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LABORATORY MANUAL

OF

GENERAL PHYSIOLOGY

BY

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PREFACE

The experiments described in this manual have been selected primarily to meet the interests of students who are taking physiology for the first time. With an almost unlimited number of experiments from which to select, care was taken to include only those experiments which had been found by experience to be workable and not too difficult and yet significant. In order to offer a rather wide selection, more experiments than can ordinarily be performed in a one-year course are included. In some colleges, teachers of general physiology prefer to introduce fundamental experiments in biochemistry into the course, since it seems necessary for a student to understand certain of the elements of biochemistry in order to appreciate fully many of the problems of general physiology. A number of such experiments have therefore been included.

This manual was designed primarily to be used in connection with the textbook "General Physiology" by Mitchell. Numerous references are made to discussions in this textbook. However, the manual may be used equally well in conjunction with other textbooks of general physiology.

The experiments have been selected and adapted from many different sources. In many cases the original source is unknown to the authors. Some of the sources that have been used are: "Elementary Course in General Physiology" by Scarth and Lloyd, "Practical Physical and Colloid Chemistry" by Michaelis, "A Laboratory Course in Physiology" by Cannon, "Practical Physiology" by Burton-Opitz, "Experimental Physiology" by Visscher and Smith, "Laboratory Experiments in Physiology" by Zoethout, "Laboratory Outlines in Comparative Physiology" by Rogers, "Introduction to Biophysics" by Burns. Experiments have also been chosen from laboratory directions prepared by Professor J. H. Bodine, by Professors W. H. Cole and J. B. Allison, and by others. The very real assistance received from these authors is gratefully acknowledged.

The authors will welcome any criticisms and suggestions that may be offered.

PHILIP H. MITCHELL,
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PROVIDENCE, R. I.,
May, 1938.

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INTRODUCTORY DIRECTIONS

1. The directions for the experiments for each laboratory session should be read in advance before attendance at the laboratory. References to discussions in the text, included in the laboratory directions, should also be read at that time. This procedure will make more time available for actual experimentation in the laboratory. The purpose of the experiment and the procedure involved should be understood before the experiment is commenced.

2. If working cooperatively in pairs, make sure that each partner sees all the results for himself.

3. Keep a notebook for recording observations in the laboratory and in it include diagrams of apparatus, special directions, results, etc. In addition, keep a better notebook in which the experiments will be written up in final form. Graphical records and tables of results should be included when possible. This notebook should be neat and well edited. Suggestions regarding the editing of the notebook and a schedule indicating the dates on which the book is to be handed in for inspection will be furnished. If possible, use a typewriter when preparing the notebook.

4. Arrange your apparatus neatly and keep it in good order. Screws, nuts and other parts that come loose or are removed should be put back in place. Report any breakage that may occur.

5. Avoid contamination of stock solutions and return solution bottles and equipment to their proper places as soon as they are no longer needed.

6. When not using the laboratory, keep your lockers and drawers locked.

7. Keep your working space clean and place discarded animal material in the special container that is provided.

8. Work thoughtfully and observe closely. Take as much time as is necessary to perform the experiments well.

LABORATORY MANUAL OF GENERAL PHYSIOLOGY

CHAPTER I

EXCITATION AND INHIBITION

1. The Frog Muscle-nerve Preparation.—Pith a frog in the following manner. Hold the animal in your left hand and press the head downward with the index finger so as to bend the body of the frog at the occipitovertebral junction. In this region note, on the mid-line and between the ears, a depression that can be readily found with the point of a probe. With a pair of sharp-pointed scissors cut a small hole through the skin just above the depression. Insert a pithing probe into this opening and sever the spinal cord. Quickly run the probe forward giving it a rotatory motion in such a manner as to destroy the brain first on one side and then on the opposite side. Then push the probe down through the center of the vertebral column to destroy the spinal cord.

Place the animal on a frog board and with scissors cut the skin completely around the lower part of the foot of one of the hind legs. Hold the cut foot with one hand and extend the cut along the ventral side of the leg and up to about the middle of the abdomen. Carefully remove the skin from the leg and discard it. The outer surface of the skin should not be allowed to come in contact with the muscles or nerves because of a toxic secretion. Place the dorsal side of the animal upward, lift the posterior tip of the urostyle with forceps and carefully cut the muscles along the sides of the urostyle. Cut off the urostyle at its anterior end being careful not to injure the nerves below. Avoid touching the nerves with metal instruments and manipulate them only by means of glass nerve hooks. Unnecessary stretching of the nerves is to be avoided. Carefully dissect out the sciatic nerve to the gastrocnemius muscle. Sever the sciatic nerve close to the spinal cord and keep it in contact with the moist tissue of the animal to prevent it from drying.

Next carefully dissect out the gastrocnemius muscle and its tendon of Achilles as follows: Cut across the tendon at its extreme lower end and sever the connective tissue between it and the ankle bones. Separate the gastrocnemius muscle from the rest of the lower limb by cutting the small amount of connective tissue that holds it. Cut transversely

across both the tibia and the fibula close to the knee. Now lay the sciatic nerve onto the gastrocnemius muscle and, with great care to avoid the nerve, cut off the thigh muscles from the femur. Sever the femur across its middle part with one clean cut of the scissors being careful not to splinter the bone. Figure 2 illustrates the appearance of the muscle-nerve preparation. The femur and the tendon respectively are used for attaching the preparation to a bone clamp and a muscle lever.

Wrap the muscle-nerve preparation in some absorbent cotton or filter paper soaked with Ringer's solution to prevent it from drying until you are ready to use it. During the course of an experiment suit-

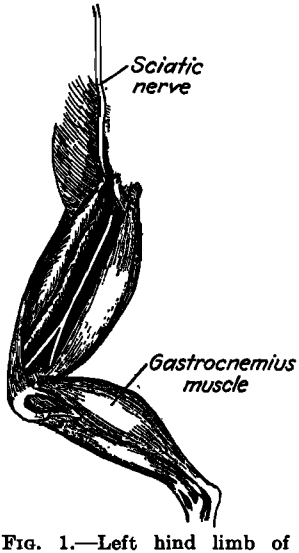


FIG. 1.—Left hind limb of frog.

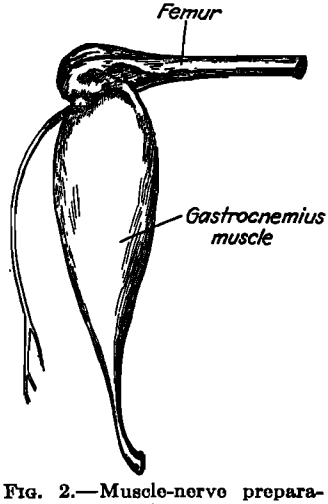


FIG. 2.—Muscle-nerve preparation.

able precautions should be taken to prevent the drying of the muscle and the nerve. In some experiments, Ringer's solution may be applied as necessary to the surface of the preparation by means of a medicine dropper.

2. Types of Stimuli.—Read the discussion dealing with stimuli in the text, pages 5 to 11. (Hereafter "the text" will refer to **General Physiology** by P. H. Mitchell, third edition.) Such reading, which will be suggested in connection with various experiments, should be done for each week's work before attending the laboratory class.

Fasten the muscle-nerve preparation to a muscle clamp by placing the femur in the clamp. Attach the clamp to an iron stand. Adjust the level of the clamp so that the nerve may lie on the bottom of a beaker placed mouth downwards.

a. Mechanical Stimuli.—Pinch the *end* of the nerve with a pair of forceps. Does the muscle respond? Strike the end of the nerve with a small glass rod. Is this an adequate stimulus?

b. Thermal Stimuli.—Touch the end of the nerve with a glass rod applying the rod gently. Is there a response? Now heat the rod and apply it as before. What is the result?

c. Chemical Stimuli.—Cut off the part of the nerve that has been used previously. Put a drop of *N* HCl on the end of the nerve. Does the muscle move? Wash off the acid with Ringer's solution and cut off the part of the nerve used.

d. Osmotic Stimuli.—Put some crystals of NaCl on the end of the nerve. Does this stimulate it? The removal of water from the nerve by the application of glycerin or by drying will sometimes stimulate it. Sometimes the twitching of the gastrocnemius muscle during an experiment is due to the drying of the nerve and can be prevented by keeping the nerve moist.

e. Electrical Stimuli.—Until ready to do the experiment, wrap the muscle-nerve preparation in a piece of absorbent cotton moistened with Ringer's solution.

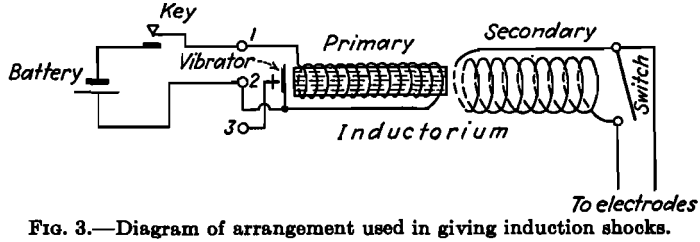


FIG. 3.—Diagram of arrangement used in giving induction shocks.

In making this test employ an inductorium, which consists essentially of a primary coil having relatively few turns of large insulated copper wire and a secondary coil having many turns of finer wire. When a current of changing intensity flows through the primary coil, the magnetic field around the coil varies, and a current is induced in the secondary coil. This effect may be obtained by starting or stopping the flow of current in the primary. A 1.5- to 4.5-volt battery is usually employed as a source of current for the primary coil, but a much higher voltage is delivered by the secondary coil. The voltage and the amperage of the secondary constantly fluctuate and their peak values may be regulated in various ways, depending upon the design of the particular inductorium employed. The instructor will explain the operation and use of the inductorium at a suitable time. In Fig. 3 is shown a diagrammatic representation of an inductorium with a simple key and a battery connected in series with the primary. Stimulating electrodes and a short-

circuiting switch are shown connected to the terminals of the secondary. When the short-circuiting switch is closed, no shock will be delivered by the electrodes, and this arrangement is convenient when one wishes to eliminate either a "make" or a "break" shock.

A single "make" shock is obtained from the secondary upon the "make" of the primary circuit, and similarly a "break" shock results from the "break" of the primary circuit. Such simple "make" and "break" shocks may be obtained when the leads from the battery are connected to the primary terminals indicated in the diagram as 1 and 2. When the leads from the battery are connected to the terminals labeled 1 and 3, closure of the key will result in a rapid series of "makes" and "breaks" of the primary circuit due to the action of the vibrating interruptor, which then comes into play. This arrangement automatically gives a rapid series of shocks (many per second) from the secondary coil and is employed to furnish a "tetanizing" current. One type of inductorium is provided with a switch that makes it possible to change quickly from simple induced shocks to tetanizing shocks. A built-in key for "making" and "breaking" the primary circuit is also a feature of one type of inductorium.

Connect a simple key and a dry cell in series with the primary of an inductorium so as to obtain single induced shocks. If the inductorium has a built-in key the extra key is unnecessary. Attach stimulating electrodes to the secondary terminals. It is to be noted that a shock is obtained only on the "make" or on the "break" of the primary circuit and not when the current is flowing steadily through the primary coil. Also the shock obtained on the "break" is always greater than the shock given on the "make" of the primary circuit. With the inductorium adjusted to furnish a "make" shock of medium intensity apply a "make" shock to the nerve after removing the absorbent cotton. What is the result? Repeat using a "break" shock. Does the response appear to be greater?

3. The Independent Excitability of Muscle and the Action of Curare. Pith a frog, cut through the skin of the dorsal part of the thigh of one leg and separate the muscles so as to expose the sciatic nerve. With a glass nerve hook, raise it gently, pass a stout thread under it and tie the thread firmly around the thigh so that all its structures, except the sciatic nerve, will be compressed. This stops the circulation of the blood below the ligature.

Inject 1.0 cc. of a 0.5 per cent solution of curare in 0.7 per cent NaCl into the dorsal lymph sac. Curare is very poisonous and care should be taken to avoid getting some of the solution into cuts on the fingers. Wait half an hour for the poison to take effect on the frog. No reflex movements should result now when the skin of the unligatured leg is

touched but they should occur when the skin on the ligatured leg is stimulated in this way. Explain.

Expose the sciatic nerve of both legs and stimulate each nerve with induction shocks using platinum electrodes. How do the results differ? Explain.

Expose the gastrocnemius muscle of the unligatured leg. Apply the electrodes to it and stimulate directly, *i.e.*, without the use of the nerve. What is the result? Was the muscle deprived of excitability by curare?

How do these observations show the independent excitability of muscle and how do they show what part of the muscle-nerve structure is acted on by curare to produce the paralyzing effect? Does curare appear to affect the central nervous system?

4. The Threshold Stimulus.—Connect a dry cell to the primary of an inductorium and include a simple switch or key[1] in series with the battery and the primary coil. Note that, to avoid undue drainage on the battery, the switch or key should be kept open except when the inductorium is in actual use. Attach stimulating electrodes to the secondary terminals. Adjust the inductorium to give its minimum shock.[2]

After making sure that the electrodes are clean, apply them to the tip of the tongue and "make" the primary circuit and a moment later "break" it. Is any shock felt on the tongue? Now slightly increase the intensity of the induced current by adjusting the inductorium. Again "make" and then "break" the primary circuit. Record the setting of the inductorium at each trial and the ability or failure to perceive shocks. Continue these tests, slightly increasing the intensity of stimulation *after each test at a certain adjustment of the inductorium.* Which shock becomes evident first, the make or the break? At what adjustment of the inductorium is the threshold stimulus (see text, page 7) obtained in each of these two cases? Ask the instructor to explain why the break shocks are greater than the make shocks in terms of induction if you are unable to do this.

5. The Graphic Method.—Examine a kymograph and learn to start and stop the drum and to regulate its speed. Remove the drum

[1] If the inductorium has a built-in key do not include another key or switch.

[2] Harvard Apparatus Co. inductorium or similar type: Slide the secondary coil away from the primary coil as far as possible and set it at an angle. The intensity of the shock is increased by decreasing the angle at which the secondary is placed and by decreasing the distance between the primary and secondary coils. When increasing the intensity of stimulation first decrease the angle until both coils are in the same plane and then decrease the distance between the coils. Note the graduations on one of the slide rods.

