac{\text{sine } i'}{\text{sine } r'}.$$

What is the constant ratio in the case of water? This constant ratio is called the *index* of *refraction* and is conventionally represented by μ.

For water,

$$\mu = \frac{\text{sine } i}{\text{sine } r} = \frac{4}{3} = 1.333.$$

(4) To determine the index of refraction of glass proceed as in the case of water. Set the instrument upon the table; the block of glass may be placed upon the body of the instrument, the polished surfaces being placed above and below. If the distance between the polished surfaces is not equal to $x\ y$, a point (y') may be located on the upper surface near the edge of the glass block by making a dot with ink where the line $x\ y$ cuts the upper surface of the block. This line is the normal.

What is the index of refraction of the glass block furnished by the demonstrator?

IV. TO DETERMINE THE FOCAL DISTANCE OF A LENS; THEN THE USE OF THE FORMULA

$$\frac{1}{o} + \frac{1}{i} = \frac{1}{F}.$$

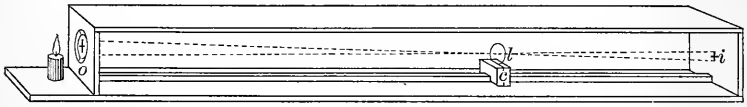
An easy method of determining the focal distance of a lens depends upon the relation of the distance of the conjugate foci to the general focal distance. This relation may be expressed thus: The sum of the reciprocals of the conjugate foci is equal to the reciprocal of the focal distance.

Now, when a lens throws upon a screen the image of an object it is evident that the distance of the object (o) represents one and the distance of the image (i) represents the other of these conjugate focal distances; so one may say: The reciprocal of the distance of the object from the lens ($\frac{1}{o}$) plus the reciprocal of the distance of the image ($\frac{1}{i}$) equals the reciprocal of the general focal distance ($\frac{1}{F}$); thus ($\frac{1}{o} + \frac{1}{i} = \frac{1}{F}$). This formula enables one to compute the focal distance after first determining by experiment the values o and i.

1. **Apparatus.** To that end one may construct a simple apparatus (Fig. 77). For the determination of the focal distance it is usual to have both object and lens movable. For our purpose this may be dispensed with, as it lends little to the reliability of the result and detracts much from the simplicity of the apparatus. Construct from half-inch pine boards a box 100 cm. or 50 cm. long and about 8 cm. high and wide (inside measurements). The box should be open at one side. The inner surface of one end may be painted white and serve as a screen; the other end should have in its center a large hole. Over this hole, on the inner surface of the upright,

fix a sheet of lead or of copper in which some figure has been cut. Construct a lens carrier (*c*), whose pointer (*p*) will indicate upon the scale (*s'*) the position of the centre of the lens. The use of the instrument will be somewhat facilitated if the distance between the surface of the screen and the surface of the lead or copper be purposely made exactly 100 cm. In addition to the above apparatus one needs the lenses whose focal distance he is so determine. He needs also a lamp or candle to place behind the metallic screen at *e*.

FIG. 77



Apparatus for determining the principal focal distance through the observation of the conjugate focal distances: *o*, object; *l*, lens; *i*, image (the conjugate focal distances *o l* and *i l* may be represented by *o* and *i*, respectively); *c*, lens carrier, which slides along the guide on the bottom of the box.

2. **Experiments and Observations.** Place a light behind the metallic screen; it shines through the figure cut through the screen. This figure is the object (*o*).

(1) (*a*) Place a lens in the carrier and so adjust it that the plane which it represents is perpendicular to the axis of the instrument and its center is in the same perpendicular plane with the index (*p*) of the carrier.

(*b*) Slide the carrier along the base until the object is sharply focused upon the screen.

(*c*) Read from the scale the distance of the lens from the image (*i*). If the instrument is made just 100 cm. between the screen and object, then the difference between 100 and the reading will be the distance of the lens from the object. Is the image erect or inverted? Explain the phenomenon, drawing geometric figure.

(2) Study the general formula.

(*a*) $$\frac{1}{o}+\frac{1}{i}=\frac{1}{F}$$

(*b*) $$F=\frac{o\,i}{o+i};\ \text{but}\ o+i=100;\ \text{therefore}$$

(*c*) $$100\ F=o\,i$$

(*d*) $$\therefore\ F=\frac{o\,i}{100}$$

From this form of the statement it is evident that the lens will throw a distinct image in either one of two positions. Demonstrate it experimentally.

(3) Determine *o* and *i* for each lens and substituting their values in the equation (*d*) determine the value of *F*. A slight deviation

may be expected between the value of F determined from the above formula and that determined directly. This deviation is due to errors in the apparatus and in the observations.

(4) **Problems.** The value of the formula $\frac{1}{o} + \frac{1}{i} = \frac{1}{F}$ is so great and its application so frequent that the student should thoroughly familiarize himself with the properties of lenses as revealed in this formula.

Solve the following problems:

(1) When the object is twice the focal distance, what is the distance of the image?

(2) When the distance of the object is greater than $2F$, how does the distance of the image compare with $2F$?

(3) When the object is at a very great distance ($o = \infty$), at what distance will the image be formed?

(4) What is the maximum focal distance that may be determined or verified with the above-described apparatus?

Discuss in detail.

V. TO LOCATE EXPERIMENTALLY IN THE MAMMALIAN EYE THE CARDINAL POINTS OF THE SIMPLE DIOPTRIC SYSTEM.

In the study of the glass lens one takes into consideration the index of refraction, and the radius of curvature of the surface of the lens. When one remembers that the eye possesses media of two different refractive indices, bounded by three curved surfaces—anterior corneal surface (radius 7.829 mm.), anterior and posterior lens surfaces (radii 10 and 6 cm., respectively)—the complexity of the problem becomes apparent.

It has been shown mathematically that a complex optical system consisting of several surfaces and media, centered upon a common optical axis, may be treated as if it consisted of two surfaces only.

Applying this principle to the eye it has been found that the several media and surfaces may be reduced to two parallel spherical surfaces whose radii are 5.215 mm. These surfaces cut the optical axis just posterior to the cornea and are only $\frac{1}{3}$ mm. apart.

To further simplify the optics of the eye it has been customary to reduce it to a simple dioptric system by assuming one refracting surface near the posterior surface of the cornea.

A Simple Dioptric System.

The simple dioptric system is one in which the ray passes from one medium into a second medium of different refractive index, the

surface of the separation of the two media being a spherical surface. In the accompanying figure (Fig. 78) the spherical surface $s's\ p\ s''$ separates the medium m, whose refractive index is 1.000 from the medium m', whose refractive index is 1.500.

Note the following *cardinal points* of a *simple dioptric system.*

The center of curvature of the spherical surface (n) in the *nodal point.*

That radius which is the center of symmetry of the dioptric system (*e. g.*, n–p) is called the *principal axis* of the system. In this axis lie the *first* and *second principal foci*, f and f', respectively. The point where the optical axis cuts the spherical surface p is called the *principal point.* The plane tangent to the spherical surface at this point is the *principal plane.* Planes perpendicular to the optical axis at f and f' are called the *first* and *second principal focal planes,* respectively. In the eye the second principal focal plane is the retina.

FIG. 78

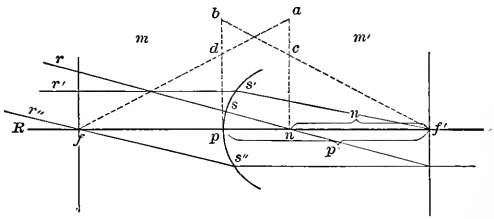


Diagram to show the cardinal points of a simple dioptric system.

1. **Appliances and Materials.** A white rabbit; support with universal clamp holder and small cork-lined burette clamps; metre stick or tape; steel or ivory rule, with millimetres subdivided if possible; hand lens; fine dividers with needle points; bone forceps; 0.6 per cent. NaCl; camel's-hair pencil; absorbent cotton.

1. **Preparation.** (1) **Mathematical.** (See Fig. 79.) We wish first to locate the nodal point in the rabbit's eye. Represent the distance from the retina to the nodal point by n; the distance from the object to the image by d; the vertical dimension of the object by o; the same dimension of the image by i. From the similar right triangles of the figure one may write:

(1) $$o : i = d - n : n;$$

(2) $$o\,n = i\,d - i\,n;$$

(3) $$n = \frac{i\,d}{o+i}.$$

Under the conditions of the experiment i is so small compared with o that it may be ignored in the denominator, and we may use the equation:

(4) $$n = \frac{i\,d}{o}.$$

2. **Arrangement of Apparatus.** (*a*) A convenient object to observe is a well-illuminated window, or one sash of a window. Measure the vertical distance between the horizontal strips of the sash.

(*b*) Arrange three or four tables end to end in a line perpendicular to the plane of a window. On the table lay off from the plane of the window the distances 5 m., 5.5 m., and 6 m.

3. **Operation.** (1) Remove an eye from the rabbit which has been chloroformed some time before and suspended by the anterior limbs.

(2) Dissect from the eye, especially from the posterior aspect of it, all of the areolar connective tissue, muscle tissue, etc., down to the glistening, smooth sclera.

(3) Wrap around its equator a band of absorbent cotton wet with normal solution.

FIG. 79

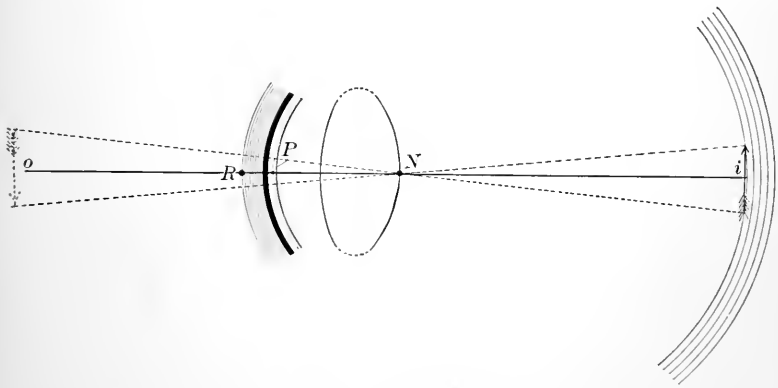


Diagram of the dioptric system of the eye: *R*, point where visual line enters cornea; *P*, principal point of dioptric system; *N*, nodal point; *o*, object; *i*, image; distance *o N* and *i N* may equal *o* and *i*, respectively.

(4) Fix the eye in the clamp with its axis transverse to the axis of the clamp, taking care to exert just enough pressure to prevent the eye from falling on being touched, *but not enough to distort it.*

(5) Fix to the clamp a thread with a bit of lead to serve as a plumb line.

4. **Observations.** (1) Adjust the support so that the eye is directed toward the object and the image is located approximately symmetrically about the fovea centralis and the plumb line over the mark 5 m. With the fine dividers measure in the image the distance between those points which were chosen as the limits of the object. The value of this measurement may be read to tenths of millimetres by laying the divider points upon the steel rule and reading with the hand lens.

(2) Make similar observations at 5.5 m. and 6 m. Each observation should be made three or four times and the average taken.

(3) Record these averages in a table ruled with columns for the values d, o, i and n.

(4) Calculate for column n the values obtained by substituting, in the formula $n=\frac{i\,d}{o}$, the values observed in (1) and (2). What is the value of n?

(5) Measure the anteroposterior diameter of the eye. How far anterior to the posterior surface of the sclera is n located? How far from the surface of the cornea? How does the ratio of these two quantities differ from that given above for the human eye?

(6) Is the image erect or inverted? Explain the phenomenon?

(7) Move the eye to within 1 m. of the object. Note that a fairly clear image may be thrown upon a posterior segment of the sphere, which is many hundred times the area of the fovea centralis.

(8) If a fine, sharp needle be thrust through the eyeball, following a course perpendicular to the optical axis and cutting it at n, what relation would this needle have with the lens? Would it be tangent to the lens; would it enter the lens, or would it pass free of its posterior surface?

For these experiments the eye may be frozen after the introduction of the needle and a vertical longitudinal section made.

VI. ACCOMMODATION AND CONVERGENCE.

In the above experiment with the excised rabbit's eye one notices a marked blurring of the image when the eye is brought near the object. Though the definition of the image is sharp at 5 m. to 6 m. or beyond, at 2 m. or 3 m. the outlines are hazy. The normal living eye is, however, able to give one the *sensation of a clear image at any distance from several inches to several miles.* That there is actually a sharply defined image upon the retina when the normal mind has a sensation of such an image there is no doubt.

One knows from his experience with optical instruments that they must be readjusted for each distance if they are to yield a sharp image for each distance.

The same thing is true in the case of the organic optical instruments with which one perceives the form, color, and space relations of the objects of his environment. *The functional adaptation of the visual organs to distance is called accommodation.*

A. Accommodation.

Experiments and Observations. (1) Take a sharp-pointed pencil or similar object in each hand; hold the upturned points in the

line of direct vision before the eye, one point being about 25 cm. distant from the eye and the other at arm's-length; make the observations with one eye, the other being closed or screened.

(*a*) Focus upon the near point. Is the image of the distant point clear?

(*b*) Focus upon the distant point. Is the image of the near point clear?

(*c*) While the eye is steadily focused upon the near point bring the distant point slowly up to a position beside the near point. One of the images is transformed from an illy defined one to a clearly defined one. Which image is it? Does one note a similar change in the definition of the image when he moves the near point out to a position beside the distant point while focusing steadily at the latter?

(*d*) Sum up the results of the experiments into a concisely formulated statement.

(2) Holding the points side by side at a distance of 20 cm., note that the points appear equally well defined.

(*a*) Direct the eye steadily at one of the points while moving the other nearer to the eye. Note the number of centimetres which it advances toward the eye before the outlines become illy defined. Reverse the act, moving the point back to its original position beside the stationary point, noting that the image of the receding point remains clear.

(*b*) Continue to carry it farther from the eye, noting that after it has been carried beyond the unmoved focused point a certain distance the outline becomes illy defined. Note the number of centimetres between the two points in this position.

(*c*) Make a similar experiment, using 30 cm. for the distance of the stationary point, and note the centimetres between the points at the limits of clear definition. In this way one may observe and measure the focal depth of the eye.

(*d*) Is the focal depth greater at 20 cm. or at 30 cm.?

(*e*) Tabulate the focal depths of the members of the class for the distances 20 cm., 30 cm., 40 cm., 50 cm., and 60 cm.

(*f*) Sum up the results of the experiment into a concisely formulated statement, and show the relation between ocular focal depth and microscopic focal depth.

(3) **Determination of the Near Point or "Punctum Proximum."** Determine the distance from the eye of the nearest point at which a pencil point or needle may be perfectly clearly seen. The exact location of the near point may be more satisfactorily determined if one look at the object through two pinholes, 2 mm. apart, in a card. At this point—the punctum proximum—the act of accommodation is brought actively into play.

(4) **Determination of the Punctum Remotum.** (*a*) Direct the eye toward some object not less than 6 m. away and describe to the

other members of the class the minute details of the object, such as slight irregularities of surface lines or other details. If an individual is able to convince his comrades that he can perceive at this distance the minute details of objects, he must be credited with normal vision. Inasmuch as he can also see with the usual distinctness more distant objects, the punctum remotum is said to be located at infinity; or, to state it in another way, the eye is able, with suspended accommodation, to bring parallel rays to a focus upon the retina.