Phipps and Bird, Inc., inductorium or similar type: The intensity of the shock is simply regulated by means of a coarse-adjustment switch and a fine-adjustment rheostat with a graduated dial.

with its supporting rod or tube from the kymograph. Always be careful not to drop the drum or allow it to roll off the table, since the aluminum walls are easily dented and bent. Place a piece of kymograph paper on the table with the glazed surface downward and with one end toward you. Apply paste or glue along the end that is farthest from you and place the drum on the paper with the top towards the right. Wrap the

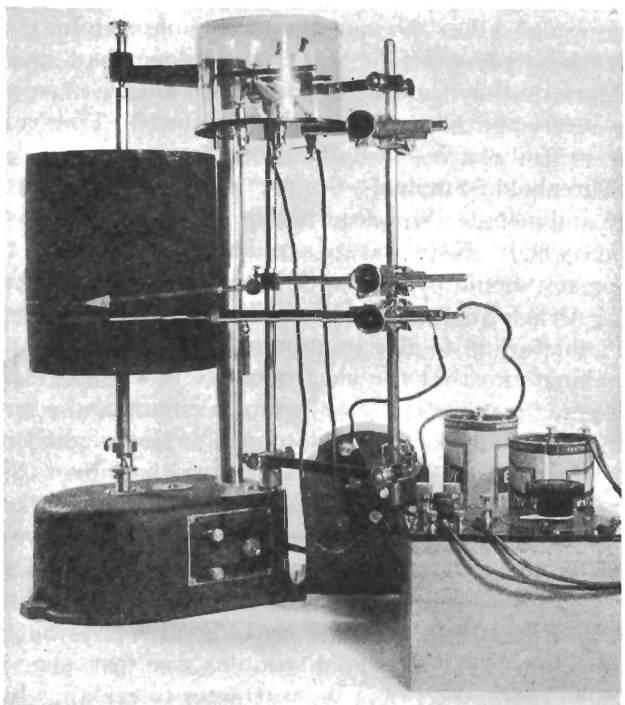


FIG. 4.—Apparatus for the graphic method.

paper tightly around the drum and seal the ends together. This procedure ensures that the ends of the paper overlap correctly.

Smoke the paper on the drum by holding the supporting rod in a horizontal position with both hands and rotate the drum in a smoky flame. A thin even layer of soot should be deposited. Return the drum to its position on the kymograph.

On an upright support mount a moist chamber, a light muscle lever, with a flexible parchment-paper or cellophane point that curves or bends toward the drum, and a time marker, as illustrated in Fig. 4. Always mount the apparatus so that the muscle lever will trace the record on the left side of the drum. This is done to facilitate the smooth movement of the point of the lever, since the drum rotates in a clockwise direction.

The lever should be placed at a tangent to the drum. Mount in the moist chamber two nonpolarizable (boot) electrodes that are well impregnated with Ringer's solution.[1]

Arrange an inductorium to give simple make and break shocks and connect the secondary terminals to the nonpolarizable electrodes. Obtain a frog muscle-nerve preparation and tie a piece of thread or fine bare wire to the tendon of Achilles, taking care not to tie onto the muscle tissue. Mount the preparation in the moist chamber by means of the muscle clamp and attach the thread or wire to the muscle lever. Adjust the height of the drum so that the muscle lever will write on the lower part of the drum. Attach a small weight (about 10 grams) to one end of a piece of thread or fine wire and pass the other end through the hole in the grooved pulley on the muscle lever and then tie, allowing the weight to hang 2 or 3 in. below the lever. Make sure that the thread or wire rests in the groove on the half of the pulley that is nearest the writing point. Bring the point of the lever to bear lightly on the surface of the smoked paper near the junction of the two ends of the paper. Make sure that the axis of the pivot of the writing lever is exactly horizontal so that the point of the lever will move vertically and not jam against or leave the paper. The movement of the lever may be tested by raising the lever with the finger near the pivot. This precaution will save much wasted effort in this type of experiments.

Adjust the after-loading screw on the muscle lever so that with the lever pointing slightly below horizontal the weight of the lever is entirely supported by the muscle, and the after-loading screw just fails to touch or support the lever. This ensures that the muscle will begin to raise the lever the instant that it commences to contract without first having to take up any slack in the thread connecting the lever and the muscle.

Set the time marker to make a mark every second or every 5 sec. Lay the nerve across the nonpolarizable electrodes using a glass nerve hook and avoiding undue stretching. Using maximum break shocks in every case, record the response of the muscle to electrical stimulation of the nerve. Make several records of the response for each of four different speeds of the drum.

Remove the drum from the kymograph and with a knife blade held with the flat side towards the drum cut the paper carefully along the sealed junction where the ends meet and place the paper bearing the

[1] To prepare the electrodes for use half-fill each one with saturated zinc sulphate solution and immerse a small zinc electrode in the solution in each boot electrode. A copper wire is then connected to each zinc electrode. It is advisable during lengthy experiments to moisten the surfaces of the nonpolarizable electrodes with Ringer's solution occasionally to prevent drying. After use the nonpolarizable electrodes should be well rinsed with Ringer's solution.

records flat on the table. In cutting the paper, great care should be taken not to scratch the soft surface of the drum. The use of a knife is unnecessary if, when the paper is first put on the drum, a piece of thread is inserted between the drum and the paper at the sealed margin so that the ends of the thread project about an inch at the top and bottom of the drum. When the paper is to be removed, the lower end of the thread is held, and the upper end is pulled so as to cut the paper.

With the point of a dissecting needle, neatly print your name, the date, the number and title of the experiment and any other data that you wish to record. Permanently fix the record by passing it through a bath of shellac diluted with alcohol and hang it up to dry. The details of technique learned in this experiment should be remembered and applied in later experiments.

6. Effect of the Strength of Stimulus on the Height of Skeletal-muscle Contraction.—Set up the apparatus as described in (5) but eliminate the time marker. Mount a muscle-nerve preparation in the moist chamber and use a piece of fine wire (about No. 32 Brown and Sharpe gauge, brass) to connect the tendon with the muscle lever. After the proper setting of the muscle lever in its initial position has been established, rotate the drum to trace a "base line." Using single make and break shocks, stimulate the nerve and record the height of contraction on a stationary drum under the following conditions. Find the threshold stimulus with break shocks by adjusting the inductorium. With the inductorium set at this position and using a stationary drum, make a series of trials with successively increasing strengths of stimuli using both make and break shocks alternately and recording the height of contraction of the muscle in each case. At first there will be no response to the make shocks, but after the threshold value for these shocks has been reached responses will be obtained. Turn the drum about 5 mm. after each test so that the different records will lie close together yet distinct from each other on the smoked paper. It is advisable to employ a uniform time interval of about 15 sec. between successive stimuli. After removing the paper from the drum, indicate by means of *M*'s and *B*'s, respectively, the responses due to the make and break shocks. Label completely and fix the record.

Repeat the experiment but this time arrange to stimulate the muscle directly as follows: Remove the wires attached to the nonpolarizable electrodes and connect one of them to the muscle clamp in the moist chamber and the other to the binding post on the muscle lever. With a short loop of fine wire connect the binding post to the bottom of the wire attached to the lever and tendon. Be sure that the electrical connections are well made and not liable to become loose. Label and fix the record as before.

How is the height of contraction related to the strength of stimulus? Is there a maximum stimulus beyond which further increases cause no increase in the height of contraction (see text, page 15)? How are the results explained without contradicting the "All or nothing" principle? (See text, page 14.)

7. Summation of Subminimal Stimuli.—A subminimal stimulus is one that calls forth no visible response, *i.e.*, it is below the threshold value. With the apparatus and muscle-nerve preparation arranged as in the last part of Exp. 6, find the threshold stimulus for a break shock and then adjust the inductorium so as to obtain a break shock that just fails to produce a response of the muscle. Then by rapidly tapping the key send in a series of make and break shocks without changing the adjustment. Be sure to adjust the after-loading screw on the muscle lever so that there is no slack in the wire connecting the lever and the tendon when the point of the lever is on the base line. What is observed? (See text, page 21.)

8. Electrotonus.—Before doing this experiment read the discussion of electrotonus in the text on page 22.

Place a muscle lever and a moist chamber with four nonpolarizable electrodes on a support. Connect two dry cells together in series by joining the positive (center) pole of one battery to the negative (outer) pole of the other. Each dry cell furnishes 1.5 volts, and the two batteries connected in series deliver 3.0 v. Connect the two batteries to the pair of nonpolarizable electrodes nearest the muscle clamp in the moist chamber by running one wire from the free negative pole of the one battery to the electrode next to the muscle clamp and another wire from the free positive pole of the battery to the adjacent electrode. Later when the nerve is laid across these electrodes a current (the polarizing current) will then pass through it in a downward direction (toward the muscle).

Connect a dry cell to the primary of an inductorium including a key in series with the battery. Run two wires from the secondary terminals to the two nonpolarizable electrodes not already connected in the moist chamber.

Mount in the moist chamber a fresh muscle-nerve preparation from a frog. In making the dissection have the length of the sciatic nerve as great as possible. Connect the tendon to the muscle lever arranged to write on a smoked drum and lay the nerve on all four of the nonpolarizable electrodes. Note the contraction of the muscle when the nerve is first placed on the electrodes connected to the battery furnishing the polarizing current. During the steady passage of the current, however, there is no contraction. The state of the nerve is nevertheless altered, and the phenomenon of electrotonus occurs. This change is shown as follows:

a. By an Altered Power of Conduction of Excitation.—Test this by finding the threshold stimulus for single induction shocks (use break shocks) applied to the nerve through the pair of electrodes farthest from the muscle, without having the polarizing current (from the two dry cells) passing down the nerve. Next test with the polarizing current on. What difference do you find? Repeat with the polarizing current off. How closely does this check with the first test? Does this constant current act as a partial or complete nerve block? If there is a complete nerve block, repeat the test using a single dry cell to furnish the polarizing current.

b. By an Altered Degree of Excitability.—Test this during the passage of the polarizing current by finding the threshold for electrical stimulation at the positive electrode (anode), at the negative electrode (cathode) and at the midpoint between them. For this test employ platinum electrodes set close together in applying the shocks from the inductorium. The threshold for electrical stimulation may be reported here in terms of the setting of the inductorium for each threshold stimulus. What is the characteristic of anelectrotonus and of catelectrotonus?

9. Stimulation of Nerves of the Arm.—For the stimulation of human nerves and muscles near the surface of the body it is convenient to use the electric current. Ordinarily electrodes may not be applied directly to the nerve or muscle to be stimulated. However, by properly applying electrodes to the surface of the skin, tissues underneath may be stimulated. It is customary to use a metallic electrode of large area to form a broad (indifferent) electrode on one side of the limb. On the opposite side of the limb a metallic rod with a rounded point forms the other electrode, and stimulation takes place in the region of this electrode, because the current density is greatest near it. A piece of cotton moistened with a strong NaCl solution is inserted between each electrode and the skin. In passing through the tissue the current spreads out and diverges as it goes from the small to the large electrode or becomes more dense and converges as it passes from the large to the small electrode. This is illustrated in the accompanying diagram. The greatest stimulating effect is obtained at the point of greatest current density.

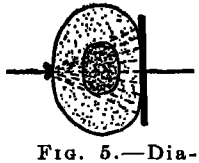


FIG. 5.—Diagram showing the path of the current in passing through the arm between a large and a small electrode.

When current is passed through the arm or other organ, the nerves are more responsive to the current than the muscles because the nerves are more irritable, *i.e.*, have lower thresholds of stimulation. Points are found on the surface of the skin where the small electrode can stimulate the nerves more readily than at other points. The former are known as the *motor points.*

Locate some motor points in the following manner: Connect a dry cell to the primary of an inductorium so as to obtain a tetanizing current. By means of sufficiently long wires attach the small and the large electrode to the secondary terminals. With a piece of absorbent cotton rub some strong NaCl solution over the skin of the inner surface of the forearm. Place the arm on the table with the palm of the hand upwards so that the arm rests on the large electrode. Insert a pad of absorbent cotton wet with the salt solution between the electrode and the arm. Wrap a small piece of absorbent cotton wet with the saline solution around the small electrode to cover its point. With the inductorium in operation, touch the skin on the inner surface of the forearm at various points with the tip of the stimulating electrode to locate motor points. Locate a point for flexion of the thumb and two points for flexion of the fingers. Explore various areas on the forearm. Find the motor point for the ulnar nerve at the elbow. Make a drawing showing where several motor points are located.

10. The Demarcation Current (Current of Injury).—Before doing this experiment read the discussion in the text on page 26.

In this experiment the demarcation current will be detected by means of a capillary electrometer, and then the injury potential will be measured with a potentiometric outfit.

As will be seen in Fig. 6, the capillary electrometer consists essentially of a column of mercury and a column of sulphuric acid solution in contact with each other in the capillary tube of a glass electrometer vessel. Purified mercury is used, and the acid solution is prepared by adding 5.8 cc. of distilled water to each 10.0 cc. of sulphuric acid (specific gravity 1.84). The electrometer is mounted vertically on a stand supporting a microscope by means of which the meniscus in the capillary may be viewed. A source of light is placed behind the electrometer. Owing to a change in the surface tension of the mercury the meniscus moves up or down depending upon the direction of the applied potential.

Two insulated wires lead from the electrometer terminals to the terminals of a short-circuiting key or switch whose contact exhibits a minimum amount of friction. The key or switch must be kept closed at all times, except when the instrument is in actual use, to prevent the loss of sensitivity and the formation of mercurous sulphate, which may clog the capillary. Two wires from the short-circuiting switch lead to the nonpolarizable electrodes, which are prepared later. A simple key is placed in one of these leads. The electrical connections are shown in the diagram. Only well-insulated wires and keys or switches should be used.

When a potential such as from a tissue is to be applied to the electrometer, open the short-circuiting switch, a second or two before applying

the potential by closing the key in the lead to the electrode. Immediately after the observation close the short-circuiting switch. Test the electrometer by opening the short-circuiting switch. The meniscus should show little or no movement when this is done. If the electrometer appears to lack sensitivity when in use the meniscus should be renewed by the instructor.

Mount two nonpolarizable electrodes in a moist chamber. These porous electrodes should be clean and impregnated only with Ringer's

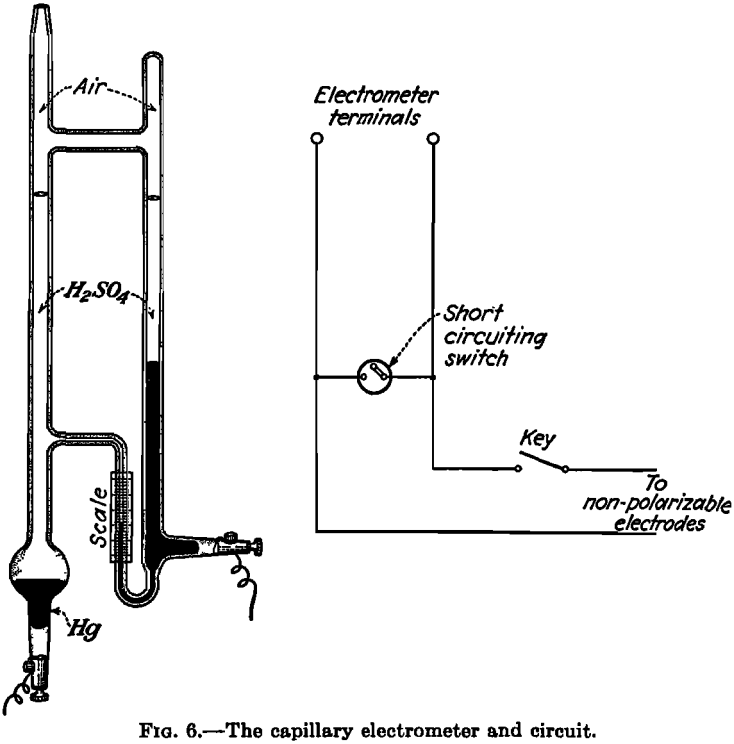


FIG. 6.—The capillary electrometer and circuit.

solution before the saturated zinc sulphate solution is placed in the leg of the boot in preparation for use. The zinc sulphate solution must not be allowed to come in contact with the outside surface of the electrode, as it is injurious to living tissues. When finished using the nonpolarizable electrodes be sure to wash them out with tap water followed by distilled water and return them to Ringer's solution.