(*b*) It frequently happens that the individual under observation fails to make out more than the merest outline of an object 6 m. away. Decrease the distance until he is able to perceive details seen by the majority of his comrades. If this distance has to be decreased to 2 m. or 3 m. the determination may be made more exact by resorting again to the needle and punctured card mentioned in (3), and carrying the needle away until it appears double.

In recording the punctum remotum,[1] write infinity (∞) for 6 m. or more, and for any distance within that record in metres and decimals thereof.

(5) How many metres from the punctum remotum to the punctum proximum in those cases where the punctum remotum is less than 6 metres?

(6) Observe the pupil closely while the subject directs the eye from a distant object to a near one. It contracts slightly. One may assume that this act of the iris is advantageous. Show from the standpoint of theoretical optics why it is advantageous.

(7) Observe from the side that when the act of accommodation takes place the iris at the edge of the pupil not only moves toward the center, but advances noticeably toward the cornea. What could produce this?

(*a*) If the edge of the iris rests upon the lens capsule, would it not be pushed farther toward the cornea incident to its contraction toward the center?

If the pupil contracted from a 3 mm. diameter to a 2 mm. diameter, how much would it advance incident to the normal curvature of the lens (radius 10 cm.)? Could this be detected by the method of observation which has been employed?

(*b*) Account for the forward movement of the pupillary edge of the iris during accommodation.

B. Adaptation of the Eye for Direction. Convergence.

Just as the eye possesses a mechanism by which it changes its refractive power for different distances, so it possesses a mechanism

[1] It must be stated here that this experiment does not make it certain that the punctum remotum is not beyond infinity. This would, however, be a pathological condition, and need not be discussed here. There will be occasion to refer to this question more in detail in a subsequent lesson.

by which it may change the direction of its visual axis from one object to another or may follow the movements of objects within the range of vision.

1. **Monocular Fixation.** Let two individuals work together, one as subject and the other as observer. Let them sit on opposite sides of the table. Let the subject close or screen one eye.

(1) Hold any object directly in front of the subject; let the subject keep his gaze continually fixed upon the object. Move the object quickly toward the subject's left, and note the fixation anew of the object in its new position. What muscle or muscles accomplished this act of monocular fixation?

(2) Move the object quickly in the opposite direction; then upward, downward, diagonally, noting the instantaneous adaptation of the eye to the new direction, recording also the muscle or muscles involved in each act. Are all the movements apparently equally ready and exact?

(3) Bringing the object to a point directly in front, 1 m. distant, note through how great a lateral movement it may be carried without inducing any discernible change in the visual axis of the eye.

(4) Bring the object to the central position and move it very slowly outward in any direction, noting whether the changes in the direction of the visual axis are equally slow and regular.

2. **Binocular Fixation.** In the above experiments it was probably noted by both subject and observer that the closed or screened eye responded to every movement of the other eye.

(5) With both eyes open and fixed upon an object held directly in front at a distance of about 1 m., let the observer move the object quickly; then slowly, right, left, up, down, and around, and observe the continuous perfect fixation of the object with both eyes.

(*a*) What muscles are involved in following an object from one's right side to his left? In each other direction in turn?

(*b*) Do all these muscles seem to act perfectly in all of the subjects examined? If not, describe any variation.

3. **Convergence.** (*a*) Let the subject direct his gaze at the tip of the observer's ear, and without warning change his point of binocular fixation to some distant object in the same line of vision. What change in the eyes of the subject is noticeable by the observer? What muscles were involved in producing the change?

(*b*) Hold an object in front of the subject and 1 m. distant. Move it directly toward the subject's eyes and note the convergence of the lines of vision of the two eyes. What muscles perform the act?

(*c*) Through how short a distance may the object be moved in the direct line of vision without causing a discernible change of the angle of convergence of the two eyes.

(*d*) From the central, 1 m. position, carry the object to a point about ½ m. to the right and ½ m. above the eyes of the subject. What muscles are involved in the act of convergence?

(*e*) Is the power of convergence apparently normal in all members of the class? If not, describe minutely any variations.

VII. MISCELLANEOUS EXPERIMENTS.

(*a*) **Scheiner's Experiment.** (1) Prick two smooth holes in a card at a distance from each other less than the diameter of the pupil. Fix two long, fine needles or straws in two pieces of wood or cork. Fix the cardboard in a piece of wood with a groove made in it with a fine saw, and see that the holes are horizontal. Place the needles in line with the holes, the one about eight inches, the other about eighteen inches from the card.

(2) Close one eye, and with the other look through the holes at the *near* needle, which will be distinctly seen, while the *far* needle will be double, both images being somewhat dim.

(3) With another card, while accommodating for the *near* needle close the right-hand hole, the right-hand image disappears, and if the left-hand hole be closed the left-hand image disappears.

(4) Accommodate for the *far* needle, the near needle appears double. Now close the right-hand hole, and the left-hand image disappears; and on closing the left-hand hole the right-hand image disappears. (*Practical Physiology*, Stirling.)

(5) Explain the phenomena, drawing figures which show just what must take place in the eye.

(*b*) **The Blind Spot.** (6) **Marriotte's Experiment.** On a white card make a black cross and a circle about three inches apart. Closing the left eye hold the card vertically about ten inches from the right eye, so as to bring the cross to the left side of the circle. Look steadily at the cross with the right eye, when both the cross and circle will be seen. Gradually bring the card toward the eye, keeping the axis of vision fixed upon the cross. At a certain distance the circle will disappear—*i. e.*, when its image falls on the entrance of the optic nerve. On bringing the card nearer the circle reappears, the cross, of course, being visible all the time.

(7) **Map Out the Blind Spot.** Make a cross on the center of a sheet of white paper and place it on a table about ten or twelve inches from you. Close the left eye and look steadily at the cross with the right eye. Wrap a penholder in white paper, leaving only the tip of the penpoint projecting, dip the latter in ink, or dip the point of a white feather in ink, and keeping the head steady and the axis of vision fixed, place the penpoint near the cross and gradually move it to the right until the black becomes invisible. Mark this spot. Carry the blackened point still farther outward until it becomes visible again. Mark this outer limit. These two points give the outer and inner limits of the blind spot. Begin again, moving the pencil first in an

upward and then in a downward direction, in each case marking where the pencil becomes invisible. If this be done in several diameters an outline of the blind spot is obtained, even little prominences showing retinal vessels being indicated.

(8) **To Calculate the Size of the Blind Spot.** Helmholtz gives the following formula for this purpose: When f is the distance of the eye from the paper, F the distance of the second nodal point from the retina (usually 15 mm.), d the diameter of the sketch of the blind spot drawn on the paper, and D the corresponding size of the blind spot:

$$\frac{f}{F} = \frac{d}{D} \text{ or } D = \frac{F\,d}{f}.$$

Determine the diameter of the blind spot (D) for each member of the class.

VIII. PERIMETRY.

In the foregoing experiments we have dealt exclusively with what is called *direct vision*—*i. e.*, with phenomena involving the formation of a clearly defined image upon the *macula lutea*. Everyone has noticed that outside the range of direct vision one may still get a pretty definite idea, not only of form, but of color as well. It is the purpose here to ascertain just how far this axis of *indirect vision* extends in every direction from the visual axis, or *to locate the perimeter of the field of indirect vision*. Various instruments have been devised, called perimeters, to aid one in perimetry.

All of these appliances have for their object the mapping of the field. In all exact methods the map takes the form of a polar map, the pole corresponding to the point where the line of vision would pierce perpendicularly the plane of the map.

1. **Appliances.** A perimeter, or ruled blackboard (Fig. 81); perimeter charts, such as shown in Fig. 82.

2. **Preparation.** A very economical and accurate perimeter may be constructed in the following manner:

Take a blackboard whose dimensions are about 1 m. by 1.5 m.; locate a point 40 cm. from one end and 50 cm. from either side. Let this be the point of fixation or the point where the line of direct vision falls upon the surface of the board.

We propose now to draw upon the board a series of circles whose distance from one another shall represent an angular distance of 10 degrees. Reference to Fig. 80 makes it evident that if the line $A\,B$ represents the plane surface of the blackboard, and if the eye be placed at O, the equal increments of 10 degrees on the quadrant become a series of increasing increments upon the surface of the board. The numbers at the right (Fig. 80) show just how many centimetres the radius of each successive circle should be, provided the distance of the eye from the board be taken at 20 cm.

After drawing the circles, draw meridians, which divide each quadrant into three to nine subdivisions. The complete blackboard chart will have the appearance and proportions shown in Fig. 81. The circles and meridians should be traced permanently in white enamel upon the surface of the blackboard. Any marks upon the board with chalk may then be erased without disturbing the perimeter circles.

The most satisfactory test objects are pieces of fresh crayon not over 1 cm. in length. They may be held in wire holders of convenient length.

FIG. 80

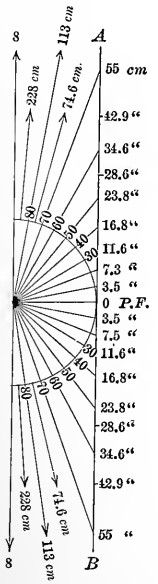
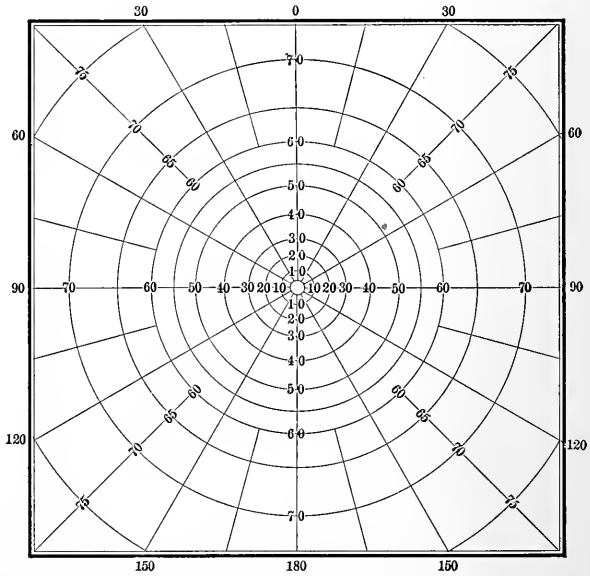


FIG. 81



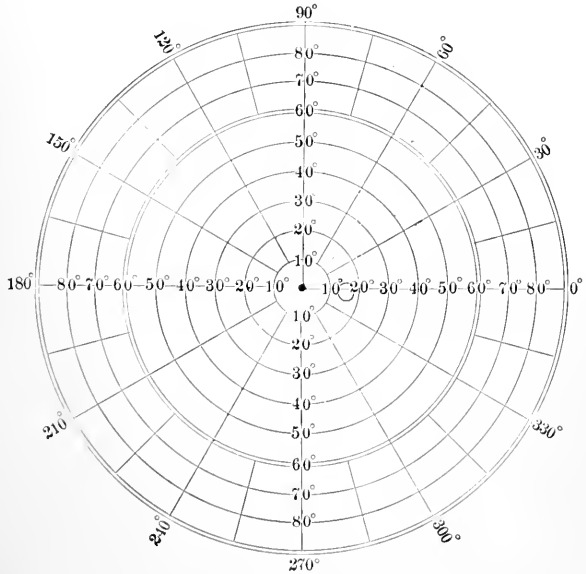
Each blackboard must be provided with a rest or contrivance to ensure that the subject's eye is 20 cm. from the surface of the board. Whether this takes the form of a rod of wood extending out from the board and so adjusted that when the subject rests the most prominent infraorbital region upon its end *the cornea will be* 20 *cm. from the center of the chart,* or whether it takes some other form that ensures the same results, is of little consequence.

3. **Experiments and Observations.** In all the observations which are subsequently indicated it is taken for granted that the visual axis is perpendicular to the surface of the chart, that the center of the chart is the point of fixation, and that the accommodation is kept uniform—*i. e.,* the eye is either uniformly focused on the center of the blackboard perimeter or uniformly relaxed; further, that the eye not under observation be closed or closely shaded.

(1) Examine the upper median quadrant by sweeping a white test object around arc 60 degrees, keeping the test object as near the surface of the chart as possible. If the subject does not see it at all, try latitude 50 degrees. Having located the circle which seems to be near the boundary, locate upon each meridian a point which indicates the limit of indirect vision in that direction. Join with a continuous line the points located, thus enclosing an area of indirect vision.

(2) Test the lower median quadrant in the same way. Is the total area covered by indirect vision in this quadrant greater or less in extent than that in the upper quadrant?

FIG. 82



Perimeter chart, upon which the blackboard perimeters are to be transcribed for permanent record.

(3) Test the upper lateral quadrant and then the lower lateral quadrant. Are these two quadrants practically equal? Is there any ready explanation why the outer two quadrants should contain such an excess of area over the inner two quadrants?

(4) **To Record the Perimeter Outline.** For this purpose one should have printed charts like the one given in Fig. 82. Note that here the circles are equidistant. They represent concentric arcs of a quadrant with 10 degrees of the circle between each two, while the circle upon the blackboard chart represents a radial projection of these arcs upon a plane tangent to the sphere at the point of fixation.

In transcribing the perimeter upon the record chart one has only

to locate the twelve or more points located upon the observation chart and join these points into a continuous perimeter.

(5) In the above experiment we have determined the perimeter for light sensation only; the subject being conscious simply of a light or white spot on a dark ground, but not certain whether the spot is circular or square.

(6) Determine and chart various color perimeters: (*a*) red, (*b*) green, and (*c*) blue.

Have the color perimeters the same general form as the white perimeters? If not, describe any noticeable variations. Which of the color perimeters encloses the greatest area? Enumerate them in order of area. Is this the order which one would expect? Give grounds for position.

(7) Take corresponding perimeter for the other eye. To use the same blackboard it will be necessary to turn it the other edge up. In what general respect do the right perimeters differ from those of the left?

(8) With the help of light perimeters of the right and left eyes, *determine the field of binocular vision.* This is the field of binocular *indirect vision.*

IX. DETERMINATION OF NORMAL VISION.

The Acuteness of Direct Vision.

1. **Appliances.** Charts printed with Snellen's test type, astigmatic chart, and test lenses of following strength: + 0.50 D., + 0.75 D., + 1.00 D., + 2.00 D., + 3.00 D., —0.50 D., —0.75 D., —1.00 D., —2.00 D., —3.00 D., +1.00 D. cyl., +2.00 D. cyl., —1.00 D. cyl., —2.00 D. cyl.; simple test frames and shade; Holmgren's worsteds.

2. **Preparation.** Preparatory to testing normal vision it is necessary to make a few general statements.

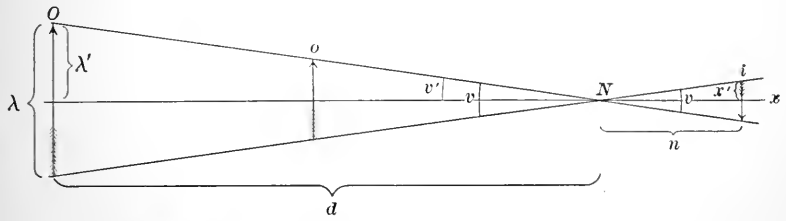
(1) **The Numeration of Lenses.** *The refractive power of a lens is the reciprocal of its focal distance.* The refractive power of a lens whose focal distance is 1 m. is, for example, only one-half as great as that of a lens whose focal distance is 0.5. Monoyer introduced the term dioptre as a unit in measuring lenses. One dioptre (1 D.) represents the refractive power of a lens whose focal distance is 1 m.; 2 D. corresponds to $\frac{1}{2}$ m.; 3 D. to $\frac{1}{3}$ m.; 4 D. to $\frac{1}{4}$ m., etc.; 0.5 D. represents the refractive power of a lens of 2 m. focal distance; 0.25 D. of 4 m. focal distance, and 0.125 D. of 8 m. focal distance. If the lenses are convex (biconvex) a plus sign is prefixed to the number—*i. e.*, +5 D. means a biconvex lens of 5 dioptres refractive power, or $\frac{1}{5}$ m. focal distance. While —5 D. means a biconcave lens of $\frac{1}{5}$ m. negative focal distance.

The use of the cylindrical lenses is frequently necessary. A cylindrical lens is a section of a cylinder parallel to its axis.

Cylindrical lenses may be convex or concave. A convex cylindrical lens capable of bringing rays to a linear focus at a distance of $\frac{1}{2}$ m. would be designated as follows: 2 D. cyl.

(2) **Test Types and Visual Angle.** The *visual angle* is that included between lines joining the extremities of an object and the nodal point, or the angle subtended by an object at the nodal point.

FIG. 83



Illustrating the *visual angle* (v) and the relation of the distance (d) to the length of the object (o) and image (i). N, the nodal point; n, the focal distance, the image being on the retina.

In Fig. 83 the object at o subtends the angle v, while the object at O, though much larger, subtends the same angle v. Now it has been determined by Snellen that the normal eye distinguishes letters subtended by an angle of 5 minutes. If we let d equal distance of object from nodal point, n equals distance of image from nodal point, i length of image, and o of object, then

$$(1) \qquad i : o :: n : d;$$

$$(2) \qquad o = \frac{i}{n} d;$$

$$(3) \qquad \text{but } \frac{i}{n} = \frac{\text{sine } v}{\text{cos. } v} = \text{tan. } v;$$

$$(4) \qquad o = d \text{ tan. } v.$$

The tangent of $5' = 0.001454$; assume $d = 1$ m. (1000 mm.), what is the height of the smallest letter discernible to the average normal eye at that distance?

At 1 m. height of letter, $o = 0.00145 + 1000 = 1.45$ mm.

Determine the height for each of the following, respectively: 60 m., 30 m., 20 m., 15 m., 12 m., 9 m., 6 m., 4.5 m., 3 m., 2.5 m., 2 m., 1.5 m., 1 m., 0.75 m., 0.50 m.

What is the size of the image in all these cases? A cultivation of the visual power of the eye may readily in the emmetropic eye bring up its definition to $\frac{1}{4}$ above the average or so that the minimum visual angle for acute vision equals $4'$.

3. **Experiments and Observations.** (I) **To Test the Form Sense.** In all of the tests here described it is understood, unless otherwise stated, that the subject sit directly facing the chart, which should be 6 m. distant and well illuminated.

(1) Let the subject put on the test frames with the left eye shaded, and direct the right eye to the letters of the line marked 6 m. These letters in their vertical dimension subtend an angle of 5′. The average normal eye will be able to recognize easily every letter in the line. Should there be any hesitation in the differentiation of *C* from *G*, of *P* from *D* or *F*, of *K* from *X*, etc., make a note of it; its significance will be apparent later.

In recording the acuteness of vision one compares the minimum angle of distinct vision in the subject under observation with the normal. If the subject reads readily at 6 m. he is credited with normal vision or with a minimum visual angle normal or unity. This is expressed in the following manner: Let *V* equal visual acuteness; *d*, the distance from the chart; *D*, the distance at which the type should be read: $V=\frac{d}{D}$. In the above case $V=\frac{6}{6}$ or 1—*i.e.*, normal vision.

(2) Suppose that the subject cannot read the 6 m. line readily, let him try the line above. If he reads that readily his visual acuteness would be $V=\frac{d}{D}=\frac{6}{9}$, two-thirds normal. It is usual, however, not to reduce the fraction, but to use the 6 as the numerator always.

(3) How shall one express visual acuteness for an individual who reads at 6 m. what he should read at 21 m.? At 24 m.? At 30 m.? At 4.5 m.? At 3 m.?

(4) How many members of the class have a visual acuteness greater than unity? May a visual acuteness above the normal be attributed in any degree to cultivation of the vision, or is it to be interpreted solely as a natural endowment?

(5) Let a subject take a seat 6 m. distant from the chart. Hold before his eye a +0.75 D. lens, it will probably make indistinct and blurred distant objects which were, without the lens, clear. (*a*) If such be the case it is likely that refraction of the eye is normal, and for our purpose it may be recorded as an *emmetropic eye.*

(*b*) If, however, the vision remains perfectly clear for distant objects, with +0.75 D. or the +1 D. lens before the eye it is evident that the refraction of the eye is not normal.

(*c*) Suppose, on the other hand, that distant objects cannot be clearly seen with the unaided eye, but with the help of concave lenses clearly seen, it is evident again that the refraction of the eye is abnormal.

(6) In case (5, *c*) where were the parallel rays focused when the concave lens was used? Where were the parallel rays focused in the unaided eye? Would it be possible for the condition to be corrected by an exercise of the accommodation? If the punctum remotum is 2 m., and if the refractive indices and curvatures of the refracting

surfaces are all normal, in what way must the eye differ from the normal eye? This condition is called near-sightedness, or *myopia*.

(7) In case (5, *b*), if a subject can read all of the letters expected of the normal eye one credits him with $V=\frac{6}{6}$; but the eye may have accomplished the result at the expense of more or less effort.

If the eye have a *punctum remotum* beyond infinity—*i. e.*, if the rays of light from a distant object are not yet converged to a focus by the time they reach the retina *in the resting eye*—it will require a certain effort of accommodation to produce a clear image. Such is the condition in the *far-sighted* person; the condition is called *hyperopia*. The term *far-sightedness* does not mean that the subject can see farther than the average individual, but that he can see far objects more easily than he can see near objects. If a subject with $V=\frac{6}{6}$ can see as clearly or more clearly when the +0.75 D. lens is in front of the eye, there is no reasonable doubt that hyperopia in some form is present.

(8) Let the subject direct the line of vision toward the *center* of the chart for testing astigmatism. It is probable that not all of the radiating lines will appear equally clear-cut and black, for most persons have a small degree of astigmatism. If the lines are unequally clear, where are the clearest ones located? Do they describe a diameter across the circle? If so, describe the location of the clear diameter, 0 degrees to 180 degrees being the horizontal diameter, and 90 degrees to 90 degrees the vertical one.

(9) (*a*) If the subject has normal vision with no astigmatism or normal vision despite a slight stigmatism, he may be given a better conception of just what a moderate degree of stigmatism is by putting a +1 D. cyl. lens before his eye; or a rather high degree of simple astigmatism by trying +2 D. cyl. or +3 D. cyl.

(*b*) How may the subject be made artificially hyperopic?

(*c*) How artificially myopic?

(II) **To Test the Color Sense.** Let the subject take the three test colors—light green, purple, and red—and choose from the mass of worsteds the colors which he considers similar ones, placing the chosen color in the class to which it belongs. It is not difficult to determine whether or not the subject has a defective color sense. If, for example, he is *red blind* he will not see the red in the purple, or related colors, but will classify these with the blues, while the reds will be confused with the greens.

X. THE RANGE OF ACCOMMODATION.

The amount of refractive change produced by the eye in adjusting for its punctum proximum after it has been at rest—*i. e.*, after it has been adjusted for its punctum remotum—is termed the range of accommodation. In a previous chapter the *punctum proximum* and *punctum remotum* were determined. It was reserved for this place to express the position of these limits of accommodation in terms of dioptres, and thus most readily determine and definitely express the *range* in simple dioptres. The relation of this to what has just preceded will be evident.

Let r equal the punctum remotum expressed in dioptres—*e. g.*, if the punctum remotum is located at infinity that would represent zero dioptres ($r=0$); if the punctum remotum is located $\frac{1}{2}$ m. distant from the eye r would equal 2 D.

Let p equal the punctum proximum expressed in dioptres—*e. g.*, if the punctum proximum is located at 10 cm. ($\frac{1}{10}$ m.) $p = 10$ D.

Let a equal the range of accommodation in dioptres; then $a=p-r$.

To apply this formula to the above example we have

$$a = 10 \text{ D} - 2 \text{ D} = 8 \text{ D}.$$

1. **Experiments and Observations.** (1) Determine the range of accommodation for each member of the class.

(*a*) Determine the punctum remotum and punctum proximum.

(*b*) Record these quantities in metres.

(*c*) Substitute these values in formula (5), expressing the distances in the corresponding dioptres—*i. e.*, using the reciprocals of the distances.

(2) **Range of Accommodation in Myopia.** (*a*) Is r positive or negative in myopia?

(*b*) Is a always less than p, or may it sometimes be greater?

(*c*) What is the average range of accommodation of the myopes of the class?

(3) **Range of Accommodation for Emmetropia.** (*a*) What is the value of r in emmetropia?

(*b*) What is the relative value of a and p in this class of cases?

(*c*) What proportion of emmetropes in the class?

(*d*) Have they all the same range of accommodation?

(*e*) Can any probable cause be assigned for any variations which may be found?

(*f*) How does the average range for emmetropes compare with the average range for myopes?

(4) **Range of Accommodation for Hyperopia.** (*a*) If the *punctum remotum* is "beyond infinity" (!), that is equivalent to saying that the eye when at rest does not focus parallel lines (from infinity) upon the retina, but the lines must be more than parallel—*i. e.*, from *beyond*

infinity; or, better, *convergent;* but if they are convergent they would meet behind the cornea. The *punctum remotum* for hyperopes is then *negative* in direction and is equal to the distance, behind the cornea, at which the convergent lines would meet if prolonged. It follows that $\frac{1}{r}$ is in the case of hyperopes negative. Our formula would then take the form:

$$a = p - (-r), \text{ or}$$
$$a = p + r.$$

Now, in determining r one may use a convex lens of such strength as to give the rays the requisite convergence. The value of the lens in dioptres is, of course, the value of r. In the hyperope a is always greater than p. As the determination of the *punctum remotum* of the hyperopic eye is a matter for the clinician to deal with, we will omit its determination here.

(*b*) If a member of the class wears glasses having the following formula for the right eye, +2 D., and if his punctum proximum is 12.5 cm. distant from the cornea, what is his range of accommodation?

(*c*) What is the range of accommodation for those hyperopes in the class whose punctum remotum may be determined from the lenses which they use?

(*d*) May variations in range be accounted for?

(*e*) Is the average range greater or less than that for myopes? For emmetropes?

(5) Tabulate the values of p and of r for the class; first, with respect to age, arranging in the first column all of the cases which range between eighteen and twenty years; in the second column twenty-one to twenty-three, and so on. Determine the average for p and for r from each age column.

(*a*) Does age within the limits of your table affect the punctum proximum? If so, how?

(*b*) Does age affect the punctum remotum as shown by your table?

(*c*) If the volume of data justifies it, make a chart showing the effect of age upon the range of accommodation. Use the values of p and r for the divisions of the axis of ordinates.

XI. NORMAL OPHTHALMOSCOPY (DIRECT METHOD).

Gould defines ophthalmoscopy as "the examination of the interior of the eye by means of the ophthalmoscope." Normal ophthalmoscopy is the examination, by means of the same instrument, of the normal eye or a model of the normal eye.

1. **Appliances.** An ophthalmoscope, with concave mirror; dark-room lamp, and Thorington's skiascopic eye or an equivalent.

2. **Preparation.** Arrange the model and the lamp so that they will be in the horizontal plane with the observer's eye. Place the skiascopic eye directly in front of the observer's eye, and the lamp a little to one side of the model.

3. **Operation.** Let the observer hold the ophthalmoscope with the right hand, mirror forward, close to the eye, directing the vision through the hole in the instrument. Throw the light, reflected by the mirror, into the skiascopic eye. Find the red reflection of the fundus, then gradually lessen the distance between the observer's eye and the model to about 2 cm. or 3 cm. The skiascopic eye will then be illuminated and the fundus with its structures will be clearly defined.

4. **Observations.** (*a*) **Adjust the Model to Represent the Emmetropic Eye.** (1) Determine with the ophthalmoscope the color of the fundus. Enumerate the structures seen.

(2) Describe the *papilla,* or entrance of the optic nerve. Is the papilla in the visual axis or to one side of it? Describe its position with respect to the visual axis of the eye and determine the most advantageous position of observer, model and instrument, to get a direct view of the papilla in the right eye; in the left eye.

(3) Describe the location of the *arteria* and *vena centralis retinæ* with reference to the papilla.

(4) The ring formed by the border of the papilla is sometimes called the *scleral ring* or the *choroidal ring.* Can this ring be distinctly seen?

(5) The *macula lutea* and the *fovea centralis* are the most sensitive portions of the retina and are in a direct line with the visual axis of the eye.

What is the most advantageous position of model, observer, and instrument in order to get a direct illumination of this part of the fundus? Describe the appearance of the structures in question.

(6) Describe the retinal bloodvessels minutely, drawing a map of their distribution.

(*b*) **The Observations of the Retina in the Hyperopic Eye.** Adjust the model for three dioptrics of hyperopia.

(7) Are the retinal bloodvessels distinct when the above-described method of observation is used?

(8) Place in a rack, before the model eye, the following lenses, with each one testing for a distinct retinal image:

$$\pm 1 \text{ D.}, \pm 2 \text{ D.}, \pm 3 \text{ D., and } \pm 4 \text{ D.}$$

With which one of the lenses is the clearest image obtained? Are all of the figures of equal size? Explain, giving a figure.

(9) In hyperopia do the rays focus in front of, on, or behind the retina? What direction do the rays take after leaving the hyperopic eye from the illuminated retina? Are they parallel, divergent, or convergent?

(*c*) **Observation of the Retina in a Myopic Eye.** Adjust the model for myopia—*e. g.*, three dioptrics.

(10) Are the retinal bloodvessels distinct?

(11) What direction do the rays from the retina take on emerging from the myopic eye: divergent, convergent, or parallel?

(12) In which of these three cases would the normal eye be able to get a clear image of the retinal structures?

(13) In which case would a correcting lens be necessary? Should one use a convex or a concave lens, and why?[1]

XII. NORMAL OPHTHALMOSCOPY (INDIRECT METHOD).

1. **Appliances.** The same as in preceding exercise, with addition of a lens of +12 D. to +20 D.