For this experiment the electrodes should be isoelectric, *i.e.*, of the same potential. First, test them by placing their tips in contact with each other and, by means of a capillary electrometer or a potentiometric outfit, determine whether or not they are isoelectric. If they are iso-

electric a capillary electrometer connected to them will show no movement of its mercury meniscus and, if a potentiometric outfit is employed, the potential difference will be observed to be zero. If the electrodes are not isoelectric, kaolin moistened with Ringer's solution should be applied to their surfaces. If an e.m.f. is still obtained, select and test fresh electrodes.

When you are certain that the electrodes are isoelectric, separate them so that they are 1 cm. apart. Carefully dissect out the gastrocnemius muscle from a frog, avoiding injury as much as possible, and do not apply any salt solution to the muscle. With scissors cut completely through and transversely across the muscle near the femur. This forms an injured end, which is then placed so that the injured surface is in good contact with one electrode. An uninjured part along the side of the muscle is placed on the other electrode. The tendon should not be allowed to touch the electrode.

Apply the potential from the injured muscle to the electrometer and observe the meniscus. Note the direction in which the meniscus moves. Remove the muscle from the electrodes but this time reverse and place it back on the electrodes so that the cut end is now on the other electrode. Does the meniscus move in the same direction as before, when the potential is applied to the electrometer? Explain. Repeat these tests using a piece of sciatic nerve.

Repeat all the above tests but use a potentiometric outfit instead of the electrometer and actually measure the magnitudes of the potentials obtained. For this work the Youden pH potentiometer manufactured by the W. M. Welch Scientific Co. for use with the quinhydrone electrode is very convenient (see Exp. 145). The instructor will explain the use and operation of the particular potentiometric outfit that is to be employed.

11. Stimulating Action of the Demarcation Current.—After pithing a frog cut through the skin of the thigh and lower part of the abdomen so that the sciatic nerve may be dissected out and left connected with the gastrocnemius muscle. Make this dissection as carefully as possible to avoid injuring the surrounding tissues. With a clean cut across the thigh remove the leg from the body of the frog after making sure that the sciatic nerve will not be injured. By means of a nerve hook, place the sciatic nerve on the cut surfaces of the muscles and loop the nerve so that the cut end of the sciatic touches the uninjured surfaces of the thigh muscles. Make and break the contact between the cut end of the nerve and the muscles five or six times by lowering and raising the nerve hook. What is the result? In what direction does the stimulating current flow? Explain.

12. The Action Current.—Before performing this experiment read the discussion in the text on page 25.

Carefully dissect out two frog muscle-nerve preparations and place them on a clean porcelain or glass test plate. Do not apply any Ringer's or other solution to them. Lay the nerve of one preparation across the muscle of the other so that it forms a loop as shown in Fig. 7.

Extend the nerve of the other preparation out in a straight line and stimulate it with adequate make and break shocks from an inductorium. What happens to both muscles? Stimulate the same nerve at its end with an adequate but mild tetanizing current. Do both remain contracted during stimulation? Explain the contraction of the muscle whose nerve was not stimulated with induction shocks.

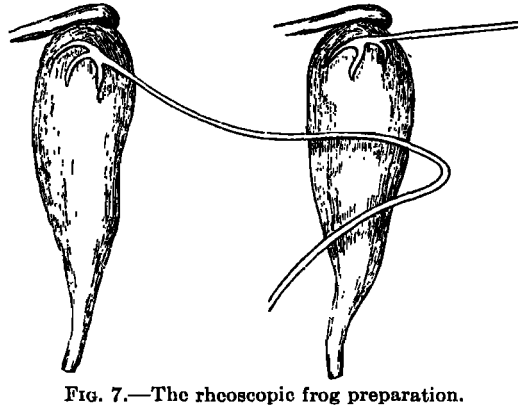


FIG. 7.—The rheoscopic frog preparation.

13. The Velocity of the Nerve Impulse.—Read the discussion in the text on pages 26 and 27 before doing this experiment.

a. Set up a spring myograph with smoked paper on the plate. Place the two left legs of the myograph against a strip of wood clamped to the table so the myograph will not move when in operation. Push the plate against the spring until the trigger-key mechanism engages with the catch that holds the plate frame from springing back. Adjust the position of the trip switch at the base of the myograph so that the handle of the switch almost touches the leg at the left end of the frame supporting the plate. Then press the trigger key to release the plate and, if necessary, adjust the spring so that the plate will move at a moderate rate when released. Arrange an inductorium with the primary circuit wired for single shocks and so connected to the trip switch on the myograph that the excursion of the plate will automatically trip the switch and produce a single break shock. If the inductorium has a built-in key for the primary circuit, the inductorium key should be kept closed throughout this experiment. The trip switch simply takes the place of the key shown in Fig. 3 (Exp. 2). Connect platinum electrodes to the secondary terminals of the inductorium.

Attach a muscle lever and a moist chamber with two nonpolarizable electrodes to a heavy stand in front of the myograph. These electrodes simply serve later to support the nerve. Make a frog muscle-nerve preparation using an animal of fairly large size and dissecting out the full length of the nerve. Mount the preparation in the moist chamber extending the nerve full length across the nonpolarizable electrodes. Attach the tendon to the muscle lever, which is arranged so that its point will bear lightly on the smoked paper at the lower left end of the plate. Make sure that the after-loading screw on the muscle lever is properly adjusted.

After closing the trip switch, apply the platinum electrodes, with the points set close together but not touching each other, to the nerve quite close to the muscle. Note this point on the nerve. Release the plate of the myograph by pressing the trigger key and thus obtain a curve of muscle contraction. It is necessary to obtain only a record of the first half of the curve of contraction.

Move the muscle lever slightly away from the myograph plate and return the plate to its original position. With the platinum electrodes removed from the nerve, again release the plate so that the point of the lever traces a line that, during the first part of its course, is coincident with the curve previously traced. This gives a "base line." Note that the point where the two lines part shows the beginning of muscle shortening. Again return the plate to its original position, being careful to avoid contact with the lever.

Examine the point of the muscle lever to make sure that it is exactly at its original position. Now apply the platinum electrodes carefully to the end of the nerve farthest from the muscle. Note this point on the nerve. Again release the plate. A second curve is obtained, but it departs from the base line later than did the first. Explain. With a millimeter rule, measure the distance between the first point of stimulation near the muscle and the second point near the other end of the nerve, taking precaution to see that the nerve is fully extended but not stretched.

Move the moist chamber and other pieces of apparatus away from the myograph and return the plate to its original position. Clamp a tuning fork, having a frequency of vibration of 100 per second, to a stand so placed that the writing point attached to one prong of the fork will touch the smoked paper at the left of the plate and below the records already taken. Apply the tuning fork starter to the tuning fork and then remove the starter to set the fork vibrating and release the plate. This traces on the paper a wavy time line. Each wave length represents a definite time interval of $\frac{1}{100}$ sec.

Remove the record and label and shellac it. When dry draw a line, parallel to the base line and a little above it, across the rising parts of the

curves. From each of the two intersections of this line with the tw curves, draw a line perpendicular to the base line across the time li . You can now find the time interval required for the transmission of the nerve impulse between the two points of stimulation. Compute the velocity of the nerve impulse in the frog sciatic nerve at room temperature.

b. If a spring myograph is not available this experiment may be performed with the use of a kymograph that is adjusted so that the drum may be spun by hand. To do this, attach to a heavy upright stand moist chamber, with electrodes, and a muscle lever, as described above. Just below the muscle lever mount a signal magnet so that the points of both lever and magnet are exactly in the same vertical plane. On a separate stand close to the first stand mount a tuning fork having a parchment-paper point on one prong so that a time line may be obtained.

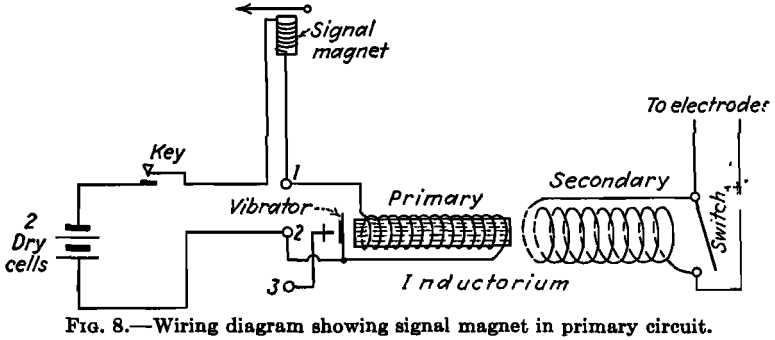


FIG. 8.—Wiring diagram showing signal magnet in primary circuit.

The point of the tuning fork should be below that of the signal magn Tie a piece of thread around the prongs of the fork so that the prongs a pulled together permitting the fork to be set in vibration when the thread is cut. Connect the signal magnet, including a simple key, and two dry cells in series with the primary circuit of the inductorium and so arrang as to give simple make and break shocks. To the secondary terminals of the inductorium connect platinum stimulating electrodes with the points set close together. The wiring diagram is shown above.

Mount a muscle-nerve preparation having a long nerve in the moist chamber as described above. Make sure that the nerve is kept moist with Ringer's solution. Press the key in the primary circuit and then, while your partner is applying the stimulating electrodes to the nerve close to the muscle, start the vibration of the tuning fork. Quickly spin the drum by hand and immediately release the key sending in to the nerve an adequate break shock.

With the muscle lever in its original position of rest, rotate the drum so as to establish a base line. The instant of stimulation is indicated by the signal magnet, and the instant of response by the muscle lever,

and the time interval between these two instants is the latent period. Note the duration of the latent period after each trial.

Repeat, but this time stimulate the opposite end of the nerve and measure the distance between the two points of stimulation as indicated in (*a*). In this second trial the latent period should be longer than in the first trial. The difference between these two latent periods gives the time necessary for the nerve impulse to travel between the two points of stimulation. Repeat these procedures several times and label and fix the records. When the records are dry draw lines on them as described in (*a*) and calculate the average value for the velocity of the nerve impulse.

14. Lillie's Iron-wire Model.—Obtain a piece of steel piano wire[1] bent in the middle to form a narrow, elongated U-shaped loop whose two ends protrude about an inch through separate holes in a cork. Fill a 50-cc. graduate cylinder with concentrated nitric acid (specific gravity 1.42) and fill another similar cylinder with nitric acid diluted with distilled water (specific gravity 1.20). Be very careful not to spill any of the acid on objects that may be damaged by it. Hold the wire by means of the cork and immerse most of it into the stronger solution of nitric acid, avoiding contact of the wire with the sides of the cylinder. Observe the surface of the wire. A film of iron oxide forms over the surface of the wire, and it then protects the wire from the acid. After this treatment for about 2 min., immerse the wire in the same manner in the weaker solution of nitric acid. This aids in increasing the sensitiveness of the passivated wire. Scratch the surface of the wire just below the surface of the acid solution with a piece of zinc rod while your partner closely observes the surface of the wire. In which direction does the surface reaction proceed? Repeat several times, transferring the wire from the one solution to the other as necessary, but always making the test with the wire in the weaker solution.

Treat the wire as before with the two solutions, hold it in the air and, while still wet with acid solution, scratch it with the zinc rod. Is the reaction faster or slower?

Test the wire for the existence of an action potential upon stimulation with the zinc rod, making use of the capillary electrometer for this purpose (see Exp. 10). Test the model to determine whether or not direct-current stimulation and faradic stimulation will cause action potentials (see the text on pages 31 to 34, inclusive).

15. The Effect of CO_2 and of Ether.—Mount a flat-jawed muscle clamp above a muscle lever on a stand and arrange for graphic recording. Connect platinum stimulating electrodes to two end terminals of a

[1] It is convenient to use a piece of No. 20 B. & S. gauge wire about 2 ft. in total length.

double-pole, double-throw switch and connect the two middle terminals of the switch to the secondary binding posts of an inductorium set to give simple make and break shocks. Dissect out a frog muscle-nerve preparation having a long piece of sciatic nerve. Clamp the femur in the flat-jawed clamp and connect the tendon to the writing lever in the usual way. Apply a small piece of filter paper wet with Ringer's solution to the inside surface of a glass gas chamber of the type illustrated below in Fig. 9. By means of a clamp, mount the gas chamber so that one of the holes near the bottom is close to and level with the femur. Pass the nerve through the two holes near the bottom and place the nerve on the electrodes. Plug up the space in the holes around the nerve with kaolin moistened with Ringer's solution. With light wires connect the two electrodes of the gas chamber to the two free end terminals of the double-pole, double-throw switch. Apply the platinum electrodes to the end of the nerve farthest from the muscle and adjust the inductorium to obtain a break shock just above the threshold value. Repeat, making the test with the electrodes in the gas chamber. Leave the inductorium set in this position.

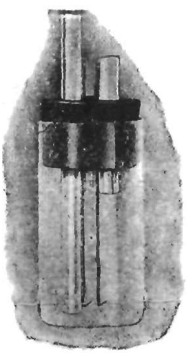


FIG. 9.—Glass gas chamber used in testing nerve irritability and conductivity. (*Harvard Apparatus Co.*)

Pass some CO_2 gas from a tank or generator into the chamber and close the inlet and outlet tubes. Continuing the use of break shocks, stimulate alternately the part of the nerve in the gas chamber and the end outside. Repeat the tests every half minute. What happens? Pass fresh air through the gas chamber and repeat the tests. Does the muscle now respond when the part of the nerve in the chamber is stimulated? Introduce a few drops of ether into the gas chamber through the long inlet tube but do not allow this liquid to touch the nerve. Repeat the tests as before. What is the result? Pass fresh air into the chamber and again stimulate the nerve at the two points alternately. Does the nerve recover?

16. The Return of Excitability after a Response.—Read the discussion on the Refractory Period in the text on pages 35 and 36.

Pith a large frog and lay it on its back on a frog board and apply clips to fasten the animal to the board. With scissors cut through the skin and pectoral girdle to expose the heart, which will be seen beating inside the pericardium. Carefully open the pericardium and locate the ventricle, the bulbus arteriosus and the two auricles. Note the auriculo-ventricular groove between the auricles and the ventricle. Raise the ventricle and notice the sinus venosus and the white crescentic line separating the sinus venosus from the right auricle. Note that the

sinus, auricles, ventricle and bulbus beat with a definite sequence. What is this order?

Inhibit the regular contraction of the ventricle by tying a "Stannius ligature." To do this wet a thread with Ringer's solution and with forceps pull it beneath the two aortas that extend beyond the *bulbus*. Then raise the ventricle and tightly tie the thread directly over the white crescentic line but do not cut the ends of the thread yet. If the ventricle does not come to a complete standstill, make a similar ligature with another piece of thread leaving the first ligature in place.