2. **Operation.** With the model of eye to be observed, the light and the observer arranged as above, direct the light reflected by the mirror into the observed eye and find the red reflection of the fundus of the eye. Hold the lens between the thumb and index finger and place it directly between the mirror and the eye under examination, and at a distance from the latter of 6 cm. to 8 cm. Be careful that the center of the lens corresponds to the center of the pupil and that the plane of the lens is perpendicular to the line of vision.

3. **Observations.** (*a*) **Observations of the Emmetropic Eye.** (1) The rays of light emerging from the observed eye are focused by the convex lens, which the observer holds, and forms an aerial image of the retina. If a +12 D. lens be used, and if its optical center be held 8 cm. from the anterior surface of the cornea, how far from the cornea will the aerial image be formed?

(2) Trace in the image all of the structures enumerated in the direct method. Is the image erect or inverted? Is the field larger or smaller than one sees in the direct method? Are the structures magnified or the reverse? Account for all phenomena representing the optics of the case with a figure.

(3) Does a change in the distance between the cornea of the model or eye and the lens which the observer holds alter the size of the image? Account for observation.

(*b*) **Observation of the Hyperopic Eye.** Adjust the model for 3 D. of hyperopia.

(4) Does an increase of the distance of the lens from the cornea cause the image of the papilla to be altered in size? Account for all phenomena.

[1] In all work with the ophthalmoscope or retinoscope it is understood that the observer's eye is emmetropic, either by nature or by correction, and that his accommodation is suspended. One may get a clear view of the retina without fulfilling these conditions, but one cannot draw reliable optical conclusions.

(*c*) **Observation of the Myopic Eye.** Adjust the model to represent 3 D. of myopia.

(5) Does the increase of the distance of the lens from the eye cause the image of the papilla to become altered in size or reversed in position? Account for all phenomena.

(6) If the position of the +12 D. lens, which the observer holds, remains the same—8 cm. from cornea—will there be any variation in the distance from the cornea of the retinal image for the hyperopic eye and myopic eye? Will the distance of the hyperopic eye be greater or less than for the emmetropic eye? Why?

(*d*) **Observation of the Human Eye.** At this point of the student's work, let him practice the direct and indirect method of ophthalmoscopy upon his comrades; after two or three days of practice he may pass to the following exercise.

XIII. SKIASCOPY.

Gould defines skiascopy as "a method of estimating the refraction of the eye by observation, through ophthalmoscopic mirror, of the movements of the retinal images and *shadows*." Synonyms: Fundus reflex test; umbrascopy; pupiloscopy; koroscopy; kertoscopy; retinoscopy, etc.

1. **Appliances.** A simple retinoscope or an ophthalmoscope with a plane mirror; Thorington's skiascopic eye or an equivalent; dark-room lamp, etc.

2. **Operation.** The observed eye and lamp are to have the same relative position as in ophthalmoscopy. Let the observer sit directly in front with the eye in the same horizontal plane with the lamp and observed eye, and somewhat more than 1 m. distant from the observed eye. Throw the light reflected by the mirror into the observed eye; rotate the mirror slowly and a shadow will be seen in the pupil of the observed eye.

3. **Observations.** (*a*) **Observation of the Emmetropic Eye.** Adjust the model to represent emmetropia.

(1) Does the shadow move in the same direction as the mirror rotates or in the opposite direction—*i. e.*, does the shadow move *with the mirror* or *opposite?*

(2) Is the movement of the shadow *quick* or *slow?*

(*b*) **Observation of the Myopic Eye.** (I) Adjust the model to represent less than 1 D. of myopia.

(3) Note that the shadow movement is with the direction of the mirror rotation, and that it is relatively quick.

(II) Adjust the model to represent a myopia of more than 1 D.

(4) Note that the shadow movement is opposite the direction of the mirror rotation, and that it is *quick* when the myopia is of low degree; *slow* when of high degree.

(5) Observe alternately the three conditions indicated above until their differences are so familiar that any one of the conditions may be readily and unerringly detected by the observer when they are arranged for him by the instructor.

(*c*) **Observation of the Hyperopic Eye.** Adjust the model to represent any degree of hyperopia.

(6) Note that for a low degree of hyperopia the shadow movement is *with* the mirror rotation and quick.

(7) Note that for higher degrees of the condition the shadow movement is *with* the mirror and *slow*.

(8) How may one differentiate a high degree of myopia from a high degree of hyperopia?

(9) Is there any difference in the size, shape, distance, or position of the shadows in these two conditions?

(*d*) **Observation of the Human Eye.** Let the student practice upon his comrades.[1]

[1] Observation of the astigmatic eye is intentionally omitted here. It belongs more especially to the clinical phase of the subject.

CHAPTER VIII.

THE PHYSIOLOGY OF THE NERVOUS SYSTEM.

I. REFLEX ACTION.

1. **Material and Appliances.** Three large, vigorous frogs; operating case; sulphuric acid, 0.5 per cent.; acetic acid, 50 per cent.; distilled water; cork board, 10 cm. square; hand basin; filter paper; six watch-glasses.

2. **Preparation.** Pith two of the frogs, taking care to sever the medulla completely; destroy the brain but leave the spinal cord intact. If there is hemorrhage plug the puncture with absorbent cotton. Lay the pithed frogs ventrum down, with legs extended, upon a moist paper. Note that if the toes be pinched the leg will not be flexed. There is no reflex response. The animal is under the influence of shock. This condition will probably last for half an hour or more. Recovery will be indicated by the drawing up of the legs, first one leg and then the other being flexed.

3. **Observations.** *a.* **Modifications of General Functions by Pithing.** (1) The pithed frogs lie upon the ventrum with legs flexed. The position simulates the normal. Make a detailed comparison of the *posture* of the pithed frogs with that of the normal frog under the bell-jar.

(2) Compare pithed frogs with normal as to the appearance of the eyes.

(3) Study the respiratory function of the pithed frogs.

(4) Is the heart, as observed through the body wall, acting with usual rate and force in the pithed frogs?

(5) Gently lower a pithed frog into a basin of cold water. Is there an adaptation to the conditions? Does the frog swim? Vary the experiment by dropping the frog from a height of six inches. Take a yard of cord and tie one end around the brachium of the normal frog. Repeat the experiments with the normal frog and note the character of response.

(6) Place a pithed frog upon a cork board; gently tip the board in any direction, noting whether there is adaptation in *equilibration*. Repeat the experiment with the normal frog. Describe differences.

(7) Lay a pithed frog on its back on the table; will it right itself? Try a normal frog.

b. **Reflex Response to Various Stimuli.** Suspend a pithed frog by a hook through its mandible. The body and legs should hang freely, and should be several inches from the table.

(8) MECHANICAL STIMULI. With forceps pinch one of the toes (not the longest) of the hind leg. Pinch the skin of the flank. Pinch the folds of skin about the anus. Note response when stimulus is varied in strength and applied to either side.

(9) THERMAL STIMULI. With a hot wire touch the skin at several points, noting response.

(10) ELECTRIC STIMULI. With single-induction shocks stimulate skin of legs, thighs, or flank, using fine platinum-wire electrodes, and touching the moist skin.

If single shocks elicit no response, use a rapid succession of shocks produced by bringing the Neef hammer into the primary circuit.

(11) CHEMICAL STIMULI. Cut some pieces of filter paper not over 2 mm square. Dip a piece into 50 per cent. acetic acid, taking care that the paper is saturated and that there is no excess of the acid.

Apply the acid paper in turn to the web of the foot; to the flank; to the ventrum; median line; to the anus. Note the character of the response, as to extent, single-sided or double-sided. After each application of acid to the skin of the frog, the acid should be thoroughly rinsed or swabbed away.

c. **The Characteristics of Reflex Response.** (12) PURPOSIVE CHARACTER OF RESPONSE. In the responses already studied the observer could easily note that the movements possessed the manifest purpose of removing the offending object. In many cases the movements involved several sets of muscles, but in every case all the muscles involved in the response acted with perfect co-ordination, and the movement was well directed toward the removal of the irritation. This is what is meant by the purposive character.

In order further to illustrate this characteristic of response, repeat Foster's instructive experiment: "Choosing a strong frog in which reflex action has been found to be highly developed; suspend it; hold the right leg firmly down, and apply a square of acid paper to the right flank. Twitchings and convulsive movements of the right leg are at first witnessed; then the left leg is brought up to rub the right flank." (*Handbook for the Physiological Laboratory*, p. 409.)

(13) THE LATENT PERIOD OF REFLEX RESPONSE. The observer will remember that in most of the above experiments the responses did not follow instantaneously upon the application of the stimulus; this was especially noticeable in the case of one of the weaker stimuli.

In order further to illustrate this characteristic, prepare five dilutions of sulphuric acid in as many watch-glasses; hold a glass so that the tip of the longest toe just dips into the acid. Note the time required to elicit a response. After each response rinse off the part stimulated and allow the animal to rest several minutes, testing

other pithed frogs in the mean time. The number of seconds required for response may be counted from a metronome or from a watch.

Strength of stimulus.		Latent periods in seconds.	
		Frog A.	Frog B.
H_2SO_4	0.05 per cent. . . .	.....	.....
H_2SO_4	0.1 " " . . .	.....	.....
H_2SO_4	0.2 " " . . .	.....	.....
H_2SO_4	0.3 " " . . .	.....	.....
H_2SO_4	0.4 " " . . .	.....	.....

(14) The Irradiation of Reflex Action. In the above-described experiment it will probably have been noted that the stronger the stimulus the more extended the response—*i. e.*, the greater the number of muscles brought into action.

When only the tip of the toe is touched to very weak acid the response will be a simple flexion of the tarsus, and this only after several seconds. When stronger acid is applied to the toe or web the crus and the femur may both be flexed, and the action is sometimes repeated.

Repeat some of the experiments, paying special attention to the variation of extent of response, with varying strength of stimulus.

Note that in some cases the response involves the opposite side, as well as higher and lower levels of the cord.

(15) Location of Reflex Centers. Take one of the pithed frogs that has been responding typically. Run a pithing probe down the spinal canal, thus functionally destroying the spinal cord.

Apply any of the stimuli mentioned above. Note results, and account for same.

II. REFLEXES IN THE HUMAN SUBJECT.

Until one's attention is called to it, he is likely to overlook the great importance of reflex action and the reflexes in the maintenance of the human body. The eyes are protected from dust and other irritable substances, the lungs from dust and irrespirable gases, through the intervention of reflex action. The food is moistened and the digestive juices started through reflex response to the stimulating influence of food in the mouth and digestive canal. The respiration, circulation, heat regulation, excretion, and various other vital processes are controlled through reflexes. The paramount importance of reflex action thus becomes apparent.

A disturbance of certain reflexes becomes a clinical symptom of considerable importance.

It is proposed here to study briefly a few of these reflexes. Their detailed discussion is left to the text-books.

1. **Appliances.** Glass rod, 20 cm. long, with rounded ends; 3 per cent. carbolic acid; beaker of water; towel; bristle mounted in handle.

2. **Observations.** (*a*) **Respiratory Reflexes.** (1) Make a bristle aseptic. Let one member of the group act as subject. Let the subject close his eyes. The observer should introduce the bristle very gently through the nostril, and, as far as possible, up the nasal passage. The response will probably be in the form of a *sneeze.*

Accidental introduction of irritating substances into the respiratory passages below the glottis causes *coughing.*

(2) Make a bristle aseptic and bend it into a semicircle. Let the subject open the mouth wide, depress the tongue, and say "Ah!" prolonging the sound several seconds. Introduce the bristle into the mouth; pass it over the tongue without touching the latter; turn the point downward back of the tongue, and tickle the glottis. A convulsive, reflex movement of the larynx, sometimes accompanied by a cough, will result.

(*b*) **Circulatory Reflexes.** (3) *Posture* influences the circulation reflexly. Let the subject remain sitting quietly while the pulse is counted through a minute. Note the number. Let the subject lie on the back upon the table. After he has rested quietly three to five minutes, take the pulse rate again. Let the subject stand and observe the rate after three to five minutes.

(4) *Respiration* influences the circulation reflexly. Let the subject sit breathing at the rate of thirty respirations per minute for two minutes. Count the pulse during the second minute. Let the subject then drop to ten respirations per minute for three minutes, and then slower, if possible, during the fourth minute, when the pulse is to be counted.

(5) *Exercise* influences the circulation reflexly. This has already been demonstrated. (See Circulation.)

(*c*) **Secretory Reflexes.** (6) The chewing of anything like paraffin or rubber incites reflexly the free flow of saliva. In a similar way the presence of food in the stomach and intestines stimulates the secretory activity of the digestive glands.

(*d*) **Reflexes of Deglutition.** (7) Let the subject open the mouth. Introduce the aseptic glass rod into the mouth without touching tongue or cheeks. Gently touch the uvula; it will probably rise. Touch the fauces, and observe the convulsive swallowing movement. Sometimes this merges into a gagging movement.

The raising of the uvula is part of a normal swallowing act. The response of the fauces may be a part of an act of swallowing, or of a protective act (gagging), according to the conditions of the stimulation.

(*e*) **Visual Reflexes.** (8) Let the subject direct his vision toward some distant object. Make a sudden movement with hand or a book

as if to strike the subject in the face. Observe the winking of the eyelids—another protective reflex.

(9) Let the subject again direct his vision toward a distant object; gently touch the conjunctiva of the eyeball with the sterilized round end of the glass rod. The convulsive winking is a protective reflex.

(10) Let the subject sit near a window, and, looking through the window, direct his vision toward some object not more than twenty feet away. Suddenly shade the eyes of the subject for a few moments; then remove the shade and observe the change in the size of the pupil. During the experiment let the subject maintain the same state of accommodation, if possible.

(*f*) **Cutaneous Reflexes.** (11) Tickle the base sole of the foot. The foot will be involuntarily withdrawn—*the plantar reflex.*

(12) Pinch skin of neck. The pupil will dilate—*the ciliospinal reflex.*

There are various other cutaneous reflexes, such as the cremasteric, abdominal, epigastric, scapular, and gluteal.

(*g*) **"Tendon Reflexes."** These phenomena are not really reflexes, though they have been called that for a very long time. They may be studied in this connection. Let the student give reasons why the responses to the stimuli are not necessarily reflexes.

(13) *Ankle Clonus.* Let the subject's leg be supported as by resting it across a chair, the subject being seated. Let the observer place the hand upon the ball of the foot and press suddenly, so as to put the tendo Achillis upon the stretch. There results a series of clonic contractions. This phenomenon is not observed in a healthy subject.

(14) *"Patellar Reflex" or Knee-jerk.* Let the subject cross the legs in a posture frequently assumed when sitting. Tap the tendon below the patella with the edge of the hand, with the back of a thin book, or with a percussion hammer. The quadriceps extensor muscle will be suddenly stretched and will respond with a quick contraction, which will throw the foot forward in a kicking motion. This phenomenon is present in health, and it may be modified in disease.

III. THE ACTION OF STRYCHNINE UPON THE NERVOUS SYSTEM.

1. **Material.** One dog; two frogs; sulphate of strychnine.