Place the end of a dissecting needle furnished with a wooden handle at the knot of the ligature around the heart. Using the ends of the thread there securely tie the needle to the heart so as to ensure that the ventricle is in contact with the needle. Connect a light wire to the needle, forming a good electrical connection, and attach the other end of the wire to one secondary terminal on the inductorium. A piece of fine bare brass wire should then be pushed through the apex of the ventricle and the end of the wire bent and wound on the adjacent part of the wire to form a small loop and to securely attach it to the heart. Connect the other end of this wire to the other secondary terminal. Place the frog board on a wooden stand and clamp the wooden handle of the dissecting needle to an upright stand so that the needle is in a horizontal position. Be careful not to tear the heart tissue. Tie a piece of thread to the wire loop fastened to the apex of the heart and attach the other end to a heart lever arranged on the upright stand to write on a smoked drum. The writing point of the lever should consist of a piece of cellophane to secure a light contact. It is necessary to carefully counterpoise the lever by means of a weight consisting of split lead shot. Apply Ringer's solution as needed to prevent drying of the heart.

Place the point of a signal magnet just below the point of the heart lever and adjust so that they both may record on a smoked drum. Connect the signal magnet, a simple key and two dry cells in series with the primary of the inductorium. Adjust the inductorium so that a single weak make shock will cause a response. Allow the drum to move slowly and send in pairs of shocks with the break preceding the make shock. Use gradually decreasing time intervals between these two shocks until no response is given to the make shock. Start with a maximum interval of 3 sec. between the break and the make, timed with a stop watch or other timer and decrease the interval by half a second on each successive trial. Allow 20 sec. to elapse between pairs of breaks and makes. Determine the shortest time interval between a break and a make shock for which the second stimulus is still effective in producing a contraction of the muscle. Following a stimulus there is a refractory period during which the tissue is not responsive to stimulation. Cardiac tissue is used here because its

refractory period is much longer than in the case of striated muscle or nerve, and therefore this period is easier to investigate in the heart. The refractory period is characterized by a great decrease in the degree of irritability.

17. Irritability Changes after Stimulation.—In this experiment employ exactly the same arrangement as in Exp. 16. It is probable that the same heart may be used as before but if it is not in satisfactory condition take a fresh one. Adjust the inductorium so that the make shock is just sufficiently strong to produce a contraction. Send in a break shock and, 7 sec. later, a make shock, the interval being timed with a stop watch or other timer. Using this interval between break and make shocks, adjust the inductorium until the make shock just fails to produce a response. Note this setting of the inductorium and record it. Allow 20 sec. to elapse between each pair of shocks.

Repeat, using a time interval of 6 sec., then 5 sec. and so on down to 1 sec. and then half a second. In each case find and record the setting of the inductorium at which the make shock just fails to cause a response. The greater the irritability of the muscle the weaker will be this make shock. Why? On paper plot the setting of the inductorium used here as a measure of irritability against the corresponding time intervals employed. What is the duration of the absolute and the relative refractory period?

18. Inhibition of the Frog's Heart by the Vagus.—Attach a counterpoised heart lever and a signal magnet beneath it to an upright stand with the point of one directly under the point of the other and so arranged that they may record on a smoked paper on a kymograph. Connect the signal magnet, a simple key and two dry cells in series with the primary of an inductorium arranged to deliver a tetanizing current. Attach platinum electrodes to the secondary terminals.

Pith the brain of a large frog and fasten the animal with clips, ventral side uppermost, to a frog board. Expose the heart and find the vagus on each side. If uncertain regarding which nerve in the region lateral and anterior to the heart is the vagus, the nerve may be tested by stimulation for a second with a tetanizing current. The heart is slowed down or stopped by vagus stimulation. Place a piece of moistened thread through the eye of a probe, which is then used to pass the thread under one vagus nerve. Leave the thread under the nerve and bring the two ends together and so use the thread to mark and lift the vagus as necessary. Repeat with the other vagus nerve to the heart. Keep the heart moist with Ringer's solution. Fasten a small S-shaped hook to the apex of the ventricle at the tip and attach the hook with a thread to the heart lever.

Obtain a record of the rhythmical contractions of the ventricle using a drum rotating at a medium speed and while doing so stimulate the vagus on one side for a second. Also record the behavior during recovery. Stimulate for 5 and then for 10 sec., obtaining the graphical record in each case, including the recovery process. Repeat using the vagus on the other side. What is the action of the vagus on the heart? Read in the text the discussion on pages 43 to 46, inclusive.

CHAPTER II

PHYSIOLOGY OF CONTRACTION

19. The Single Muscle Contraction.—*a.* Set up a spring myograph with smoked paper on the plate and adjust the tension of the spring so that the plate may move rapidly when released by the action of the trigger key. Leave the plate in position ready to be released. In front of the myograph, mount, on a heavy stand, a moist chamber with two nonpolarizable electrodes and a muscle lever beneath it. Attach a small weight to the muscle lever. Close below the lever mount a signal magnet. Clamp a strip of wood to the table against the two legs at the left end of the myograph to prevent any movement of the myograph base. Connect the automatic trip switch of the myograph, the signal magnet and two dry cells in series with the primary circuit of the inductorium so that a single induced break shock may be obtained when the plate carriage trips the switch. Join the secondary terminals of the inductorium to the nonpolarizable electrodes. If the inductorium has a built-in key in the primary circuit, it should be kept closed throughout the experiment.

Pith a frog and obtain a muscle-nerve preparation. Securely fasten a thread to the tendon, mount the preparation in the moist chamber and lay the nerve across the electrodes. Tie the lower end of the thread to the hook on the muscle lever. Adjust the lever and, if necessary, also the muscle clamp so that the thread is in a vertical position and not touching the margin of the hole in the floor of the moist chamber. Make sure that the axis of the pivot of the lever is horizontal and that the point of the lever will move lightly against the smoked paper in the proper manner. Test the adjustment of the after-loading screw as in previous experiments. Adjust the signal magnet so that its point will bear lightly on the smoked paper exactly under the point of the muscle lever and in the same vertical line. By opening and closing the trip switch, test the circuit to ensure that the muscle and the signal magnet both respond properly. When everything is properly arranged press the trigger key of the myograph to release the plate and obtain a curve of contraction.

Move the points of the muscle lever and signal magnet slightly away from the myograph and return the carriage and plate to the original position against the spring. Return the point of the muscle lever to the smoked paper, making sure that it is set in its original position. Again

press the trigger key to release the plate and in this way trace a base line. Take the stand bearing the moist chamber, lever, etc., away from in front of the myograph and return the plate and carriage to the original position against the spring. In front of the myograph place another stand bearing a tuning fork with a writing point on one prong. Place the point just below the base line at the left end of the plate after putting a starter on the fork. Set the tuning fork in vibration and immediately press the trigger key so that a time line is traced as the plate moves. If the tuning fork has a frequency of vibration of 100 per second, each wave length will correspond to a time interval of $\frac{1}{100}$ sec.

After the record is labeled, fixed and dried, draw four lines across the time line perpendicular to the base line and passing through the following points: (*a*) the point on the signal-magnet line that indicates the instant of stimulation, (*b*) the beginning of the rise of the muscle-contraction curve, (*c*) the crest of the curve and (*d*) the point where the curve returns to the base line. Measure on the time line the duration of the latent period, the phase of shortening and the relaxation phase. What is the duration of the entire contraction process in this case including all phases?

b. If a spring myograph is not available, the experiment may be performed by two people with the use of a kymograph in the following manner: Mount a moist chamber with two nonpolarizable electrodes on a support placed in front of a kymograph adjusted so that the drum with smoked paper may be revolved by hand. Below the moist chamber clamp a muscle lever with a parchment-paper point and place a signal magnet beneath it. Tie a thread around a tuning fork to bring the prongs together. One prong should have a writing point. Using a separate stand if necessary, place the tuning fork below the signal magnet and close to it. Align the points of the lever, signal magnet and tuning fork so that they are in the same vertical line and will record properly on the paper.

Connect the signal magnet, two dry cells and a simple key in series with the primary coil of an inductorium so that single make and break shocks may be obtained. This circuit is the same as that shown in Fig. 8 (Exp. 13). If the inductorium has a built-in key in the primary circuit, do not include a second one. Join the secondary terminals of the inductorium to the nonpolarizable electrodes.

Mount a frog muscle-nerve preparation in the moist chamber and connect the tendon to the writing lever with a piece of thread. Attach a small weight to the lever and, if necessary, raise the moist chamber on the stand a little to remove any slack that might exist in the thread attached to the tendon. Make a final adjustment of the muscle lever to ensure that it is tangent to the drum and capable of tracing a smooth,

continuous curve. Keep the muscle and nerve moist with Ringer's solution.

Adjust the inductorium so that a break shock will produce a good contraction of the muscle. Press the key or switch which short-circuits the secondary terminals and close the key in the primary circuit. Open the short-circuiting key and immediately have your partner cut the thread on the tuning fork with scissors and, while the fork vibrates, spin the drum rapidly by hand and instantly open the key in the primary circuit. Your partner should quickly stop the drum before it makes a second revolution. Repeat until each partner has a good record. Treat the records as indicated in the last paragraph under (*a*) in the directions for this experiment.

20. Effect of Load upon the Work Done by Muscle.—The term *loaded* is applied to a muscle when it is allowed to contract and to lift a load that it is supporting before contraction occurs. However, one can arrange so that the muscle does not support the load before contraction occurs but only supports it after contraction starts and while the load is being lifted. In this case, the muscle is said to be "after-loaded" preceding contraction. An initial applied tension will cause the muscle to do an increased amount of work upon contraction. This will be shown in a later experiment on isometric contraction. Isotonic contractions will be employed in this experiment, and the load will be increased without placing the muscle under initial tension.

Prepare a kymograph for graphic recording. On a support in front of the kymograph, mount a heavy muscle lever so that it may record on the paper. Extend the length of the lever by attaching to it an aluminum writing lever or straw with a parchment-paper point. About 6 in. above the lever, mount the insulated clamp holder that is supplied with the heavy muscle lever. Some heavy muscle levers and clamp holders are supplied with tripod supporting frames as single units. In such a case the tripod is simply placed on the table with or without a wooden stand beneath the tripod. Note the spring lever (isometric lever) on the same base as the heavy muscle lever. Loosen the screw holding the isometric lever, push this lever over to one side and tighten the screw again. Place a flat-jawed clamp in the clamp holder with the jaws downward. Connect a dry cell and a simple key in series with the primary coil of an inductorium to obtain single make and break shocks.

Mount a frog gastrocnemius preparation, without the sciatic nerve, with the femur held tightly by the flat-jawed clamp above the muscle lever. Connect the tendon securely to the hook on top of the heavy muscle lever, using a fine brass wire. Wrap absorbent cotton moistened with Ringer's solution around the muscle. Adjust the aluminum writing lever or straw so that the distance from the pivot of the lever to the point

is a simple multiple of the distance from the pivot to the hook on top of the lever. Take up any slack in the wire by raising the clamp holder or the flat-jawed clamp. Turn the after-loading screw that supports the lever until the muscle is just at its resting length. Connect one secondary terminal of the inductorium to the binding post on the heavy muscle lever and the other terminal to the binding post on the flat-jawed clamp. Adjust the inductorium to give a maximum make shock. Fasten a weight pan to the long hook or chain suspended from the muscle lever. Remove the absorbent cotton from the muscle. Rotate the drum once to form a base line.

Stimulate the muscle with a single make shock without changing the adjustment of the inductorium and record with the drum stationary. Prevent break shocks from reaching the muscle. Rotate the drum 5 mm. and add a 10-gram weight to the pan. Stimulate as before and record and then move the drum 5 mm. Repeat using 20, 30, 40, 50 grams and so on until the lever is not lifted by a contraction of the muscle.

Now elevate the flat-jawed clamp holding the femur to stretch the muscle without raising the lever from the after-loading screw. Stimulate with the same intensity as before and record. Does the muscle now lift the load and cause the lever to rise? With a millimeter rule measure the distance from the pivot to the writing point and also that from the pivot to the hook on top of the lever. Label the record, indicating the weights used. Shellac the record and allow it to dry.

Measure the heights in millimeters to which the lever point was raised on each contraction and compute the actual height that each load was lifted. The work done by a muscle during a single contraction is equal to the load multiplied by the distance it is lifted. If the load is expressed in grams and the lift in millimeters, then the work is given in gram-millimeters. Take the data obtained in this experiment and calculate the amount of work done during each contraction. Plot on graph paper the height of contraction against the load. On the same paper plot the work done against the load. With what weight does the muscle give the highest contraction? With what load was the greatest amount of work done? Read the discussion in the text on pages 62 to 63.

21. The Isometric Contraction.—When a muscle is allowed to contract without being permitted to decrease its length the process is known as an isometric contraction. In recording isometric contractions the muscle is arranged so that it contracts against a spring. With the arrangement employed, there is practically no shortening of the muscle, and the energy of contraction is used to develop tension.

Prepare a kymograph with smoked paper on the drum for graphic recording. Mount a heavy muscle lever and isometric lever combined on a support in front of the drum and attach the insulated clamp holder

to the support about 6 in. above the lever. If the clamp holder and combined heavy muscle lever and isometric lever are furnished with a tripod frame supporting them, mount the tripod on a small wooden stand on the table. Note the flat steel spring (the isometric lever) on the same base as the heavy muscle lever. Attach an aluminum writing lever with parchment-paper point to the end of this spring by means of two spiral rings. Turn the heavy muscle lever upward and then backward so that it is out of the way. Loosen the milled screw that holds the isometric spring to the base and, if necessary, turn the spring so that the long hook or chain on the spring is upward. The position of the spring should be adjusted so that the long hook or chain is directly above the hole in the base. Place a flat-jawed clamp with the jaws downward in the clamp holder. Arrange an inductorium to give single make and break shocks including a key and dry cell in series in the primary circuit.

Dissect out a frog gastrocnemius muscle with femur attached neglecting the nerve. Fasten the femur securely in the flat-jawed clamp and connect the long hook or chain on the spring to the tendon by means of an S-shaped pin passed through the tendon. Raise the flat-jawed clamp to bring the muscle in position, placing it under very slight tension.

Note that the clamp holder is insulated. Connect one secondary terminal of the inductorium to the binding post on the flat-jawed clamp holding the femur and join the other secondary terminal to the binding post on the base of the lever. Adjust the point of the lever to write on the smoked paper and record a short base line about 2 in. in length. Set the lever point at the beginning of this base line and stimulate the muscle with a maximum make shock. Make five or six records of the isometric contractions over this base line, eliminating the break shocks.

Do not change the elevation of the drum but raise the position of the flat-jawed clamp slightly to place the muscle under some tension. This raises the point of the lever. Record another short base line at the end of the first. When the spring is standardized as described later, the distance between these two lines will be a measure of the tension under which the muscle starts to contract. Set the point of the lever at the beginning of this second base line and make a few records of isometric contractions along the base line. Repeat these operations, increasing the tension on the muscle in stages until no upward movement of the lever point is obtained.