2. **Preparation.** Make a solution of sulphate of strychnine, 0.01 grm. to 10 c.c.; also concentrated solution, 0.2 grm. to 10 c.c. pithed frogs. Do not fasten the dog to the dog board. Set up electric apparatus to obtain tetanizing current.

3. **Experiments and Observations.** (1) Hypodermic injection of 2 mg. strychnine per kg. of the dog. This dose is invariably lethal, even with early antidotal treatment.

(*a*) Record the condition before and symptoms as they arise after exhibition of the drug, especially with reference to:

(I) Muscular activity. Describe convulsions.

(II) Respiration. How affected by reflexes.

(III) Circulation. Rapidity and rhythm of heart.

(IV) If death occurs, which stops sooner, the circulation or respiration?

(*b*) Formulate results.

(2) Ligate thigh of frog, except sciatic nerve, at junction with body. Sever all structures, except nerve and femur, just below ligature. Separate cut surfaces with rubber tissue to prevent diffusion of the drug. Turn the frog over and make a median abdominal incision. Pressing viscera aside, pick up the sacral plexus of nerves going to the uninjured leg. The sacral plexuses may be readily recognized, lying on each side of the median line. Pass a thread loosely around the nerves, so as to quickly find them when wanted. Inject into dorsal lymph space 0.0001 grm. strychnine.

(*a*) What part of the frog is reached by poison? What part is protected from it? Illustrate by diagram.

(*b*) Were strychnine a convulsant through its action on the sensorium, would the legs be equally convulsed? If it acted on the spinal cord? If it acted on the motor nerves? If it acted on the muscles directly?

(*c*) Are both legs convulsed?

(*d*) To what parts in the reflex arc have you limited the action of the strychnine?

(3) Using as a guide the thread formerly passed around it, pick up the sacral plexus and sever it high up.

(*a*) Does this strychnine reach the motor nerve and the muscles of the uninjured leg?

(*b*) If strychnine were a convulsant through its action on either the motor nerves or the muscles, or both, would the uninjured leg still participate in the convulsions?

(*c*) Demonstrate that muscles, sciatic nerve, and sacral plexus below the point at which it was severed are still intact by stimulating distal portions of the latter.

(*d*) To what elements of the reflex arc have you limited the possible action of strychnine?

(4) Expose the heart of a frog and ligate the aortæ at the base. Operation as follows:

Freely expose sternum by + shaped incision and laying back of flaps. Remove lower half of sternum with scissors, taking care not to injure vessel in abdominal wall, which comes just to the tip of sternum. Freely incise exposed pericardium, bringing heart into view. Grasp apex of heart with forceps, taking care not to use force enough to cut through ventricular wall, and draw heart down and

forward. This gives ready access to bulbus arteriosus and aortæ. With an aneurysm needle pass fine thread around latter, taking care not to injure auricle, and ligate.

With scalpel cut through occipito-atlantoid membrane from side to side, and bend head forward. Remove posterior wall of upper end of spinal canal by inserting smaller blade of strong scissors into spinal canal and cutting, taking care not to injure cord. Allow a drop of the concentrated solution of strychnine to fall directly upon cord; or with fine hypodermic needle inserted 1.5 cm. into the arachnoid space inject two drops of the solution.

(*a*) What effect has ligation of the aortæ on the circulation?

(*b*) Would stoppage of the circulation prevent the drug from reaching the peripheral terminations or trunks of the sensory nerves? Motor nerves? Muscles?

(*c*) Where, then, must strychnine act to produce the observed symptoms?

(*d*) Would cessation of the circulation delay the action of strychnine on the cord by slowing the rate of its absorption by the latter?

(5) After observing results in experiment (4), destroy first the upper, then the lower, portion of the cord by passing a wire down the spinal cord.

(*a*) How does destruction of the upper part of the cord affect the convulsions?

(*b*) What is the result of the destruction of the entire cord?

(*c*) Do the results agree with those of previous experiments?

NOTE. Destruction of the upper part of the cord during the preparation of the animal may take place; if so, the upper limbs will not take part in the convulsions.

(6) **Further Observations and Comparisons.** (*a*) Compare the general effects of strychnine and curare in the dog.

(*b*) Compare results obtained in experiments consisting of ligating the thigh of a frog, except the sciatic nerve, and injecting, in the one case strychnine, in the other curare.

IV. THE ACTION OF CURARE UPON THE NERVOUS SYSTEM.

1. **Materials.** One dog; two frogs; normal saline solution; curare; dry cells; inductorium; hand electrodes.

2. **Preparation.** Prepare the following solution: sodic chloride, curare, 0.2 grm. to 10 c.c., in acidulated 20 per cent. alcohol. Pith frogs. Do not fasten the dog to the board, but simply restrain him. Set up electric apparatus so as to obtain single induction shocks.

3. **Experiments and Observations.** (1) Give a hypodermic injection of 0.01 grm. per kg. curare to the dog.

(*a*) Record the condition of the dog[1] just before and every ten minutes after injections of curare, with special reference to:

(I) Muscular activity.

(II) Respiration, number and depth.

(III) Circulation, rate and rhythm of heart beat.

(IV) Which stops sooner, respiration or circulation?

(*b*) Formulate the total effect of curare upon the animal.

(2) Ligate the thigh of a frog, except the sciatic nerve near the knee-joint. Inject into the dorsal lymph space 0.002 grm. curare.

(*a*) What elements enter into the formation of a *reflex arc?*

(*b*) What motor phenomena would result from increased irritability of any part of the reflex arc?

(*c*) What motor phenomena would result from lessened irritability or destruction of any element in the reflex arc?

(*d*) What effect has the ligature of the thigh on the distribution of the curare?

(*e*) How do the reflex arcs, of which the gastrocnemii are the motor ends, differ with regard to the distribution of the curare? What part of the reflex arc is protected from curare in the ligatured limb?

(*f*) Describe the relative reaction of the gastrocnemii to stimuli (chemical, mechanical, electric) applied to the various parts of the body and limbs.

(*g*) Is the sensorium intact? Is it reached by the curare?

(*h*) Is the cord intact? Is it reached by curare?

(3) Expose the sciatic nerves, near the body, in the frog, used in the experiment. Stimulate them.

(*a*) What elements in the reflex arc enter into consideration in this experiment?

(*b*) Which of these elements are exposed to, which protected from, the poison?

(*c*) Are both sciatics reached by curare?

(*d*) Is there a difference in the reaction of the gastrocnemii to the stimuli applied to the sciatic nerves?

(*e*) To what elements of the reflex arc have you limited the possible action of the curare?

(*f*) Have you proven that curare does not affect the nerve trunks?

(4) Expose gastrocnemii by cutaneous incision. Stimulate the muscles directly.

(*a*) Is there a difference in reaction to stimuli?

(*b*) If a muscle in a poisoned animal reacts to direct stimuli, but not to indirect stimuli, though the nerve fibres be proven to be intact, on what element in the reflex arc must the poison act?

[1] On dog: Voluntary muscles first paralyzed, then semivoluntary, *e. g.*, respiration. Students have maintained life for forty minutes by artificial respiration after respiratory paralysis, the heart's action being normally strong after complete respiratory paralysis.

(*c*) Why should you not use curare as an anæsthetic if the poisoned animal does not react to painful stimuli?

(5) Make two muscle-nerve preparations as described on page 48. Dip the nerve of one and the muscle of the other into curare solution. The parts of the preparation not immersed should be kept moist with normal saline solution. After several minutes mount specimens in the myograph. Stimulate the nerves and note:

(*a*) The relative reaction of the gastrocnemii to indirect stimulation.

(*b*) Does this bear a resemblance to any previous experiment?

(*c*) How do results compare with those of previous experiments?

(6) Stimulate the same muscles directly?

(*a*) Relative reaction.

(*b*) Taking this in connection with the preceding experiment, where have you proven that curare acts?

(*c*) How do experiments (5) and (6) compare with experiments (3) and (4)?[1]

V. THE ACTION OF VERATRIN UPON THE NERVOUS SYSTEM.

1. **Materials.** Sulphate of veratrin; one dog; three frogs.

2. **Preparation.** Prepare a solution of veratrin, 50 mg. to 10 c.c. Pith frogs. Restrain dog, but do not fasten to board. Set up myograph and induction coil, the latter arranged for single-induction shocks.

3. **Experiments and Observations.** (1) Give subcutaneous injection of 1 mg. per kg. veratrin to the dog.

(*a*) Describe symptoms as they arise.

(*b*) Summarize.

(2) Place thread around the sacral plexus of the pithed frog so as to easily find it, as described under strychnine. Inject 0.003 grm. veratrin into dorsal lymph space.

(*a*) Describe symptoms referable to reflexes.

(*b*) Note particularly the difference between a *forcible* contraction and a *prolonged* contraction.

(3) Sever the sacral plexus around which the thread has been passed.

[1] Failure in experiments (5) and (6) may result from insufficient immersion of muscle in curare solution, capillary attraction resulting in the curare reaching muscle supposed to be free from poison, and drying of parts not immersed in solution. Of these the first is by far the most frequent cause of failure, and the sheath of the muscle rendering the absorption of poison a slow process. It may be overcome by making a few slight incisions in sheath, or injecting a drop of the curare solution directly into the muscle.

The immersed nerve preparation often fails through death of nerve.

Failure of experiment (2), and consequently (3) and (4), may result from ligature around thigh being not tight enough to prevent diffusion of curare into gastrocnemius.

(*a*) How do contraction of the legs in response to direct stimuli compare?

(*b*) Has severing the sacral plexus altered the *duration* of the contraction of the muscles supplied?

(*c*) If veratrin still produces its typical effects, to what elements in the reflex arc have you limited its action?

(*d*) Compare the effect of severing the sacral plexus in a frog poisoned with veratrin with that of a frog poisoned with strychnine.

(4) Ligate the thigh of a pithed frog at the junction with the body, not including in the ligature the sciatic nerve. Sever all tissues just below the ligature except the nerve and the femur. Carefully separate the cut surfaces with rubber tissues so as to prevent diffusion of the drug. Inject 0.003 grm. veratrin into the dorsal lymph space.

(*a*) By means of a diagram show the distribution of the poison.

(*b*) Compare the contraction of the legs, noting particularly the difference in the *duration* rather than the difference in the force of contraction.

(*c*) If the protected limb reacts normally, to what elements in the reflex arc have you limited the possible action of veratrin.

(*d*) Compare results with similar experiment with strychnine.

(5) From the frog used in experiment (4) make two gastrocnemii preparations. Fasten in myograph by means of femurs and stimulate them directly, making tracings of contractions.

(*a*) Compare tracings.

(*b*) To what elements have we limited the action of veratrin?

(*c*) Suggest an experiment which would limit the action to one element.

(6) Very cautiously sniff veratrin. Describe the sensation.

(7) General observations and comparisons.

(*a*) Review your notes on the action of curare, strychnine, and veratrin upon the reflex arc.

(*b*) How would you prove that a drug paralyzed by its action on the spinal cord?

(*c*) How would you prove that a drug destroyed reflex activity by its action on some part of the sensorium?

VI. SENSATION.

The phenomena of reflex action and the function of the several elements of the reflex arc have been studied. It will be remembered by the subject on whom were observed the phenomena of human reflexes that he was conscious of all the stimuli, though he was unconscious of the response until it had already been effected.

The sensory element of the reflex arc—the dendritic element of the afferent spinal neuron—carries from the periphery to the spinal

cord messages or impressions of stimuli. There is no conscious sensation in the cord. Motor centers in the cord may send out impulses to muscles, thus producing the reflex responses.

If the brain is intact the impression travels through the cord to the sensorium, where it becomes a *sensation.* In the mean time the cord may have returned motor impulses of reflex action. These motor impulses pass away from the central nervous system and therefore never reach the sensorium—never give rise to sensations. The efferent reflex impulses cause action of end-organs—*e. g.,* muscles. One is unconscious of the impulse, but he is conscious of the action through sensory impressions coming to the brain from the organs in activity. The relation between sensation and reflex action has been set forth.

The relation between sensation and voluntary action is somewhat less direct. When an animal takes food into the digestive tract it is in response to the sensation of hunger. When he seeks shelter it is in response to sensations of uncomfortable exposure. These are direct voluntary response to sensation.

When one prepares food for a future meal or builds a shelter, he anticipates the coming sensation of hunger or exposure and forestalls it. Here we have an intervention of reason or of instinct, inducing a series of voluntary actions.

Voluntary action is, then, either directly or indirectly dependent upon sensation.

Finally, sensation is the source not only of all activity, but of all knowledge. Its importance in any study of the nervous system then becomes paramount.

Besides the *auto-objective sensations*—hunger, thirst, suffocation, fatigue, pain, shivering, and tickling—there are the distinctively *objective sensations:* touch, posture, temperature, smell, taste, hearing, and vision.

1. **Appliances.** Dividers; millimetre rule; two beakers; two 20d spikes, and towel.

2. **Observations.** *a.* **Tactile Sensation.** (1) To test the *acuteness* of the tactile sense as well as the power of *localization.* Take a pair of dividers whose points may be approximated to a millimetre or less. Let the subject of the observations be blindfolded. Apply the points to the tip of the ring finger so as to bring the line between the two points transverse to the axis of the finger. Press the points gently and draw them over the skin for a distance of 1 mm. If the subject feels two points, bring them nearer together and repeat the experiment; presently the points will have been brought so near together that they can no longer be distinguished as two, but the sensations are merged into one. The crucial point has been passed. The greater the acuteness of tactile sense, the nearer the points may be brought together and yet be felt as two points. What is the limit

of acuteness—*i. e.*, how near together, expressed in millimetres, may the points be felt as two?

Place the dividers upon any portion of the subject's hand and test the acuteness of touch; the subject will be able to describe accurately where the points touch the surface and the more accurately the more acute the tactile sense as tested in the above manner.

(2) In a similar way test the acuteness of tactile sensation in the following locations:

Left fourth finger—tip; palmar surface of third, second, and first phalanges; dorsal surface of second and first phalanges.

Left hand—mid-palm, mid-back.

Left wrist—flexor surface, extensor surface.

Left forearm—flexor surface, extensor surface.

Left upper arm—flexor surface extensor surface.

Left scapular region.

(3) Tabulate results for left and right side in case of Mr. A.

(4) Tabulate results for left and right side for at least two individuals.

(5) Make a careful study of the results with a view to answering the following questions, which answers may be formulated in a series of conclusions:

(*a*) Do different parts of the same individual possess the same acuteness of tactile sensations?

(*b*) Do symmetrically located points in the same individual possess the same acuteness of tactile sensation?

(*c*) Is there any appreciable variation of acuteness of tactile sensation in homologous points of different individuals?

(*d*) Is there any difference in the acuteness of tactile sensation between the flexor and corresponding extensor surfaces?

(*e*) Is there a progressive decrease of acuteness as one passes from tip of finger up along the anterior limb?

(*f*) Formulate any other conclusions which may be based upon the observations.

b. **The Temperature Sense.** (6) Map out upon the flexor surface of the forearm a 3 cm. square field, dividing it into 100 squares each 3 mm. square. Draw a similar map on paper. Fill one beaker with chipped ice and water, and another with water at 60° C. Put a spike in each beaker.

Wrap the cold spike in a towel in order that it may maintain its temperature as long as possible. Place its point gently on one of the squares of the skin map; if it feels cold to the subject make a "*c*" in the corresponding square of the paper map. Slip the cold point from square to square of the skin, noting those squares which give the sensation of cold and recording same on paper map.

(7) After finding the cold areas, determine in a similar manner the areas which give a sensation of heat for the warm spike.

(8) Test blank areas to determine whether their tactile sense is more acute than that of the hot and cold spots.

(9) Formulate conclusions in answer to the following questions:

(*a*) Are all portions of the skin equally sensitive to temperature change?

(*b*) Are all portions of the skin equally sensitive to cold?

(*c*) Are all portions of the skin equally sensitive to heat?

(*d*) What percentage of the space in the skin map is sensitive to cold? To heat?

(10) Place a cold coin on the palm of the hand; the same coin at same temperature on the back of the hand.

In which of these two positions does the coin feel the larger? Why?

VII. SENSATION (Continued).

c. **The Sense of Equilibrium.** Through the sense of equilibrium one is able to balance the body when sitting, standing, walking, or riding. In order to study some of the phenomena of equilibration try the following experiments:

(1) Apply a bandage to a subject's head in the horizontal plane. Slip a very sharp pencil (a pen or a needle will answer), point up, behind the bandage in such a way that the point will be held firmly upright by the bandage and register the movements of the head.

Smoke a kymograph drum; after the drum cools slip the paper off the drum without cutting it.

Arrange two horizontal arms, adjustable as to width and height, but held parallel by construction of apparatus. Slip the cylinder of carboned paper over the arms and separate them until the paper is stretched and held in two parallel sheets with horizontal surfaces above and below.

Adjust the carboned surfaces so that when the subject stands erect the point of the pencil (pen or needle) will just touch the lower surface.

Let the subject take a position beneath the carboned surface, standing erect and as still as possible. Any deviations from the erect position will be traced upon the carboned paper. At the end of one minute close the observation. The paper bears an accurate record of the equilibration of the subject.

(2) Shift the paper a few inches, exposing a fresh field. Let the subject again take position, standing as still as possible this time with closed eyes. The observation lasts one minute as before.

(3) Repeat the observation, letting the subject stand upon one foot: (*a*) with eyes open; (*b*) with eyes closed.

(4) Choose another subject as different as possible from the first in stature. Compare his tracings with those of subject number one.

(5) How many elements enter into the more or less complex perception of equilibrium? Has the tactile or pressure sense of the soles of the feet anything to do with it? Has the muscular sense or sense of muscle tension any part to play? What role does vision play? Are there other factors? Formulate conclusions.

d. **The Muscle Sense.** This might better be called the sense of muscle tension. It enters largely into the maintenance of equilibrium. It is an important factor in all voluntary movements because through it one gauges the strength of motor impulse to be used in each movement.

(6) Take two wooden cylinders of exactly the same shape and size, but differing in weight by an appreciable amount. Let the several members of the group weigh the cylinders in their hands, determining which is the heavier.

(7) Take two wooden cylinders alike in weight, but differing in size. Let the members of the group test them and describe their sensations. Account for the sensation.

e. **Gustatory Sensation.** Prepare the following solutions:

(I) 10 grams of cane-sugar in 1 litre distilled water.

(II) 1 centigram sulphate of quinine in 1 litre distilled water.

(III) 1 gram glacial acetic acid in 1 litre distilled water.

(IV) 10 grams dry sodium chloride in 1 litre distilled water.

(8) *To determine the acuteness of taste,* take a uniform quantity of the solution into the mouth at each observation. A convenient quantity is 4 c.c. Rinse the mouth with distilled water, or with boiled water, before each test.

Any person with normal taste is able to detect the taste of the standard solutions. In order to determine how much weaker the solution may be and yet be detected, make dilutions of the standard solutions, recording the final results in number of parts of water to one of the substance in question.

(9) Tabulate for the class and determine average strength of each solution that marks limit of acuteness.

(10) Vary the experiments by modifying temperature of solutions (10°, 20°, 30°, and 40° C.). Note latent period. Note whether subjects habitually use tobacco.

(11) Formulate a series of conclusions from the data collected.

(12) *To determine localization of sense of taste—i. e.,* to find whether there are areas of the gustatory region which are especially sensitive to particular stimuli; bitter, for example.

Through the aid of a probang apply to different limited areas of the tongue, palate, fauces, and buccal mucous surface either the standard solution or somewhat stronger solutions of the same substances.

Is the tip of the tongue equally sensitive to the four different tastes? The edges? The dorsum? The back?

(13) Draw a map of the tongue, locating those areas most sensitive to the four tastes, severally.

f. **Auditory Sensation.** (14) *To test acuteness of hearing,* determine how far the subject can hear a watch tick when the timepiece is held at the level of the head, and some distance to one side. Record distance in centimetres.

(15) *To determine the highest pitch* discernible by the ear test the subject with a Galton whistle, recording the number of vibrations per second audible.

g. **Visual Sensation.** (See Chapter on Vision.)

VIII. FUNCTION OF SPINAL NERVES.

It is intended here simply to outline a technique which may be used in making tests of any efferent nerve trunk.

In testing a spinal nerve trunk one has the choice of stimulating the anterior root—the efferent or motor root—or of stimulating the whole trunk. If one stimulates the anterior root only there is no need of cutting the nerve root next to the cord, as all impulses are efferent.

In testing a nerve trunk, it is, however, necessary to cut the nerve and stimulate the distal end only, if one wishes to observe the action of the motor fibres. If the trunk were not cut, some of the fibres, being afferent and sensory, would carry impressions to the cord and elicit a reflex response which would seriously complicate the observation of the direct efferent impulses from the point of stimulation to the motor distribution of the nerve. Results to be of any value, therefore, must be gotten through the electric stimulation of distal ends of cut nerve trunks.

1. **Appliances.** Dry cell; inductorium; contact key; short-circuiting key; three wires; shielded electrodes with wires; small dog or large rabbit; chloroform or ether; clippers; operating case.

2. **Preparation.** Fasten animal to holder, anæsthetize, clip the throat, and set up electric apparatus for single-induction shocks.

3. **Operation.** Dissect out any nerve trunk which it is proposed to test. Take, for example, one of the several trunks of the brachial plexus; take the fifth cervical.

Make a cutaneous incision along the outer margin of the sternocleidomastoid muscle. Separate the subcutaneous tissues and deeper tissues to expose the cervical nerve trunks as they emerge from between the scalene muscles. Identify the fifth cervical trunk and separate it from the surrounding tissues sufficiently to permit the introduction of the shielded electrode beneath it. Tie a ligature around the nerve close to the spinal column and cut the nerve beyond the ligature.

4. **Observation.** (1) Lay the distal end of the cut-off nerve upon the electrode. Stimulate with single-induction shocks of optimum strength. Watch carefully the response of the muscles and repeat the stimulus until the movement is perfectly understood. What is the general movement?

(2) Palpate the muscles as the stimulation is repeated and determine the individual muscles which respond.

(3) In what order do the several muscles contract? Is the movement due to a single muscle or to several acting together?

(4) Vary the experiment by tetanizing the muscles. Are any new facts discovered?

In a similar way any nerve trunk may be stimulated and the response studied.

CHAPTER IX.

THE PHYSIOLOGY OF THE MUSCULAR SYSTEM.

THE physiology of contractile tissues was treated under General Physiology. This chapter should follow that, and presupposes a knowledge of the human skeleton and skeletal muscles.

I. ANIMAL MECHANICS.

Animal mechanics is the application of the laws of mechanics to animal motion. The bones are used as levers; the articular surfaces of bones usually serve as fulcrums, while the power is exerted by the muscles. In a vast majority of cases the bones represent levers of the third class—in which rapidity of motion is attained at the expense of power. In other words, the arrangement of the bone-muscle organs is such that a contraction of a muscle—moderate in extent and rate of motion—is manifested by a movement of the limb which is much in excess, as to extent and rate, of the movement of the power.

In solving problems in animal mechanics the principal factors to be considered are: (1) The relative length of the two lever arms; (2) the relative size of the muscles involved in any movement; (3) the direction in which the power acts, and (4) the weight to be moved.

a. **Problems in Animal Mechanics.** Two typical problems in animal mechanics are the following:

1. Determine, in a particular case, the tension exerted upon the tendo Achillis in supporting the weight (60 kilograms) of the subject upon the ball of the foot.

2. How much tension was there on the biceps tendon in the subject upon your dissecting table when he held a 10-kilo iron ball in the most advantageous position? This is a typical problem and its solution will make the difficulties to be encountered apparent. It will also show that nothing more than an approximate solution can be attained without an extended and detailed study.

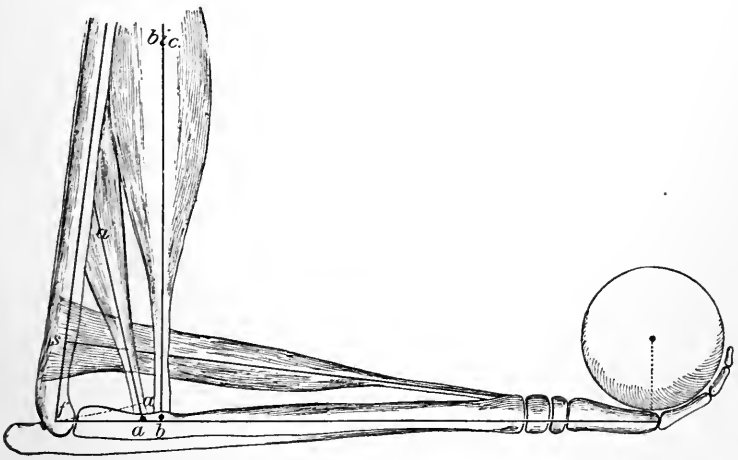
Solution. The principal muscle involved in the required action being the biceps, the most advantageous position is the one in which that muscle exerts its power in a line perpendicular to the lever. Placing the subject's arm as nearly as possible in that position, one takes the following measurements: (1) The long arm of the lever;

this would be from the center of articulation between the humerus and the ulna to the center of the 10-kilo ball, which would be, approximately, to the end of the metacarpal bone (36 cm.).

(2) The short arm of the biceps lever; this would be the distance from the center of the insertion of the biceps to the fulcrum—the center of articulation (6 cm.).

(3) The short arm of the lever for the brachialis anticus. If the brachialis anticus were exactly parallel to the biceps the short arm would be the distance from the insertion to the fulcrum (5 cm.), as in the biceps; but it is not parallel. A line drawn from the fulcrum perpendicular to the axis of the brachialis anticus $f\ a'$ is shorter than the line $f\ a$. The angle between the brachialis anticus and the biceps is approximately 10 degrees; therefore the angle $a'\ f\ a$ would be approximately 10 degrees; then $a'\ f$ is the cosine 10 degrees, or 98 per cent. of the radius $a\ f$ (5 cm.), or 4.9 cm. (Fig. 84).

FIG. 84



Mechanics of flexion of the forearm. (The upper a is to be understood as a'.)

(4) The power arm of the supinator longus is the perpendicular distance from the fulcrum to the line of force of the supinator longus, and is represented by the line $f\ s$, which is 4.8 cm. Now the carpal and digital flexors which take origin from the humerus act as forearm flexors after having flexed the carpus and digits. In the action under consideration they would not be brought into forcible action as carpal and digital flexors. We may, therefore, ignore them and confine our discussion to the three muscles mentioned above.

In the action of the biceps the long arm is 36 cm. and the short arm 6 cm.; in the action of the brachialis anticus the long arm is 36 cm. and the short arm 4.9 cm.; in the action of the supinator

longus the long arm is 36 cm. and the short arm 4.8 cm. Reducing these to per cent. ratios we have: For the biceps, which we will designate as *b*, 16.6 per cent. leverage; for the brachialis anticus, which we will designate as *a*, 13.6 per cent. leverage; and for the supinator longus, which we will designate as *s*, 13.3 per cent. leverage.

But there is another important consideration: Fick has demonstrated that when the fibres are parallel the strength of two muscles is proportional to the areas of their cross-sections (Hermann's *Handbuch der Physiologie*, i. p. 295). The average ratio of the diameter of the three muscles in question is 4 : 2 : 1, respectively. This means that with the same leverage the biceps would lift four times as much as the brachialis anticus and that the brachialis anticus would, with the same leverage, lift four times as much as the supinator longus.

We have now discussed the relation of these three factors as to *leverage* and as to *relative power* exerted.

As to leverage one may say: The power of the three muscles varies in proportion to biceps leverage (*bl*), brachialis anticus leverage (*al*), supinator longus leverage (*sl*), respectively; or, mathematically expressed, *P* varies as *bl : al : sl* or varies as 16.6 : 13.6 : 13.3. As to cross-section one may say: The power varies in proportion to the respective cross-sections (*s*), or *P* varies as *bs : as : ss* = 16 : 4 : 1. Now when any function varies with two or more variable factors, its variation when influenced by the action of all of these factors at once would be represented by the product of the several variables. Then the power varies as the leverage times the cross-section of each of the muscles when all act together; or, expressed mathematically, *P* varies as $b(l\times s) : a(l\times s) : s(l\times s)$.

$b(l\times s)=16.6\times 16=265.6$, or 79.7 per cent. of the total power exerted; $a(l\times s)=13.6\times 4=54.4$, or 16.3 per cent. of the total power exerted; $s(l\times s)=13.3\times 1=13.3$, or 4.0 per cent. of the total power exerted; total = 333.3, or 100.0 per cent.

But the weight supported by the action of these muscles is 10 kilos. If the biceps does 79.7 per cent. of the total work, it would support 7.97 kilos. What would be tension upon the tendon of the biceps when it is supporting 7.97 kilos at the end of its lever? One need only to use the 16.6 per cent. leverage ($7.97\div 16.6$ per cent.) to find that the tension would be 47.8 kilos. A similar process shows that the approximate tension upon the tendon of the brachialis anticus is 12 kilos, and upon the tendon of the supinator longus 3 kilos.

b. **The amount of contraction of a muscle** bears a fairly constant ratio to the resting length of the muscle. This law of muscle physiology was discovered and demonstrated by Ed. Fr. Weber (*Mechanik der menschlichen Gehwerkzeuge*, 1851) and was cited by Strasser (*Funktionellen Anpassung der Quergestreiften Muskeln*, 1883) as an example of the adaptation of muscle tissue to the mechanical requirements of the body. Weber showed that the maximum

contraction of which a muscle fibre is capable is approximately 47 per cent. of its resting length. Both Weber and Strasser looked upon this as the factor which determines the length of the muscles, and the location of their points of origin and insertion. In all of the skeletal muscles the tension of the contracting muscle is greater than the weight lifted. The farther the insertion of a muscle from a joint (fulcrum), the less the tension upon the muscle and the greater the amount of contraction or shortening necessary; but the inherent structure of striated muscle tissue seems to set 47 per cent. as the limit of the extent of its contraction. The fact that all skeletal muscles actually do contract that much (varying, however, in special instances from 44 per cent. to 62 per cent.) indicates that the position of the origin and insertion or the length of muscle tissue (excluding tendon) between the origin and insertion; or, more likely, that both of *these structural features have been determined by the laws of selection and now represent in all highly organized animals the most perfect mechanical adjustment consistent with the inherent properties of muscle tissue.*

(1) Make a gastrocnemius preparation; measure the length of its contractile tissue; mount it in the myograph; load it moderately; stimulate it with optimum strength of stimulus, and determine from the height of the myogram the actual shortening of the muscle. What relation does this shortening sustain to the total length of the contractile tissue?