Remove the muscle, turn the flat spring completely over and set it in place again. Push the long hook or chain through the hole below and attach a weight pan to it. Adjust the point on the lever and rotate the drum to draw a short base line. Rotate the drum to bring the lever point to the beginning of this base line. Add a 50-gram weight to the pan and record a second base line under the other after vibration of the

spring has stopped. Set the lever point at the beginning of this base line and add a 100-gram weight. Draw a third base line under the second line. Repeat using 150, 200 and 250 grams. This standardizes the tension of the spring and it is now possible to determine the amount of tension developed by the muscle during isometric contraction. Label and shellac the record. What was the maximum tension developed by the muscle?

Arrange in the form of a table the values of the initial tension in grams and the corresponding values for the tension developed by the muscle during contraction. How does the initial tension affect the degree of tension developed during contraction?

22. The Effect of Temperature on the Contraction of Muscle.—Arrange a kymograph for graphic recording and mount a heart lever with a parchment-paper point on a support in front of it. Place a small wooden stand on the table and under the lever. Set an 800-cc. beaker on the stand, place a muscle warmer (Fig. 10) in the beaker and clamp its handle below the heart lever to the same support. The metallic handle of the muscle warmer should have a piece of heavy rubber tubing slipped over it to insulate it from the support. Mount a time marker to record below the heart lever. Arrange an inductorium to deliver maximal break shocks, using a single dry cell and a key in series in the primary circuit. Connect one secondary terminal of the inductorium to the metallic handle of the muscle warmer and join the other secondary terminal to the metallic handle of the heart lever, making sure that good electrical connections are made. Care should be taken to ensure that these two leads are not short-circuited through the metallic support.

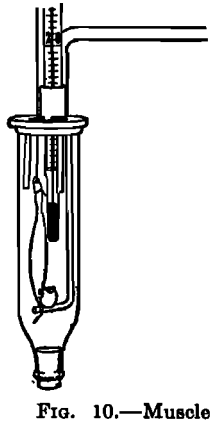


FIG. 10.—Muscle warmer. (*Harvard Apparatus Co.*)

Set the wooden stand and beaker aside temporarily and remove the glass vessel from the muscle warmer. Obtain a gastrocnemius-muscle preparation from a frog, disregarding the nerve. Make a small hole through the tendon with the point of a dissecting needle. Push the end of a fine brass wire about 8 in. in length through this hole and fasten the end of the wire securely to the tendon. Attach the femur to the tip of the L-shaped rod in the muscle warmer, using a piece of fine brass wire. The wire connected to the tendon should be then passed through the hole in the cover and connected to the heart lever about 1½ in. from the pivot so that the point of the lever will rise when the muscle contracts. Counterpoise the lever by attaching a small weight to it on the recording end close to the pivot and adjust the lever so that it may record properly.

Place a few drops of Ringer's solution in the bottom of the muscle-warmer vessel.

Fill the beaker with chipped ice and place it under the muscle so that the warmer is immersed in the ice. Set the wooden stand under the beaker. Insert a thermometer into the muscle-warmer vessel using a piece of rubber tubing to hold it in place. When the temperature inside the muscle warmer has fallen to about 0°C., start the time marker and with the drum rotating at its maximum speed stimulate the muscle with a single break shock and record the contraction. Secure another record at once. Record base lines.

Carefully warm the contents of the beaker by applying the flame from a Bunsen burner to the walls of the beaker. When the temperature inside the muscle warmer has risen to 10°C. again make two separate records. Allowance should be made for the fact that the temperature of the muscle lags behind that of the air in the muscle warmer. Repeat at 20, 30 and 38°C. Then, using a slowly moving drum, gradually heat the muscle warmer up to 60°C., recording continuously.

Label and shellac the records. Place your records in your final notebook in order of increasing temperature effects. What is the effect of temperature on the muscle contraction?

23. The Effect of Veratrine on Muscular Contraction.—Pith the brain of a frog with the minimum amount of bleeding. Insert a small plug of cotton into the hole made by the pithing probe. With a Luer syringe and needle slowly inject into the dorsal lymph sac about 2 cc. of a 1 per cent solution of veratrine sulphate. Also inject a quantity of the solution under the skin covering the gastrocnemius muscles. Then set up a kymograph with smoked paper, a moist chamber with two nonpolarizable electrodes, a muscle lever and a time marker for graphic recording. Connect the secondary terminals of an inductorium to the nonpolarizable electrodes and wire the primary circuit to obtain single make and break shocks.

Fifteen minutes after making the injection, mechanically stimulate the skin of one hind leg by pinching with forceps to cause reflex action. The legs become extended and remain so for several seconds because of the slow relaxation of the extensor muscles. Do not stimulate the animal more than two or three times as the veratrine effect diminishes with activity of the muscles. Make a muscle-nerve preparation, using this animal, and mount it in the moist chamber connecting the tendon with the muscle lever. Place the nerve on the nonpolarizable electrodes and adjust the muscle lever to record using a drum revolving at a medium speed. Start the time marker and stimulate, using a single, strong, break shock, and record the contraction.

Note that the phase of contraction is apparently normal, but the phase of relaxation is very prolonged. Repeat to obtain several records

of contraction. If a veratrinized muscle is made to contract several times, the effect passes off. Stimulate the muscle until the normal type of curve is obtained. Allow the muscle to rest for a few minutes, keeping it moist with Ringer's solution and then stimulate and record again. Does the effect reappear? Label and shellac the record. Describe and discuss the results (see pages 63 to 64 in the text).

24. The Effects of Repeated Contractions and of Fatigue on a Muscle. Prepare a kymograph for graphic recording and on a support in front of it mount a moist chamber, without electrodes, and a muscle lever. On two upright stands, set up a flat-spring, vibrating interrupter with the clamp holding the spring near the binding post on the spring. Connect the electromagnet of the interrupter, a built-in switch on a metronome

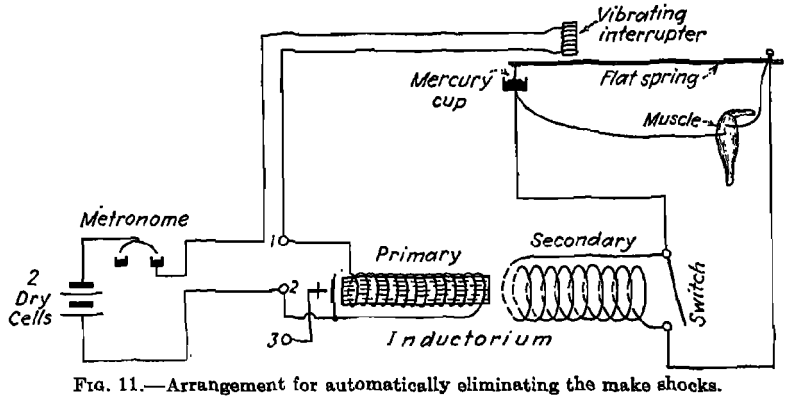


FIG. 11.—Arrangement for automatically eliminating the make shocks.

and two dry cells in series with the primary coil of an inductorium so as to obtain single shocks. The purpose of the interrupter here is to automatically eliminate the make shocks. Join one secondary terminal of the inductorium to the mercury-filled cup on the interrupter and the other secondary terminal to the binding post on the end of the flat steel spring. It is advisable to attach a clamp above the mercury cup to the support so that the end of the flat steel spring bearing the contact point may bump against it on the upward swing. This clamp should be insulated with tape where the spring hits it. This arrangement damps the vibrations of the spring. Connect one binding post on the moist chamber to the mercury cup and another to the binding post on the end of the steel spring. If the inductorium has a built-in key in the primary circuit, the key should be kept closed throughout the experiment. The wiring diagram is shown above.

If a vibrating interrupter is not available, the experiment may be performed employing both make and break shocks with the following arrangement: Connect the built-in switch of a metronome and a dry

cell in series with the primary coil of an inductorium adjusted to give single make and break shocks. If there is a built-in key in the primary circuit of the inductorium it must be kept closed. Join the secondary terminals of the inductorium to two binding posts on the underside of the moist chamber.

Irrespective of which of these two methods of stimulation is employed, proceed as follows: Obtain two pieces of fine, bare, flexible brass wire, each about 5 in. in length, and wind them on a pencil to spiral them. Connect an end of each to a binding post on the upper side of the moist chamber so that the current from the inductorium may reach them. Mount a frog gastrocnemius preparation in the moist chamber and join the tendon to the muscle lever with a thread. Place a 10-gram weight on the lever and adjust the after-loading screw so that the muscle supports the lever and weight. Take the two free ends of the fine wires attached to the moist-chamber binding posts and push them through the muscle close to the femur so that they are about 5 mm. apart. Bend the ends of the wires over so that they will not pull out of the muscle and adjust them to relieve any tension. The muscle is to be stimulated directly so as to avoid fatigue effects in the neuromuscular junctions. Avoid stimulating the muscle in making adjustments so that the phenomenon of treppe may be obtained later. Wind the metronome and set it so that one shock per second will be given.

Make a final adjustment of the writing lever and record a base line near the bottom of the smoked paper. With the writing point set on this base line and the drum revolving at a slow speed, short-circuit the secondary of the inductorium and start the metronome and also the vibrating interrupter if one is used. Open the short-circuiting key and stimulate the muscle automatically until it is completely fatigued.

Allow the muscle to rest for 5 min., keeping it moist. Stimulate it again as before. Compare the records.

Label and shellac the records. What effects do the first few stimuli have on the height of contraction? What symptoms of fatigue are shown?

25. Compound or Tetanic Contractions.—Read the discussion in the text on pages 71 to 74, inclusive, before doing this experiment.

Prepare a kymograph for graphic recording and on a support in front of it mount a moist chamber, without nonpolarizable electrodes, and a muscle lever. With wires join the secondary terminals of an inductorium to two binding posts on the underside of the moist chamber. Procure two fine bare brass wires about 5 in. in length, spiral them and connect them on the upper side of the moist chamber to the two binding posts joined to the secondary terminals. Connect a flat-spring vibrating interrupter and two dry cells in series with the primary coil of the induc-

torium. The clamp holding the spring should be attached close to the binding post at one end of the spring. Make an electrical connection between the metal clamp attached to the electromagnet and the binding post at one end of the flat spring. The arrangement of the primary circuit is shown below.

The flat steel spring should be so adjusted that when at rest its contact point will just touch the surface of the mercury and when vibrating will make and break the primary circuit at regular intervals.

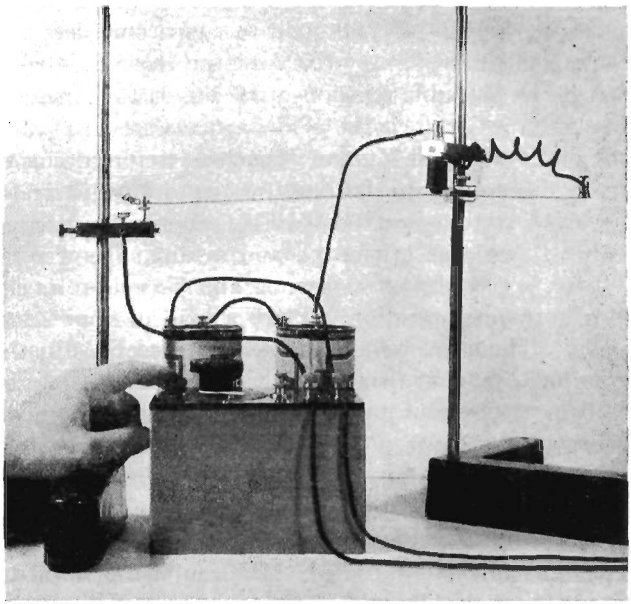


FIG. 12.—Arrangement for making and breaking the primary circuit at different frequencies.

If the inductorium has a built-in key in the primary circuit keep it closed during the experiment. Adjust the inductorium to give maximal make and break shocks.

Mount a frog gastrocnemius-muscle preparation, without the nerve, in the moist chamber and connect the tendon to the muscle lever with a piece of thread. Attach a light weight to the lever and adjust the lever to record on the smoked paper. Take the two free ends of the fine brass wires on the moist-chamber binding posts and push them through the muscle near the femur so that the wires do not touch each other. Close the short-circuiting key across the secondary terminals. Adjust the kymograph so that the drum may move at a high speed when everything is ready and start the vibrating interrupter. Allowing the drum to rotate, open the short-circuiting key for about 3 sec. and obtain a record. Immediately after closing the key, stop the drum.

Readjust the vibrating interrupter so that the spring will be clamped about 2 in. closer to the end making contact with the mercury. Since the vibrating part is now shorter, the spring will vibrate at a higher frequency and will therefore make and break the primary circuit more frequently. Take a tracing as before.

Again decrease the length of the vibrating portion of the spring by about 2 in. Readjust the interrupter and take a record. Continue this process until you have records of the effects of stimuli administered at five or six different frequencies. A final record should be taken with the interrupter working rapidly enough to cause complete tetanus, *i.e.*, a smooth prolonged contraction. Examine the records, label and shellac them. What is the probable relationship between the single contraction and complete tetanus?

26. Work and Fatigue of Human Muscle.—Before doing this experiment read the discussion in the text on pages 75 and 76. Note the diagram there illustrating how the Mosso ergograph is arranged when in use. Set up the Mosso ergograph and arrange the writing point so that it may trace a record using a kymograph placed on its side with its long axis in a horizontal position. Place a load of about 2 to 3 kg. in the weight pan. The exact weight should be noted. Adjust the clamps on the ergograph with your arm in position and over the middle finger slip the loop on the cord to which the weight is attached. Start a metronome beating at a rate of one beat per second. Set the belt with the scale at the zero point on the ergograph. Flex the finger bearing the loop in rhythm with the metronome and allow the drum to rotate at a slow speed while the record of the contractions is being obtained. Continue until the finger is fatigued. Determine the total height that the weight is lifted by taking the reading on the scale on the movable belt. Compute the amount of work done by multiplying the sum of the heights by the weight lifted. Express the amount of work in kilogram-meters.

27. Tetanic Contractions of Human Muscle.—Prepare a kymograph with smoked paper on the drum and fasten an L-shaped spring ergograph to the table by means of a clamp so that an aluminum writing lever attached to the horizontal spring on the ergograph may record on the paper. A point of parchment-paper or suitable material should be attached to the aluminum lever. Arrange an inductorium to give a mild tetanizing current and connect the secondary terminals to brass electrodes such as are used in unipolar stimulation.

Place the hand in position in the ergograph and put the adjustable rod in a vertical position on the index finger with the curved rest on the finger. The pointed end of the rod should be placed in one of the holes on the underside of the horizontal spring. Raise the index finger and,

if necessary, put the pointed end of the rod in a different hole nearer to the writing point so that the point of the lever may be raised fairly easily. On the forearm place the flat brass electrode inserting a cotton pad wet with salt solution between the electrode and arm. Fasten the electrode in place. Cover the point of the brass-rod electrode with a small piece of cotton wet with salt solution and tie the cotton on with thread. This forms the stimulating electrode.

Using a fast drum and with the inductorium in operation, have your partner touch the skin of the hand in the ergograph with the stimulating electrode placed close to the angle between the thumb and index finger so that the abductor indicis is stimulated. Obtain graphical records of several contractions while the stimulating current flows for 4 or 5 sec. in each case. Is complete or incomplete tetanus obtained?

28. Contraction of Smooth Muscle.—Before doing this experiment read the discussion on visceral muscle in the text on pages 77 to 82, inclusive. Set up the apparatus as described in the first paragraph of Exp. 22, on the effect of temperature on the contraction of a muscle. However, use a flexible cellophane point on the heart lever and arrange the inductorium to deliver a tetanizing current of medium intensity.

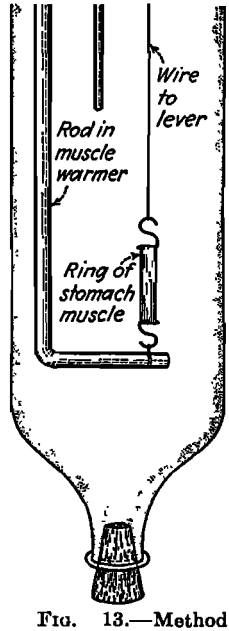


FIG. 13.—Method of mounting ring of stomach muscle.

After removing the glass vessel from the muscle warmer connect a bent S-shaped pin to the tip of the L-shaped rod in the muscle warmer by means of a fine bare brass wire. Connect the end of a piece of similar wire about 8 in. in length to another S-shaped pin. Pass this wire through the hole in the muscle-warmer cover with the pin downward and wind the end of the wire around the heart lever close to the pivot and on the opposite end from the writing point, making a good electrical connection.

Pith a large well-nourished frog, open the abdomen and from the central part of the stomach cut a ring of tissue about 7 mm. wide by making two transverse cuts completely across the stomach. Slip the ring of stomach muscle onto one S-shaped hook and then onto the other being careful not to pass the pin into the tissue. The arrangement is shown in Fig. 13. Counterpoise the heart lever with a suitable weight. Adjust the elevation of the lever so that it is held in a horizontal position by the wire going to the ring of stomach tissue. The lever point should be adjusted to bear lightly on the smoked paper. Place a few drops of

Ringer's solution in the glass vessel of the muscle warmer and put the vessel in place on the warmer. Place water having a temperature of 30°C. in the large beaker which should be set on the wooden stand so that the muscle warmer is immersed in the water. Insert a thermometer into the muscle warmer and start the drum at a slow speed.

Record any spontaneous contractions that may occur, taking care to keep the muscle moist. If these contractions fail to appear in about 12 or 15 min., put a few drops of 0.5 per cent barium chloride solution on the muscle. Make a mark on the paper when the solution is applied and record a few contractions.

Wash the tissue by applying Ringer's solution or replace the tissue with a fresh piece and with the tetanizing current stimulate the tissue for 4 or 5 sec. Record the contraction. Determine the length of the latent period. Repeat several times. The processes of contraction and relaxation are slow and may be followed by eye. What is the duration of contraction and of relaxation? Label and shellac the records.

CILIARY MOTION

29. Microscopic Examination of Cilia.—Before doing the experiments on cilia read the discussion in the text on pages 84 to 87, inclusive. Destroy the brain and spinal cord of a frog with a pithing probe and, with a scalpel, scrape some of the cells from the membrane on the roof of the frog's mouth. Place the scrapings in a drop of Ringer's solution on a clean glass slide. Put a cover slip on top of the drop and examine the cells under the low and then the high power of a microscope. Adjust the light so that the cilia may be seen in motion. Make a sketch to show the typical arrangement of cilia on an epithelial cell. The action of cilia may also be seen very well on a piece of tissue from the gill of an oyster or clam.

30. Functional Activity of Cilia.—Place the frog, which has already been pithed, on its back on a cork sheet and with scissors divide the lower jaw on the mid-line and carry the incision backwards through the pharynx and esophagus to the stomach. Pull aside the flaps thus formed and pin them down at the margins to the cork sheet. Cut away any viscera that might interfere and apply Ringer's solution to the mucous membrane that has been pinned down, draining away any excess solution. Sprinkle a little powdered charcoal onto the tissue at the roof of the mouth. In what direction and how far does it move? What function of cilia in the frog is thus shown?

31. The Effect of Temperature on Ciliary Action.—Cut out a small plate of cork about 2 mm. thick, 5 mm. wide and 8 mm. long. Obtain 50 cc. of Ringer's solution at room temperature but actually determine its temperature. Pour this solution slowly over the ciliated epithelium

used in the preceding experiment and drain away any excess solution. Immediately place the small cork plate with its smoothest surface downward flat on the ciliated surface. When the plate begins to move, find how many seconds it takes to travel a distance of 1.0 cm. Now slowly pour about 50 cc. of Ringer's solution warmed to 30°C. over the tissue and drain as before. At once, return the cork plate to the same position that it had at the beginning of the test. How much time is required to move it over the same distance? How does such a rise in temperature affect ciliary motion?

32. Work Performed by Cilia.—From the frog used in the preceding experiment, dissect out the ciliated membrane from the roof of the mouth to about the middle of the stomach. Moisten a sheet of cork with Ringer's solution and on it stretch the membrane out flat with the cilia upwards. Pin the tissue down at the margins avoiding undue stretching. Place the cork sheet so it forms an angle of about 30 deg. with the surface of the table and keep it in this position by means of a suitable support. The mouth end of the tissue should be at the bottom of the incline.

Select a point near the mouth end and a point near the stomach end of the tissue, between which the small cork plate used in the preceding experiment may be propelled by the cilia. Measure the vertical distance between these two points in millimeters. Place a 10-mg. weight on the cork plate and set this plate at the selected point at the mouth end of the tissue. Measure the time in seconds taken by the load to travel between the two points. Repeat, using, in turn, 20-, 50-, 100-, 200-, 300- and 500-mg. weights. Find the largest weight that may be moved. The work done in each case is obtained by multiplying the vertical distance through which the weight is lifted by the particular weight used. Calculate the amount of work done with each weight expressing the data in terms of milligram-millimeters of work per second. With what weight is the greatest amount of work done per unit of time? Present the data in the form of a table or graph.

33. Stimulus for Ciliary Action.—Place the sheet of cork bearing the ciliated membrane used in the preceding experiment flat on the table. Heat the point of a dissecting needle until it is quite hot and towards one side or end of the tissue push the point into the ciliated surface. Sprinkle powdered charcoal over the cauterized region and observe the distribution of the powder after a short interval. Note that ciliary motion does not exist in a triangular area, one point of which is at the heated spot. The base of the triangle lies towards the mouth end of the tissue. This indicates an interdependence among neighboring cells for ciliary action. See the discussion in the text on pages 84 to 86, inclusive.

34. The Effect of Ether on Ciliary Motion.—Wash the ciliated surface used in the previous experiment with Ringer's solution and remove any

excess solution. Measure the time necessary for a small piece of cork to travel between two points about 2 cm. apart on the tissue. Then cover the membrane with a large inverted beaker. Place some ether on a small piece of filter paper and put the paper under the beaker but not on the tissue. At the end of half a minute remove the beaker and paper and then measure the time taken for the cork to travel the same distance as before. What effect has ether on the rate of ciliary motion?

CHAPTER III

REFLEXES AND TROPISMS

35. The Reflex Frog.—Before doing this series of experiments read the discussion in the text on pages 110 to 112, inclusive.

Arrange an inductorium to deliver a tetanizing current, using platinum electrodes in stimulating. Fasten a hook-shaped pin in a flat-jawed clamp attached to a stand. With care, pith a large frog to destroy the brain, finally pushing the pithing probe slightly towards the spinal cord to ensure destruction of the medulla. As far as possible avoid loss of blood and plug the incision with cotton to check bleeding. Place the lower jaw of the frog on the hook allowing the animal to hang downward without coming in contact with any object. Apply the tetanizing current to one foot and observe. What position is assumed by the animal when placed in a vessel of water? Is there any evidence of spinal shock?

Place the animal dorsal side upward on the table and note its position. At the end of several minutes reflex responses will begin to appear. When signs of recovery become evident pinch a toe with forceps. Does the leg move? Note the attitude of the body, the breathing movements, behavior when placed on its back, whether or not the eyes are open and the quiescence. Are any movements made except in response to stimuli applied to the skin? Pinch the skin with forceps on various parts of the body and record the nature of the responses which occur. At intervals during this series of experiments, momentarily dip the animal in water to keep the skin moist.

36. Unilateral and Crossed Reflexes.—Suspend the reflex frog used in the previous experiment by the jaw from the hook as before. The animal should not be allowed to touch any object.

When the frog becomes quiet, gently pinch one of the hind toes with forceps. Repeat with a harder pinch. Is there a difference in response? Now pinch a toe of the other foot in the same manner. Are these responses unilateral? Pinch one toe firmly with forceps, holding the toe so that the foot can not be withdrawn. Is the other foot moved so as to show a crossed reflex?

37. Varying Intensity of Stimuli.—It will have been noted during the preceding experiments that the greater the intensity of stimulation the greater is the number of muscles brought into play, and accordingly the more complex is the response. The intensity of stimulation also

has an effect on reflex time, *i.e.*, the latent period of the response, as can be shown by the following experiment.

Obtain sulphuric acid solutions containing, respectively, 0.1, 0.2, 0.3 and 0.4 per cent of H_2SO_4. Put 75 cc. of each in a separate 100-cc. beaker. Label each beaker indicating the strength of acid. Obtain a stop watch or other timing device and fill an 800-cc. beaker with tap water.

Place the beaker containing 0.1 per cent acid so that one foot will be immersed up to a chosen point without coming in contact with the beaker. Find the time in seconds between the instant of immersion and the beginning of withdrawal of the foot from the acid. Do not leave the foot immersed longer than 2 min. if it is not withdrawn by the animal. Immediately wash off the acid by dipping the foot into the beaker full of tap water. Wait 2 min. and repeat the experiment, being careful to immerse up to the same point as before. Is the first result duplicated? Record the average of two observations. This time interval measured is the reflex time.

Allowing a 2-min. interval between consecutive tests and washing the foot immediately after each trial, determine the effect of each of the other three acid solutions. Does the reflex time become shorter with increasing acid concentration? In your final notebook present the results in tabulated form.

Arrange an inductorium to deliver single make and break shocks and tie two pieces of fine bare brass wire about a centimeter apart around one foot of the frog. Connect these two wires, which are to act as electrodes, to the secondary terminals of the inductorium. With the frog still suspended as before, stimulate the skin of one foot with a weak break shock of sufficient strength to produce a movement of the leg. Measure the reflex time and repeat 2 min. later. Now stimulate, using a strong break shock and obtain the reflex time. Repeat 2 min. later. How does the reflex time vary with the intensity of stimulation? Leave the apparatus and frog set up so that they may be used in the next experiment, which should be performed at once.

38. Summation of Excitation.—Use the same frog and arrangement of apparatus as in the preceding experiment (37) and adjust the inductorium to find the threshold of excitation for the reflex action, using a break shock. In making the adjustment, allow 15 sec. to elapse between stimuli. Then slightly decrease the intensity of the induced current so that a subthreshold stimulus for the reflex movement of the leg may be obtained. Slight local muscular contractions, however, may be obtained when this subthreshold stimulus is applied. With the inductorium adjusted to this position apply a series of four or five break shocks in rapid succession. It is not necessary to eliminate the make shocks since they are weaker than the break shocks. Repeat a few times. Is

there any evidence that the effects of preceding stimuli are "stored up" and added to those of later ones? Are there any signs of fatigue? Leave the frog and apparatus in position for the next experiment.

39. Inhibition of a Reflex.—With the frog and the apparatus still arranged as in the preceding experiment (38) adjust the primary circuit of the inductorium so that a weak tetanizing current may be obtained. Place 75 cc. of 0.2 per cent H_2SO_4 solution in a 100-cc. beaker and fill a large beaker with water. Adjust the inductorium so that the tetanizing current applied for 3 sec. will elicit a reflex response. Allow the animal to rest for 2 min.

Hold the beaker containing the acid solution so that the hind foot without the attached electrodes is immersed in the acid solution up to a definite point. The foot should not be allowed to come in contact with the beaker. Note the time in seconds before the foot is reflexly withdrawn. Immediately immerse the leg in the beaker full of water to wash off the acid.

Allow the frog to remain quiet for 2 min. Again immerse the same foot as before in the acid solution but at the same time apply the tetanizing current to the other hind leg continuously until the foot is removed from the acid solution. Again note the time necessary for the withdrawal of the foot from the acid solution and then wash the leg. In the event that withdrawal of the foot does not occur within 30 sec. discontinue the electrical stimulation. Note what then happens. Explain.

At the end of 2 min. repeat the test without electrically stimulating the other foot and again measure the reflex time. Are the sensory endings of the skin still responsive to the acid? Wash again. Repeat this series of tests. Keep the frog to use in the next experiment.

40. Coordinated, "Purposeful" Reflexes.—Wet small pieces of filter paper, about 5 mm. in diameter, with 35 per cent acetic acid solution. With forceps drain one of the pieces and, with the reflex frog suspended from the hook as before but with the wires removed, apply the paper to the skin on the inner side of the frog's thigh. Describe the responses, especially as regards coordination of movements directed towards removal of the paper. Immediately wash the frog by immersion in a vessel full of tap water and continue to do so after each test.

After 2 min. for recovery of the frog, repeat the test holding the foot of the leg to which the paper with acid is applied. Do you obtain a crossed reflex? Allowing 2 min. after each test, try the effect of application of the acidified papers to other parts of the skin. Do the responses differ and do they show adaptation according to the place of excitation? Do the responses seem to be "purposeful"? Keep the frog for the next experiment if it is in good condition.

41. The Site of Fatigue.—Use the animal employed in the preceding experiment (40) or, if it is not in satisfactory condition, obtain another

reflex frog. Carefully make an incision in the skin of the left leg to expose the gastrocnemius muscle. Expose the sciatic nerve on both sides of the animal and run a piece of thread moistened with Ringer's solution under each nerve. Keep the frog suspended from the hook by the lower jaw. Attach stimulating platinum electrodes to the secondary terminals of the inductorium and arrange the primary so that a tetanizing current may be obtained. Adjust the inductorium to deliver a current such that the left leg shows reflex action when the electrodes are applied to the right sciatic nerve. Continue to stimulate this nerve until no contractions of the muscles of the left leg are given.

Immediately place the electrodes on the left sciatic nerve and stimulate with the tetanizing current. What happens? Stimulate the left sciatic nerve continuously until the muscles of the same leg stop contracting. Then immediately place the electrodes on the left gastrocnemius muscle to stimulate. Is there contraction of the muscle fibers? Did fatigue appear first at the periphery or in the central nervous system? Was there evidence of fatigue in the nerve trunk? Was there primary fatigue of the muscle? Was the motor end plate fatigued?

42. Action of Strychnine.—Carefully pith the frog brain, including the medulla, and plug the incision with cotton to check bleeding. Allow the animal to recover from spinal shock. With sharp-pointed scissors make a small hole in the skin on the middorsal line. With a medicine dropper inject into the dorsal lymph sac several drops of a 1.0 per cent solution of a strychnine salt such as the sulphate. After a few minutes, notice the heightened sensitiveness to stimuli. To what extent do the reflexes show irradiation? Test the effects of mild stimuli by blowing on the skin, touching the skin lightly, tapping the table near the animal, etc. Describe the spasms produced. Do they show a maximum response to a minimal stimulus? How has the coordination of reflex movements been affected by strychnine?