(2) Determine approximately the ratio of shortening to length of contractile tissue in the human biceps.

c. **Problems in Human Locomotion.** (1) **The Muscles Used in Locomotion.** Let a person stand erect with heels together; let him take several steps forward and stop in a position similar to the one which he had at the beginning. What is the mechanism of *starting?* What muscles are involved in starting? What is the mechanism of *locomotion?* What muscles are involved in locomotion? What is the mechanism of equilibration while walking? What muscles are involved in maintaining the equilibrium while walking? What is the mechanism of *stopping?* What muscles are involved in stopping? How is the equilibrium maintained during the process of stopping? What muscles are involved in the maintenance of equilibrium while *standing?* How does running differ from walking in respect to the *starting*, the *locomotion*, the *equilibration*, and the *stopping?*

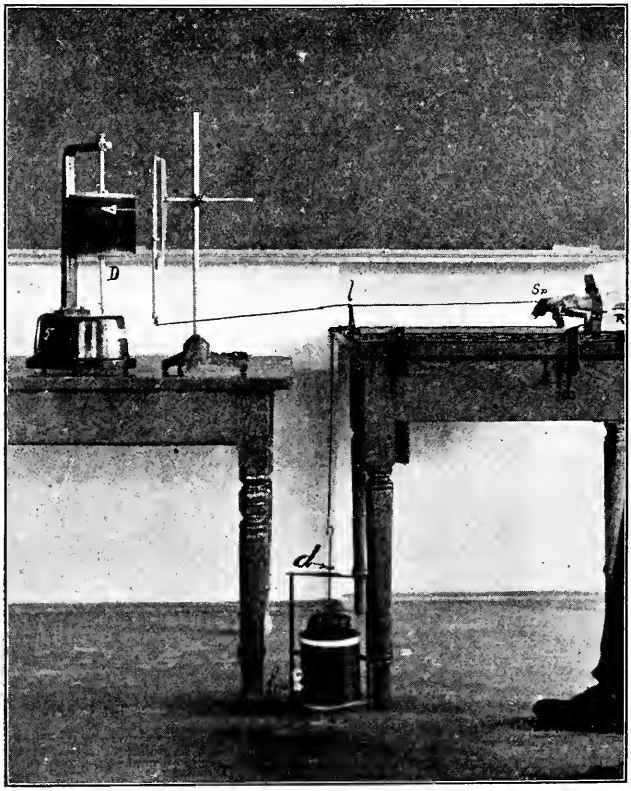
(2) **The Energy Involved in Locomotion.** How far is the body lifted at each step when one walks over a level surface? When one walks up an incline of 30 degrees? When one walks down an incline of 30 degrees? Does one do work while walking down hill? If so, how may it be computed? If not, why does one become fatigued in descending an incline? How much energy will a 70-kilo man expend in walking 1 kilo on a level road? (Suppose the man to be 172 cm.

in height, and to have a pubic height of 88 cm.) A part of the energy will be expended: (1) in lifting the body; (2) a part in maintaining equilibrium; (3) a part in overcoming resistance. Express in kilogram-metres the amount in (1). How could (2) be determined?

II. ERGOGRAPHY.

The term ergography applies to that field of physiology which deals with problems of work done by individual muscles or groups

FIG. 85



The air-cushion ergograph.

of muscles in the human subject. The instrument used is the ergograph. This instrument, first devised by Mosso, has undergone many modifications in the hands of Lombard, Hough, Bolton, and others.

It has been demonstrated that normally the muscle works under the following conditions: (1) contraction with load; (2) relaxation without load or with a very light load; (3) rest without load. This cycle may be repeated for thousands of times in a day; but so long as these conditions are filled the practised muscle can work for three to five hours without fatigue, unless the cycle of changes is too rapidly repeated, or the load abnormally heavy.

These are some of the problems that may be studied in the field of ergography.

(1) How much work can be done by the flexors of the third digit of the right hand before fatigue becomes absolute—*i. e.*, before fatigue makes it impossible to do more work in any continuous series of contractions. Conditions: *load*, 5 kilo; *rate*, 1 every second.

(2) How much work can be done by same muscle when load is 3 kilos? 2 kilos?

(3) How much work can be done with 5 kilos and rate 1 every half-second?

(4) Determine the optimum load and rate.

1. **Appliances.** Ergograph or any instrument which fulfils the above conditions (Fig. 85 shows such an instrument); kymograph and tracing apparatus; metronome to mark the time.

2. **Observation.** Adjust the splint to the middle finger and the arm to the arm rest. Fasten the 5-kilo weight to the splint; adjust the tracing apparatus and kymograph in such a way that the contractions every two seconds will result in a tracing with a straight abscissa, with lever strokes for the contractions about $\frac{1}{2}$ mm. apart. Set the metronome to beat seconds.

Let the subject contract once per second to a moderate extent, and keep it up regularly until fatigued.

To determine the work done proceed as shown in Lesson XIII., p. 56.

Use similar technique for various problems, varying only the details.

APPENDIX.

1. NORMAL SALINE SOLUTION.

This solution or, as it is also called, normal salt solution or physiological salt solution, is so much used in the physiological laboratory that it should be made in considerable quantity and always easily accessible.

FORMULA.

Common salt (c. p.)	30 grams.
Distilled water	5 litres.

It is convenient to keep the solution in a siphon bottle. It is thus protected from dust and evaporation, and is always easily accessible.

2. FROG BOARDS.

There is probably no more satisfactory or economical frog board than a piece of dressed soft pine 15 cm. by 30 cm., and 1 or 2 cm. in thickness. Some prefer to use cork board, which comes in pieces 10 cm. by 25 cm. and $\frac{3}{4}$ cm. in thickness. In the case of either, two or three coats of orange shellac should be given to the boards before they are put into use.

3. THE PHYSIOLOGICAL OPERATING CASE.

A convenient case, and one which will be sufficient in the simple experiments presented in this book, contains the following instruments: one medium scalpel; one small scalpel with narrow blade; one medium scissors; one microscopic scissors; one medium dissecting forceps; one microscopic forceps with curved, serrated jaws; two serre-fine forceps with stiff springs and serrated jaws; one grooved director and aneurysm needle; one silver probe; one blunt needle for pithing frogs, and two dissecting needles.

The case may be of leather or leatherette. Such a case may be used nearly as much in the histological as in the physiological laboratory.

4. GALVANIC CELLS.

For general use in the physiological laboratory there is probably no galvanic element superior to the Daniell cell (named after Prof. J. F. Daniell, of King's College, London). Much the most convenient and economical size is the quart or litre cell, whose porous cup measures 5 to 6 cm. in diameter and 10 to 12 cm. in height. If more current is needed than is furnished by one of these cells it is very easy to join two or more of them into a battery.

In large laboratories it will be found expedient to devote a table to the galvanic cells. This table should be provided with a supply of copper sulphate and of 10 per cent. sulphuric acid in large siphon bottles similar to the one suggested for normal salt solution, except that instead of the short tube for equalizing pressure one may insert a filter through which at the end of the laboratory period the student may return the liquids.

The accumulation of zinc sulphate in the acid makes the renewal of acid necessary from time to time. The deposit of metallic copper upon the copper plate reduces the copper sulphate solution in strength. It may be kept replenished by an excess of crystals of that salt in the large supply jar. A very practical method of amalgamating the zinc plates is to have a jar containing 10 per cent. sulphuric acid with mercury in the bottom; as the plate is immersed the acid attacks it and cleans it so that the mercury readily clings to it and may be rubbed over the surface with a cloth. Another method which is preferred by some is as follows:

Dissolve 75 grm. of mercury in a mixture of 150 c.c. strong nitric acid and 300 c.c. strong hydrochloric acid. Keep this amalgamating solution in a ground-glass-stoppered jar. To amalgamate a zinc plate one needs only to dip it for a few moments into the solution, remove it, rinse under the spigot, and rub with a cloth.

At the end of each laboratory period the cells should be emptied, the zinc plates rinsed and drained, and the porous cups left in a tray of running water, or at least in a considerable excess of water.

5. DRY CELLS.

For a part of the work in electro-physiology, particularly electric stimulation with induction shocks, the common dry cell may be conveniently used.

The dry cell becomes rather easily polarized and must, therefore, be used on an open circuit only. Used in this way, it will maintain its strength through a laboratory period and will recover its original condition in the rest which intervenes between laboratory periods.

Most dry cells consist of a zinc cup or can enclosing ammonium chloride usually mixed with plaster of Paris. In the midst of the cell is a carbon plate surrounded by manganic dioxide.

When the two plates (zinc and carbon) are joined in circuit outside of the cell the NH_4Cl attacks the zinc, forming $ZnCl_2$ and liberating NH_4 and H at the carbon plate. This tends to polarize the cell after it has been in use; but during the rest the H combines with O liberated from MnO_2.

6. TO CURARIZE A FROG.

On experiments of the irritability of muscle tissue it is necessary to in some way suspend the activity of the irritable nerve fibres which are supplied to every muscle. In certain other experiments it may be advisable thus to remove the influence of the nervous system. Curara (also spelled curare, curari, urari, woorara, woorari, wourali, etc.), an arrow poison used by South American aborigines, is the means usually employed to accomplish the end desired. The way in which the curare exerts its influence is made the subject of study in another place. Make a 1 per cent. solution by pulverizing 1 grm. of commercial curare and dissolve it in 100 c.c. of distilled water. It need not be filtered unless intended for use with a hypodermic syringe. If kept in a ground-glass-stoppered bottle, in a cool place, it will retain its efficiency for months.

The most convenient method of curarizing a frog is to inject with a narrow-pointed pipette 1 to 3 drops of the solution, through a minute, ventral, cutaneous incision.

The drug will begin to take effect in a few minutes. The maximum effect may be delayed some time.

7. TO PREPARE THE KYMOGRAPH FOR WORK.

Remove the cylinder, stretch a sheet of the prepared glazed paper tightly upon the surface, place it upon such a stand as the one shown in Fig. 33; set the drum to rotating and bring the triple gas flame under the drum. In a few moments it will be evenly covered with a film of carbon, which is as sensitive to touch as a photographer's plate is to light.

8. A FIXING FLUID FOR CARBON TRACINGS.

Gum damar	160 grm.
Benzole	q. s. ad 2000 c.c.

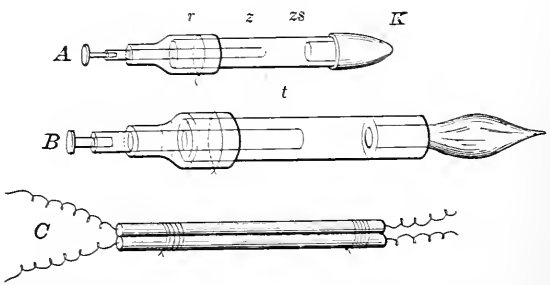
If this solution be kept in a large museum jar in the laboratory, the removed sheet bearing the tracings may be dipped *in toto* or it may

be subdivided and dipped in sections. Let the tracing be lowered quickly into the solution and after a few seconds taken out and drained. If it be now laid upon a sheet of filter paper (or a newspaper) it will be dry in a few minutes.

9. NON-POLARIZABLE ELECTRODES.

The Du Bois-Reymond non-polarizable (N. P.) electrode is made as follows (Fig. 86): *t*, glass tube of about 4 mm. lumen; *z*, zinc rod with binding screw (the zinc rod must be amalgamated before use in an electrode); *r*, rubber tube clasping both glass tube and zinc rod; *zs*, saturated solution of sulphate of zinc, introduced with a narrow-pointed pipette; *K*, kaolin plug, made by working China-clay powder into a stiff paste with normal salt solution.

FIG. 86



Electrodes: *A*, kaolin electrode; *z*, zinc rod; *zs*, saturated solution of $ZnSO_4$; *t*, glass tube; *r*, rubber tube; *K*, plug of plastic kaolin; *B*, v. Fleischl's brush electrode, in which a camel's-hair brush is substituted for the kaolin plug; *C*, hand electrodes, made by pushing the common battery wires through rubber tubing—for insulation—and binding together with thread.

The electrodes should be filled at each time of using, and the parts may be "assembled" in the order and manner enumerated in the description.

The Fleischl brush electrode differs from the foregoing in substituting the brush of a camel's-hair pencil for the kaolin plug. This variation of the non-polarizable electrode is somewhat more difficult to prepare, but is more convenient for certain uses.

Porter's Non-polarizable Electrode. This electrode is boot-shaped and is the most convenient form for general laboratory use. The electrode is of porcelain with glazed leg and unglazed foot.

To prepare the electrode soak it in normal saline solution for an hour or two to fill the pores of the unglazed portion with the salt solution. Fill the foot of the boot with a saturated solution of zinc sulphate. The amalgamated zinc rods may be dropped into the legs of the boots and the electrodes are ready for use.

After the laboratory period pour out the $ZnSO_4$ and put the boots in running water or in a considerable excess of water for twelve to

eighteen hours to remove any $ZnSO_4$ which has gained access to the porous portion of the electrodes.

If one has not the zinc rods at hand he may readily prepare an efficient non-polarizable electrode as follows: (1) Take 5 cm. of No. 16 copper wire; make one end perfectly clean and bright. (2) Dip the bright end into molten chemically pure zinc. The zinc adheres to the wire, and if the dipping be repeated two or three times the lower 1 cm. of wire will have a thick coating of zinc. (3) Take a glass tube 10 cm. long and with a 4 mm. lumen; draw it in the middle to about two-thirds its original diameter; cut into two. Before assembling the parts, that part of the copper wire not covered by zinc, excepting the tip, must be painted with Brunswick black or any varnish, and the zinc must be amalgamated. With this electrode, as with the preceding, zinc sulphate, kaolin, and 0.6 per cent. NaCl are used.

10. THE FROG-HEART LEVER.

A lever for recording upon the kymograph the movements of a frog's heart may be constructed very simply from such materials as a cork 2 cm. in diameter, a straw 30 cm. long, a piece of parchment or celluloid for a tracing point, and a few pins. In the smaller end of the cork cut a groove wide enough to receive the straw and leave a space of 1 mm. on either side. The groove should have the sides cut perpendicular to the end of the cork and should be 1 cm. deep. Pass a pin or fine needle through the cork in such a way as to cross the groove perpendicularly and about 2 or 3 mm. away from the end of the cork. Partly withdraw the pin and pass it through the straw or lever, ensuring free play of lever in the groove turning on the pin as a fulcrum. Let 6 cm. of the straw be on the side of the fulcrum and 24 cm. on the other. To the short arm a counterpoise may be fastened, and to the long arm a slender cork foot may be fastened about 4 cm. from the fulcrum and long enough to reach within $1\frac{1}{2}$ cm. of the table when the cork fulcrum stands upon the table and the lever is horizontal. To the distal end of the long arm fasten a long, slender tracing point of parchment or celluloid.