With a probe introduced through the opening previously made for pithing, destroy the spinal cord of the frog. Does the animal now respond to reflex excitation? Where and how does strychnine take effect? (See pages 116 and 117 in the text.)

43. Inhibition of Equilibrium Movements.—Place a normal frog on the table and turn it on its back holding it gently for a few moments. Observe that it will not remain in this position but at once turns over to right itself. Tie rather tightly a thick piece of string around each upper arm. This does not cause any interference with the animal's movements. Now place the frog on its back on the table and hold it quiet for a few seconds. The animal will remain in this position. Try moving it and pulling it by the leg. Explain. Are the respiratory movements altered?

CHAPTER IV

THE CORRELATING ACTION OF THE NERVOUS SYSTEM

44. Reaction Time.—The time interval between the instant of stimulation and the initiation of the response is known as the *reaction time.* In measuring simple reaction time, one employs a single type of sensation and one kind of stimulus, and accordingly choice and discrimination do not appear as complicating factors. In man the average simple reaction time has been found to be, for sight, 0.20 to 0.22 sec.; for hearing, 0.15 to

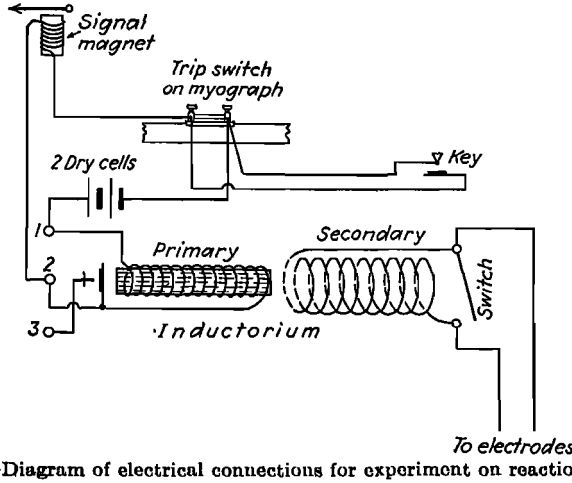


FIG. 14.—Diagram of electrical connections for experiment on reaction to touch.

0.16 sec.; for touch (cheek), 0.14 to 0.15 sec.; for touch (skin of finger), 0.15 to 0.18 sec.

a. Reaction to Touch.—Set up the spring myograph and arrange a signal magnet to record on the smoked paper on the plate. Connect the signal magnet, the automatic trip switch on the myograph and two dry cells in series with the primary of the inductorium. Adjust so that single make and break shocks may be obtained. Connect the two terminals of the trip switch on the myograph to the two binding posts on a simple key so that the primary circuit may be closed by the trip switch or by the key. Attach platinum stimulating electrodes to the secondary terminals of the inductorium. The wiring diagram for these connections is shown above.

Mount an ordinary tuning fork with parchment-paper point so that a time line may be recorded under the signal magnet. Tie a thread around the tuning fork to bring the ends of the prongs almost together. The fork is conveniently set in vibration by cutting the thread with scissors at the proper time.

Work in pairs and have one person seated so as not to be able to see the myograph. This person, the subject, holds the platinum electrodes with the left hand and places the tips of them against the cheek when everything is ready. The right hand is used to operate the simple key connected to the trip switch on the myograph. The other person, the operator, closes the automatic trip switch on the myograph, tells the subject to place the electrodes against the cheek, sets the tuning fork in vibration and, at his discretion, presses the trigger key to release the myograph plate. The subject, as soon as the break shock is felt, closes the key as quickly as possible to determine the reaction time.

A kymograph may be substituted if a spring myograph is not available. In such a case use a simple key in place of the trip switch on the myograph. The operator then sets the tuning fork in vibration, closes this simple key, tells the subject to apply the electrodes to the cheek, spins the drum by hand and immediately opens the simple key.

Determine the time interval between stimulation and response. Repeat three or four times and calculate the average reaction time. What errors may be involved? Repeat using a stronger shock. Does this alter the reaction time? Let the subject then act as operator and the operator act as subject.

b. Reaction to Sight.—Use the same arrangement as before but disconnect the wires at the binding posts of the primary of the inductorium. Insert another signal magnet in its place but have the wires long enough so that this signal magnet may be mounted on a stand in front of the subject who is not permitted to watch the myograph or kymograph. The subject watches for movement of the white writing point on the signal magnet which should be adjusted to produce no sound. Test the circuits.

If a spring myograph is used, the operator closes the trip switch on the myograph, sets the tuning fork in vibration and, at his discretion, presses the trigger key to release the myograph plate. The subject responds as quickly as possible by closing the key (use the same hand as before) as soon as the signal-magnet point is seen to move.

If a kymograph is used, the operator sets the tuning fork in vibration, closes the simple key, spins the drum by hand and immediately opens the simple key. The subject responds as indicated above.

Repeat these operations several times and find the average reaction time. The subject and the operator should then exchange places.

c. Reaction to Sound.—In place of the signal magnet introduced into the circuit in the above experiment (*b*, Reaction to Sight), connect a telegraph sounder or other instrument that will produce a sound upon the opening of the circuit. Proceed as in the preceding experiment (*b*, Reaction to Sight), except have the subject respond to the sound. The eyes of the subject may be kept closed. Repeat several times to obtain the reaction time to sound. The subject and the operator should then exchange places.

45. Reaction Time with Choice.—With the apparatus arranged as in the preceding experiment (44) under the heading (*a*, Reaction to Touch), modify the circuit by connecting a double-throw switch in place of the simple key shunting the trip switch on the myograph. This permits the circuit to be closed when the switch is thrown to the right or to the left. The switch should be connected as indicated below (Fig. 15).

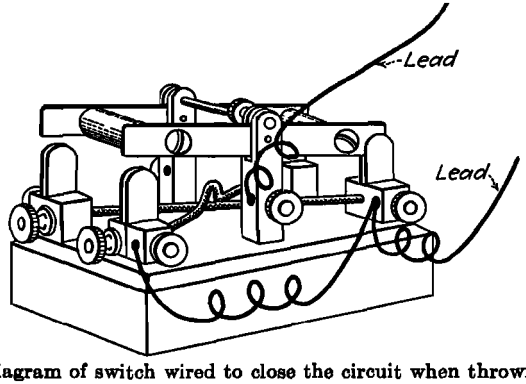


FIG. 15.—Diagram of switch wired to close the circuit when thrown either way.

The subject should sit and hold the stimulating electrodes with the left hand so that the points are against the cheek. He should close his eyes and hold the handle of the double-throw switch with the right hand. He is instructed to throw the switch to the left if he receives a strong shock and to the right if he receives a weak one. The correct adjustment of the inductorium for a strong stimulus and that for a weak one must be determined beforehand. The operator manipulates the apparatus as in the preceding experiment (44) and sends in a weak or strong shock without warning the subject in advance which it will be or when it will be given. Repeat several times and compare this reaction time with that obtained when no choice is involved. Now let the subject and the operator exchange places.

46. Series of Impulses from Central Nervous System.—It is believed that, to produce contractions of our voluntary muscles, the central nervous system discharges series of impulses into the muscles.

Set up a Porter ergograph by clamping it to the table. Attach an aluminum lever or straw with a parchment-paper point to the flat spring on the ergograph. Arrange the recording point to write on smoked paper on a kymograph drum. Place the wrist under and against the spring close to the upright support on the ergograph. Keeping the elbow so that it does not touch the table, lift the spring upward, using considerable force. Place a tuning fork with parchment-paper point on one prong to record just above the level to which the writing point of the spring was raised. Lift upward on the spring as before and maintain the contraction. Your partner should set the tuning fork in vibration and spin the drum to record the vibrations of the tuning fork and spring. Determine the rate at which the muscle vibrates.

CHAPTER V

RECEPTORS

47. The Weber-Fechner Law.—Read the discussion on page 174 in the text.

Obtain two small boxes of the same dimensions and furnished with tops. In the bottom of each place a layer of cotton to prevent rolling of the shot that will be added.

Blindfold your eyes, extend your arm forward palm upward and have your partner place each box, in turn, upon your hand after a 10-gram weight (consisting of pieces of shot) has been added to each box. Gain an impression of the weight of the box with its contents in each case. You should not be able to detect a difference in the weights of the two boxes. While you are holding one box your partner should remove from or add to the other box one or more pieces of shot and then quickly exchange boxes. If you do not notice a difference in the weights the test should be repeated with the removal or addition of a larger amount of shot. Continue until you can detect a distinct difference. Your partner should check your judgment of the weights by changing the order in which the weights are given to you without informing you of the order of presentation. What is the least change in weight that you can detect? Obtain the average of several trials.

Increase the weight of the boxes to 50 grams each and repeat the tests. What is the least change in weight that can now be detected? Are your results in agreement with the Weber-Fechner Law?

a. PRESSURE

48. Distribution of Receptors for Touch.—On the inner surface of the wrist near the palm, mark off a square 1.0 cm. on each side. In your notebook make a similar area. Rest the hand on the table, close your eyes and have your partner explore the area marked off with the tip of a bristle that is pressed against the skin just enough to cause it to bend each time. The pressure should be applied in the same manner on each trial. Indicate when the sensation of touch is experienced and have your partner record on the paper with the square the corresponding points at which the sensations are felt. Are the touch receptors uniformly distributed in the area investigated? Mark off a similar area

in the palm of the hand and repeat. How does the distribution of the receptors in this case compare with that obtained for the wrist?

49. Touch Localization.—Sit with your eyes closed and arm resting on the table. Hold a sharpened pencil in the other hand and have your partner press down firmly on the skin of the inner surface of your lower arm for a moment with the point of a pencil leaving a depressed mark. Try to place the point of your pencil directly over the mark, pressing firmly with the pencil for an instant as soon as you feel certain that the mark has been located exactly. Your partner should then measure the distance in millimeters between the two marks. Make at least six trials in this region and find the average error in locating the correct spot stimulated. Repeat the tests on the skin of the tip of the finger, the palm of the hand, the forehead and the back of the neck. Compare the results in the different cases.

50. Discrimination.—Sit with the arm resting on the table, close your eyes and have your partner touch the skin on the inner surface of your lower arm with one or both points of an esthesiometer. A pair of drawing compasses having blunt points may be used in place of an esthesiometer. The distance between the two points on the instrument is varied and you should not be told in advance whether one or both points are to be applied to the skin. Find out by tests how close the points may be brought together and still be recognized as two points touching the skin. Repeat the tests on the skin of the back of the hand, the tip of the index finger, the forehead and the back of the neck. Record your results and compare.

51. Afterimage.—Place a rubber band around the head across the forehead. The band should be fairly tight. After 5 min. remove it and describe the sensation that follows.

b. PAIN

52. Distribution of Pain Receptors.—Mark off on the inner surface of the wrist a square each side of which is 1.0 cm. long. Draw a similar area in your notebook. Apply a piece of absorbent cotton soaked with water to the area on the wrist for about 5 min. to soften the skin. Apply water as needed during the experiment. Place the point of a needle to the surface of the skin and press enough to produce a sensation of pain. Explore the area in a systematic manner recording on the paper the locations of the points that give pain sensations when stimulated. Distinguish between touch and pain sensations. Are the pain spots and the touch spots identical?

53. Referred Sensation.—Place the tips of the fingers over the ulnar nerve at the elbow and move them back and forth while applying pressure. This should cause a slightly painful, tingling sensation. Is the sensation referred to the elbow or to the hand?

c. HEAT AND COLD

54. Distribution of Heat and Cold Receptors.—On the back of the wrist mark off a square, each side of which is 2 cm. in length, place a metal probe or pointed rod in cold water for a minute, dry it quickly and with the dull point explore the area in the square for the existence of cold spots. Keep the probe cool and with ink mark the position of each spot found.

Immerse the probe or rod in hot water so that it will give a sensation of warmth when removed and applied to the skin but avoid having it hot. Proceeding as before, locate the positions of the warm spots in the same area. Mark these spots with ink of a different color. Which of these two types of receptors is the more numerous within the area? Cover the square with a piece of tissue paper and on the paper mark the positions of the spots to obtain a record.

55. Intensity of Thermal Sensations.—Place warm water in a small beaker and apply the bottom of the beaker to the back of the hand and then to the palm of the hand. In which position is the greater sensation of warmth obtained? Apply the side of the beaker to the cheek and to the mid-line of the forehead and compare. Similarly, place the beaker against the skin on other parts of the body. In which positions is there a greater feeling of warmth?

Obtain a large vessel full of water that feels hot. Immerse the fingers and then the elbow in the water. Compare regarding the degree of warmth that is felt.

56. Adaptation.—Immerse one index finger in hot water that does not cause a painful sensation and at the end of 2 min. immerse the other index finger. Is the same sensation of warmth obtained from both fingers? The finger that is immersed first becomes adapted earlier.

Close your eyes, keeping the fingers immersed in the water and have your partner add hotter water to the vessel. Raise the fingers so that the water level on the fingers is kept constant as the water is added. Which finger now feels hotter? Does the temperature increase seem to be the same for both fingers? Explain.

57. Contrast.—Fill a beaker with cold water, another with lukewarm and a third one with hot water. Immerse one index finger in the cold water and the other index finger in the hot water simultaneously for 2 min. Then place them in the lukewarm water. Does the temperature of both fingers feel the same? Explain.

58. Summation of Stimulation.—Obtain a large beaker and half fill it with hot water that is not painful when applied to the skin. Place the finger tips of one hand in the water and slowly immerse the entire hand. Is the feeling of warmth intensified as more and more of the hand is immersed?

59. Afterimage.—Immerse a flat strip of metal in cold water and, when it is cool, quickly dry it and press the flat side against the forehead for about 30 sec. Does the sensation of cold persist after the stimulus is removed?

d. TASTE

60. Distribution of Taste Receptors.—Obtain a dry crystal of sucrose and place it upon the tip of the tongue. Count the number of seconds that elapse before the sweet sensation is obtained. Repeat, placing a crystal of sucrose upon the middle portion of the tongue. Then repeat, testing the posterior area. From which part of the tongue is the most distinct sensation of sweet obtained?

With a camel's-hair brush apply each of the following solutions in turn to different parts of the tongue,

(*a*) 0.001 *M* quinine sulphate (bitter).
(*b*) 0.1 *M* sucrose (sweet).
(*c*) 0.1 *M* NaCl (salty).
(*d*) 0.01 *M* tartaric acid (sour).

In each case note which part gives the most distinct sensation and compare the distribution of the various taste receptors.

61. Threshold for Taste.—Place in the mouth 5.0 cc. of a 0.001 *M* sucrose solution and note whether or not a taste of sweet is obtained. Rinse the mouth out with water and make the test with sucrose solutions of the following concentrations, 0.005 *M*, 0.01 *M*, 0.1 *M* and 1.0 *M*. Similarly, find the threshold for quinine sulphate, NaCl and tartaric acid, rinsing the mouth after each test.