To adjust such an apparatus for tracing the movements of the frog's heart one has only to place the cork fulcrum beside a frog prepared as described on page 75, in such a position as to bring the lower end of the cork foot into the required position upon the heart. When adjusted fix in position by passing pins through the edges of the cork fulcrum into the cork plate beneath.

If one prefers to have permanent heart levers as a part of the laboratory equipment they may be constructed as indicated in Fig. 43.

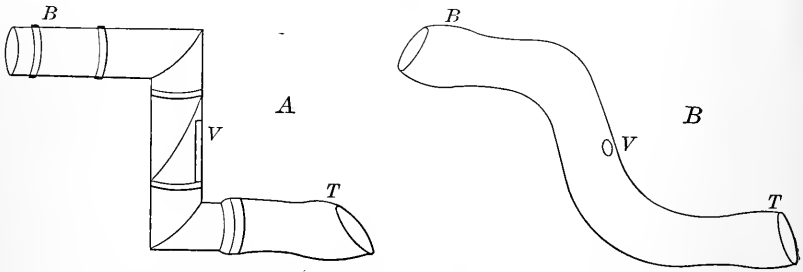
Prof. Porter, of Harvard, has devised a very fine heart lever which may be preferred to either of the forms described above.

11. THE RESPIRATORY CANNULA.

In experiments involving the opening of the thoracic wall, artificial respiration must be maintained. A bellows worked by hand or by machinery will be required. The valves of the bellows open inward only; therefore, some vent or escape must be furnished, otherwise the air pressure would rise too high within the lung and the animal would breathe the same air repeatedly.

To accomplish the desired result and to avoid the last-mentioned difficulties Prof. Ludwig long ago devised the respiratory cannula shown in Fig. 87, *A*.

FIG. 87



Respiratory cannulæ: *A*, that of Ludwig, metallic; *B*, that of the author, of glass.

If one does not have at hand a Ludwig respiratory cannula he may quickly and easily prepare one from glass that will answer all purposes. Several of these of different sizes should be prepared so that when needed one may select a size best fitted to the animal under operation. Fig. 87, *B*, shows the construction of the glass respiratory cannula.

12. TAMBOURS (RECEIVING AND RECORDING).

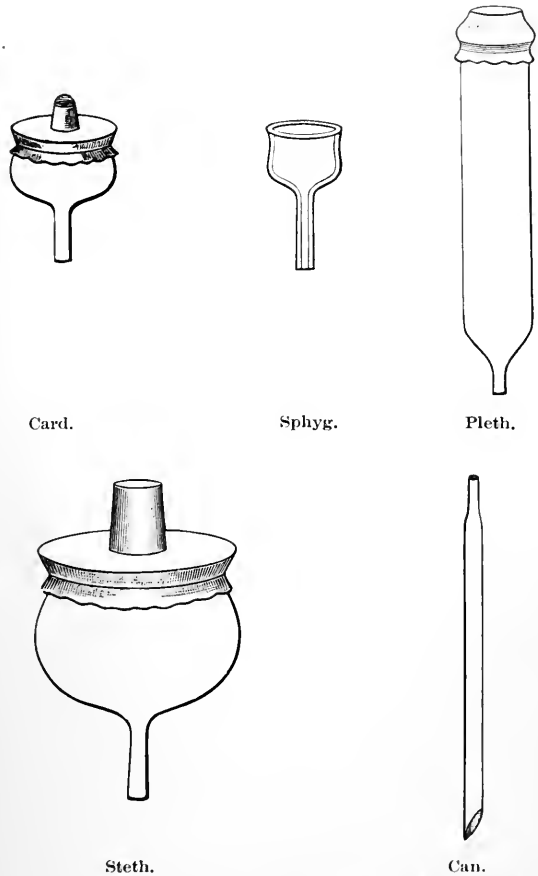
Next to the kymograph the tambour is the most important piece of apparatus in the physiological laboratory. They are used almost daily and in most observations of changing form or pressure. Each completely outfitted table should have the recording tambour in three sizes and receiving tambours of various shapes and construction.

The recording tambour must vary in size according to the desired excursion of the middle of the membrane, and that, in turn, will vary with the amount of air involved in the to-and-fro movement in the pressure tube and tambours. If one is to trace the movements of the human thorax in respiration he will want a capacious receiving tambour and a large recording tambour, while the tracing of a sphygmogram requires a very small recording tambour.

The recording tambour consists essentially of a shallow pan 1 to 6 cm. in diameter, from which a tube leads to the pressure tube

connecting the receiving tambour. Across the pan is stretched, not too tightly, a sheet of very light dentist's rubber-dam, which is tied on with thread. Upon the middle of the tambour membrane there rests the foot of the tracing lever. Through this foot every movement of the membrane is communicated to the tracing lever. The tracing

FIG. 88



Receiving tambours for various purposes. First four for the cardiograph, sphygmograph, digital plethysmograph, and stethograph, respectively. The last one (Can.) is a thoracic cannula for use in determining intrathoracic pressure.

lever is delicately pivoted to an arm which extends up by the side of the pan and which is joined to the tube or to the pan, making of the tambour and lever, with its supports, one apparatus. Only the lever holder should be metallic, while long, light straws or reeds with tracing points of parchment or celluloid may be inserted into the

metallic holder. Each tambour should have a set of levers of varying lengths—say, 10 cm., 20 cm., and 30 cm. Fig. 56, page 106, shows one form of recording tambour.

The receiving tambours must be constructed with a view to their adaptation to each particular experiment. The receiving tambour of the cardiograph (Fig. 88, Card.) should be of medium size and should have the membrane stretched rather tightly. The stethographic receiver should be far more capacious, having perhaps twice the linear dimensions of the cardiographic tambour, and the membrane should be only moderately stretched. The recording tambour should be of the largest size and should be fitted with a short lever.

The sphygmographic receiver should be only about 2 cm. in diameter. When used for the carotid pulse no membrane is used. To trace the radial sphygmogram a membrane with a button is used as described in the text.

The plethysmographic receiver is used to determine the varying size of the finger incident to circulatory changes. The finger is inserted through the rubber collar. The record is made by the smallest recording tambour.

The cannular receiver is used for taking changes in intrathoracic and intra-abdominal pressure.

13. THE MANOMETER TAMBOUR.

A very large number of research problems require the recording blood pressure of the animal (rabbit or dog) under observation. In the physiological or in pharmacological laboratories, where such observations are in progress almost daily, it is not difficult to get satisfactory results with the classical apparatus, which consists of a mercury manometer whose proximal tube (*p*) is joined through the medium of the pressure tubing (*Pt*) to the cannula (*C*), the pressure tubing being interposed at (*T*) by a T-tube, one limb of which passes to the reservoir containing one-half solution of $MgSO_4$ or some other agent for retarding coagulation. Into the distal limb of the manometer (*d*) there is fitted, in the classical apparatus, a float which rests on the mercury, following more or less accurately the variations in the lever, and carrying a vertical rod which slides through a guide in the upper end of the distal limb, and bears at its upper extremity a horizontal reed, bearing at one end a tracing point.

There are two serious difficulties with this float. First, it is likely to fail to work properly just at the time when you are most anxious that there should be no interruption in your observation, though if the apparatus is in almost daily use this difficulty is not a serious one. The serious objection against the float is that it does not follow accurately the movement of the mercury. The mercury starts up a little before the float does, and the float itself has so much inertia

that the actual movements of the mercury are not shown at all by it. In order to overcome this difficulty, and to be able to record accurately the movements of the mercury meniscus, the following variation of the classical apparatus was contrived:

A small tambour was joined to the distal end of the manometer by a piece of pressure tubing and supplied with a very delicate tracing lever which magnifies the movements of the membrane ten to twenty times, as desired, the levers being variable in the construction of the instrument. The surface of the tambour should not be larger than 15 mm. in diameter. With this ratio between the two surfaces and the levers multiplying ten to twenty times, the most beautiful arterial tracing can be obtained, showing not only the respiratory and percussion waves, but also the dicrotic wave clearly superimposed on each cardiac wave. (See Fig. 52, page 92.)

This apparatus as above described has one limitation, which may, for certain kinds of work, seem to be a disadvantage. The pressure tubing leading from the distal end of the manometer to the tambour (*Tb*) is not attached to the manometer until after the rise of the mercury in the distal tube following the removal of the clamp (*Cl*). After the tambour is attached to the manometer every movement of the mercury is shown by corresponding movements of the tracing point (*t*). Should there be a sudden rise or sudden fall of pressure, it will be instantly and clearly shown by corresponding rise and fall of the tracing. Should there be, however, a very gradual rise or a very gradual fall, owing to physiological changes in tonus of the blood-vessels, for example, or, perhaps, by the gradual action of some drug on the animal under observation, this will not be shown by corresponding rise and fall of the tracing. It will, however, be shown by the stand of mercury in the distal limb of the manometer, and this may be easily read off or noted from time to time.

To offset this slight disadvantage one has the two advantages above mentioned, namely, accuracy of the tracing of all the quicker movements of the mercury and the assurance that one's apparatus will always work.

14. THORACIC CANNULÆ.

A cannula for transmitting the air pressure from the pleural or mediastinal or abdominal cavity may be easily constructed as follows: Take a piece of ordinary soft and thin-walled glass tubing about 10 cm. in length and 3 cm. lumen. Grind one end diagonally sharp as shown in Fig. 88, Can.

15. THE STETHOGRAPH.

In order to record graphically the movements of the chest one may use various mechanical devices. The most simple device, and a most

effective apparatus, when only the time relations and the character of movements are matters of concern, is the instrument which involves the use of two tambours, a receiving and recording tambour. The latter one is described above (12).

A receiving tambour may be constructed especially for this purpose as follows: Take a large thistle tube, cut off its funnel with 8 or 10 cm. of the tube, stretch across it loosely a piece of thin sheet rubber and fasten this tightly as shown in Fig. 88, Steth. To the middle of the rubber diaphragm fasten a long cork, using glue or sealing-wax.

The stethograph complete consists of a thoracic frame, as shown in Fig. 58, and of the tambours, the recording tambour being held by an extra support as usual.

The thoracic frame is very simply constructed of pieces of half-inch glass-pipe supported by a heavy stand and clamps as shown in the figure.

The receiving tambour is held in a clamp, its location upon the bar being readily adjusted. The width of the frame and its height are both easily adjustable.

16. THE CHEST PANTAGRAPH.

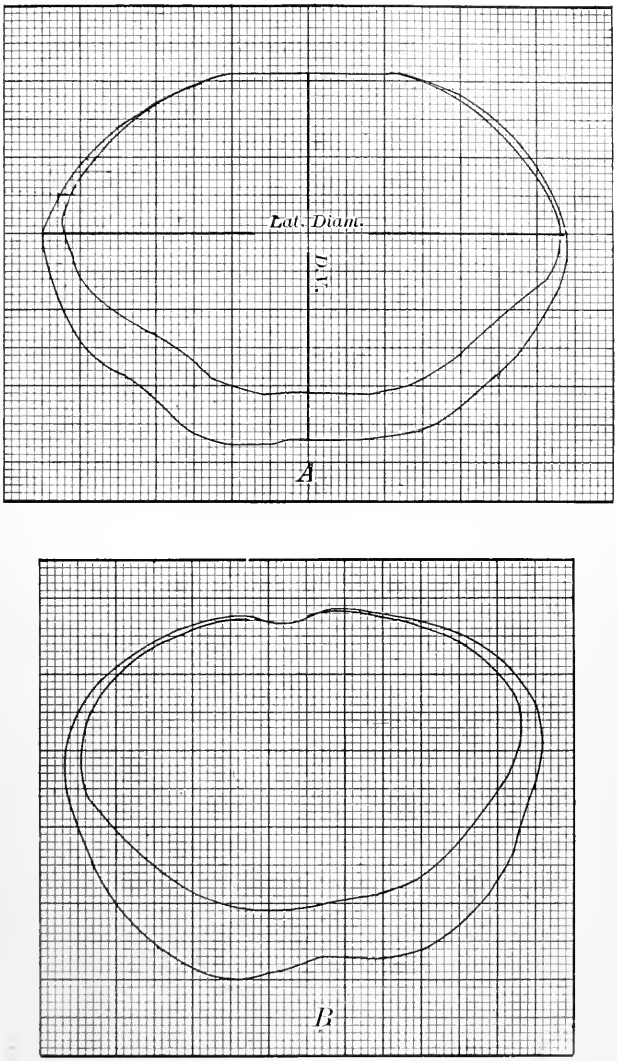
Fig. 59 shows the instrument, which is constructed of brass or wood with brass or steel semicircle. The joints *a*, *b*, *x*, and *y* move easily in the plane of the instrument. The semicircle, 40 inches in diameter, rotates at *x* around the diameter *t x*. The point *f* is fixed to a table. With *f* a fixed point all movements of *t*, the tracing point, are accompanied by corresponding movements of *r*, the recording point. The triangles *f*, *r*, *b* and *f*, *t*, *a* are similar triangles in all positions of the instrument *fb* : *fa* : : *fr* : *ft*; but *fb* : *fa* : : 1 : 5; therefore, the distance *fr* is always one-fifth the distance *ft*.

The object of the semicircular arm is, of course, to permit the tracing point *t* to be carried around the thorax. The seat upon which the subject sits is adjustable in height and back and side supports for the waist, so that the upper part of the body is not allowed to waver from side to side, distorting the contour. If the subject to be examined sit beside the table on which the instrument is fixed; if the seat be adjusted in height to bring the plane of the thorax to be examined into the plane of the instrument—*i. e.*, on a level with the top of the table; if a sheet of millimetre paper be fixed to the table under the recording pencil *r*; and if the tracing point *t* be swept around the thoracic wall, a record of the chest contour will be traced upon the paper.

The accompanying Fig. 89 shows two such contours from healthy, well-developed young men. Two millimetres in the figure equal one

centimetre of actual measurement. The inner contour is that of forced expiration, while the outer one is that of forced inspiration.

FIG. 89



Contours of chests, taken with chest pantagraph.

In contour *A* the increase of lateral diameter by forced inspiration is 2 cm., while the increase of dorsoventral is 3 cm. In the same

contour the cross-sectional area of the thorax in the plane of the ninth rib is represented by 25.52 of the larger squares containing 25 square cm.; total area equals 637.5 square cm., while the cross-sectional area of the chest in forced expiration is 517.5 square cm. Forced inspiration shows an increase of 120 square cm., or about 23 per cent., over cross-sectional area of forced expiration. Furthermore, both contours show a prominence in the right side (left of the figure), possibly due to stronger musculature on that side.

17. THE PNEOMANOMETER.

This instrument may be easily constructed in the laboratory. Take a piece of heavy glass tubing 7 to 9 mm. lumen and at least 160 cm. in length. Bend it as shown in Fig. 61. A covered filter may be attached as shown in the figure if there is any tendency for the mercury to be thrown out.

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