62. Contrast in Taste.—Take some 0.001 *N* sulphuric acid into the mouth and, without swallowing, expel it from the mouth. Rinse the mouth with water and note the taste now obtained from the water. Explain.

e. OLFACTORY

63. Location of the Olfactory Receptors.—Connect a piece of rubber tubing of small diameter to the tip of a glass funnel and place the funnel over a small opened bottle of perfume resting on the table. Insert the end of the tubing into the lower posterior part of one nasal cavity and close the other nostril. Inhale through the nose and try to detect the odor. Repeat with the end of the tubing placed in the upper anterior part of the nasal cavity. Which region gives the most distinct olfactory sensation?

64. Fatigue of the Olfactory Mechanism.—Close one nostril and with the other smell tincture of iodine or oil of cloves in a bottle held close to the nose. Expire through the mouth and find the time interval necessary

for olfactory exhaustion to be produced. Determine the time necessary for recovery. Repeat the test several times and obtain the average values.

Fatigue the olfactory mechanism with tincture of iodine or oil of cloves and then inspire the volatile material from oil of peppermint or perfume. Is it now possible to smell the second substance? Explain.

65. Relationship between Taste and Smell.—Sit with your eyes closed while your partner places small pieces of apple and raw potato on your tongue with forceps without telling you in advance which is to be used. Hold the nostrils closed while the tests are being made and try to identify the material placed on the tongue by taste alone. Does the flavor of food depend in part upon the sense of smell?

f. EQUILIBRIUM

66. Compensating Movements.—Place a large beaker in an inverted position on a frog board and put a normal frog under the beaker. Rotate the board slowly and observe the movements of the head and other parts made in an effort to compensate. Tilt the board to and fro along the transverse axis of the animal's body. Repeat along the longitudinal axis. Observe the compensatory movements.

67. Effect of Vision on Equilibrium.—Stand upright and raise one foot about 12 in. from the floor. Try to maintain this position for a minute with the eyes open. Rest and then repeat but close the eyes. Do you now experience difficulty in maintaining equilibrium? Explain. See the text on page 151.

68. Compensatory Movements in Man.—Sit on a swivel chair or revolving stool with your eyes closed while your partner rotates the chair or stool fifteen times in 30 sec. Allowing the rotation to cease without aid, indicate the instant you feel that rotation has stopped. Does your partner who acts as observer agree that this represents the actual instant of stoppage? Explain. Do objects in the room appear to move after rotation has ceased? The sensation of turning is known as vertigo.

Bend your head forward at an angle of 30 deg. and close the eyes while your partner rotates you towards the right in the same manner as before. This time the rotation should be stopped suddenly. Immediately open your eyes and look straight at some object at a distance. Your partner should observe the jerky movement of your eyes (nystagmus) noting its direction and duration. Repeat with the rotation performed in the opposite direction.

Sit as before, but with the head erect, close the eyes, extend the right arm and with the index finger touch your partner's finger which is to be kept in front of you in a stationary position. Then raise your arm until

it is in a perpendicular position and lower it to again touch the finger of your partner. Repeat with the left arm. After you have been rotated ten times at the rate of 1 r.p.s., repeat these tests and continue them until able to respond in the normal manner. After each unsuccessful attempt to locate the finger without error your partner should bring your finger to the correct position. To what extent and in what direction is an error made? How long does it take to return to normal? Is the error made in the same direction with both arms? Repeat the tests but let your partner bring his finger to yours after you have put your finger in the position where you think his is held.

After you have been rotated ten times at the rate of 1 r.p.s. and then suddenly stopped, stand upright, with feet together, while your partner observes in which direction you tend to fall. Keep your eyes closed. Your partner must save you from an actual fall. Repeat with the rotation in the opposite direction.

g. HEARING

69. Threshold of Hearing.—In preparation for these experiments read in the text pages 190 to 195, inclusive.

Sit in a quiet room, plug the right ear with cotton, close your eyes while your partner holds a watch at various distances opposite your left ear and find the maximum distance at which the ticking of the watch is audible. The distance may be measured with a yardstick. Repeat the test with the other ear. At the threshold point does the intensity of the sound seem to vary?

70. Bone Conduction.—Hold the handle of a vibrating tuning fork between the teeth. From where does the sound seem to come? Close one ear. Does the source of the sound seem to be located as before? Repeat, closing both ears. Any difference? Explain.

With the tips of the fingers close both ears while holding a ticking watch between the teeth. Does closure of the ears permit the ticking to be heard more or less distinctly? Now close only one ear and note from which ear the ticking seems to be more distinct.

71. Localization of Sound.—Sit in a quiet room with the eyes closed while your partner holds a ticking watch in various positions with respect to your head but at a uniform distance. The watch should be placed above, in front, behind and toward the sides of the head. Attempt to state the location of the watch when held in the various positions. In which positions is the localization of the sound the most accurate? Repeat the tests when one ear is closed.

72. Auditory Fatigue.—Rest the handle of a vibrating tuning fork upon the top of the head and as soon as the sound is no longer audible remove it. After an interval of 5 sec. return the fork to the original

position on the head. Is the sound now heard again after the period of rest? Repeat the test with the tuning fork held at the side of the ear at a uniform distance from the head. Explain.

Obtain a Y-tube having a piece of rubber tubing a foot long connected to each of the two corresponding branches. Place the ends of the two rubber tubes into the openings of the ears and listen while your partner sets a vibrating tuning fork close to the opening of the Y-tube so that the intensity of the sound is equal in both ears. Then instruct your partner to remove the fork immediately, pinch one rubber tube to an ear to close it and 5 sec. after removal of the fork have it returned to the original position close to the Y-tube. When the sound from the fork can be scarcely heard open the rubber tube that has been closed. The sound will be heard more distinctly in one ear. State which it is and explain.

h. VISUAL

73. The Blind Spot.—In preparation for this series of experiments read pages 198 to 215, inclusive, in the text.

On a piece of paper make a large dot about 7 mm. in diameter and 7 cm. to the left of it draw a small cross mark. Close the left eye and with the right eye fixate the cross mark while you hold the paper about 18 in. from the face with the dot towards the right. The dark dot will be seen in the outer field of vision. Slowly bring the paper towards the face keeping the right eye focused on the cross mark and note the position at which the dot disappears. Bring the paper closer and note that the dot reappears again. Explain. Why do we not usually observe this effect?

Pin a sheet of paper to a board or wall so that it is mounted in a vertical position and on a level with the eyes when you are seated before it. Clamp a rod in a horizontal position onto a stand and adjust the height of the rod so that it touches the chin while the eyes are 40 cm. from the paper. Place a small black dot near the left margin of the paper about midway between the top and bottom. Attach a pin with a large black head to the end of a straw or thin strip of wood. Sit with the chin touching the horizontal rod, cover the left eye and fixate the dot on the paper with the right eye while your partner moves the head of the pin along the surface of the paper to map the projected boundary of the blind spot. Start with the pin in the outer field of vision towards the right and have it moved slowly to the left along the horizontal meridian of the eye. Mark on the paper the point at which the pin head will just disappear and also the point where it reappears farther to the left. Midway between these two points draw a vertical line on the paper. From this mid-point draw several lines evenly spaced and radiating outward. As your partner moves the head of the pin along these lines,

one after the other, mark on the paper the points where the pinhead disappears and reappears while your eye is kept focused on the dot. Draw a continuous line through the various points on the paper which are found in this way. What is the shape of the blind spot?

74. The Yellow Spot (Macula Lutea).—Fairly close to the blind spot on the retina there is an area that lacks blood vessels. This area (the yellow spot) contains a pigment that absorbs blue and green rays.

Obtain a flat bottle filled with a concentrated solution of chrome alum. Shut the eyes for about a minute and look through the solution at a white cloud or sheet of white paper held towards the light. What colors are observed? Do you notice a rose-colored spot? The solution allows the passage of blue, green and red rays whereas the pigment in the yellow spot transmits only the red in this combination of wave lengths. Is the rose-colored spot in the direct or indirect field of vision?

75. The Visual Angle.—Two straight lines drawn from opposite extremities of an object through the optical center of the lens (nodal point) form what is known as the visual angle. This is illustrated in Fig. 80 in the text. From this it is evident that as objects move away from the eye they would have to increase in size in order to subtend the same visual angle. For example, an object giving a certain visual angle at a distance of 5 yd. from the eye would have to be ten times larger at 50 yd. to be seen just as well.

On a blackboard draw two heavy parallel lines 1.0 cm. apart and, while looking at the lines, walk backward away from the blackboard to the point where you just fail to see the lines as two separate lines. With a meter stick measure the distance between this point and the lines on the blackboard. Calculate the visual angle necessary to distinguish two points as separate. The nodal point in the lens of the eye is about 15 mm. in front of the retina. Calculate the distance between the images of the two lines on the retina, obtained at the distance where the lines were still seen as separate but nearly fusing into one line. Is the minimum size of a discernible image on the retina dependent upon the distances that the photosensitive elements are apart?

76. Visual Fatigue.—Read the print on this page with the eyes at a distance such that a little difficulty is encountered in recognizing the words. When the print becomes blurred, close and open the eyes several times quickly and then attempt to read. Does the rest cause any improvement in vision?

77. Accommodation.—Look at a distant object while holding a piece of wire mesh about 6 in. in front of the eyes. Now close the eyes for a few seconds and upon opening them note whether it is the distant object or the mesh that is seen more distinctly. Focus on the mesh and, while doing so, close the eyes and then make the test as before. Is the result

the same as before? Focus alternately on the distant object and on the mesh. Is an effort required in doing this? Would nearsightedness cause different results than those obtained by a person with farsightedness or normal vision?

Make two pinholes about 2 mm. apart in a thin card or sheet of paper and, with the holes close to the eyes, look through them and observe that they overlap somewhat. Hold a pin in a vertical position about a foot in front of the eyes so that it comes into the field of vision within the overlapping area of the fields of the two holes. Look at a distant object through the holes and note the double appearance of the pin. Move the pin toward the left and toward the right and note that it is seen as single outside the overlapping area. The pin appears as double because its image is not in focus on the retina when the eyes are looking at the distant object. In this case would the true image of the pin tend to fall before or behind the retina? Now hold the pin at arm's length and bring it within the field in the overlapping area. Look at a distant object and then focus on the pin. Does it become single in appearance when you accommodate in this way? Try this with the pin at various distances from the eyes.

Again hold the pin at arm's length and focus on it until it appears as single. Slowly move the pin toward the eyes, keeping it in focus until no longer able to do so. Find the nearest distance of the pin from the eyes at which it may still be seen distinctly as single when you focus upon it. Your partner may aid in measuring the distance. Find the average of three measurements. This distance is known as the *near point* of distinct vision. What muscles and properties of the lens are involved in accommodation?

Look at a distant object and then at a near object. While doing so let your partner observe the pupils of your eyes and report the changes which occur. Do the eyeballs change in position? How do these changes aid in accommodation?

78. Projection of Visual Sensations (Monocular).—Make a pinhole in a sheet of paper and place the hole about 2 cm. in front of the eyes. Hold a pinhead upward quite close to one eye (between the eye and the hole) and bring it upward into the field of vision occupied by the hole. What appears to be the orientation of the pin and how does it seem to move? Now repeat but place the pin on the far side of the hole. Explain.

79. Binocular Projection.—Attach one end of a thread about a yard long to the wall at the level of the eyes. About a foot from the opposite end tie a straight pin to the thread and hold the thread at the bridge of the nose so that the pin is about 10 cm. from the eyes. The thread should be kept taut and horizontal. Look steadily at the head of the pin

and note the appearance of the thread. While doing so hold another pin directly before or behind the pin on the thread. Do the double images of the pins unite? Move the pin in your hand away from the other one along the thread while fixating the other pin. What impressions do you receive regarding depth in space?

80. Adjustments in the Size of the Pupil.—Allow a strong light from the window or lamp to fall on the eyes and then look at a dark surface away from the light while your partner observes the pupils of your eyes. Place your hand over one eye for a minute and upon removing it have the reaction of the pupil observed. Cover one eye again and allow your partner to observe the pupil of the other. What happens to the pupil of the open eye when the other is uncovered?

Look through a pinhole in a sheet of paper at a white well-illuminated surface such as that on a reading lamp. Close the other eye and note the size of the illuminated field. Upon opening the eye that has been closed note that the field becomes reduced in size. Now close the eye again and observe that the field increases in size. The changes in the size of the field are due to alternate constriction and dilation of the pupil.

81. Alternate Predominance of Retinas.—Place in a stereoscope a card having a vertical strip of blue in the center of one field as seen through the stereoscope and a similar strip of yellow in the corresponding part of the other field. Look at the strips through the stereoscope intently and notice that after a time the colors will appear to alternate. While your partner indicates an interval of 1 min., count the number of alternations that occur.

In a similar manner observe the lines on a card ruled so that there are about 20 horizontal lines evenly spaced in the left field and the same number of vertical lines in the right field. Note that after an interval the lines will alternate between horizontal and vertical. Count the frequency of alternation as before.

82. Afterimages (Positive and Negative).—Close the eyes and cover them with the hands. At the end of a couple of minutes look directly at an unshaded electric light for about 10 sec. and cover the eyes as before at once. Note the positive afterimage and its duration. After a time there is a reversal of the light and dark areas.

Place a piece of white paper about 1.0 cm. square on a black background and look at its center for about a minute. Then look at a white surface such as a sheet of paper about a foot from the eyes. Do you observe a negative afterimage? Look at a white surface several feet away and note whether or not the afterimage appears to be larger.

Repeat the last test using, in turn, red, yellow and blue squares on a white background. What is the color of the negative afterimage in each case?

CHAPTER VI

THE ORGANIC CONSTITUENTS OF LIVING MATTER

83. Separation of Egg Albumin and Egg Globulin.—Separate the white from the yolk of an egg. Put the yolk into a flask, cover it with ether, stopper it and set it away for later use in the study of lecithin. Strain the white through a cloth to remove the membranes that form invisible partitions in the egg white. Dilute the strained material with ten times its volume of water. Note the resulting precipitate. It is ovoglobulin. Let it settle and filter the supernatant liquid through a plaited paper. Then filter the rest of the material through a fresh, wet plaited paper. Wash the precipitate with water. Reserve it for use in Exp. 93. Evaporate about 100 cc. of the albumin-containing filtrate in a shallow dish at a temperature below 60°C. This can be done over a steam radiator or water bath with the flame turned low. The resulting dried material is nearly pure albumin. It can be used in Exp. 94.

84. The Biuret Test on Albumin.—To about 5 cc. of albumin solution (filtrate, preceding experiment) add an equal volume of 10 per cent KOH and, drop by drop, add dilute (about 0.5 per cent) $CuSO_4$ until no further color change can be obtained. Look for a violet color that is given by all proteins in this reaction. Excess of $CuSO_4$ gives the blue color of copper hydroxide, which conceals the violet and thus spoils the test. Although this test is given by all proteins and is thus of much practical value, it is also given by other substances, *e.g.*, biuret from which the test gets its name. Try this either on some biuret solution furnished you or on one prepared by heating a few crystals of urea in a dry test tube and dissolving the resulting fused mass in water.

85. Millon's Test.—To about 5 cc. of albumin solution add 5 to 10 drops of Millon's reagent[1] (a solution of metallic mercury in HNO_3, diluted with water); boil and note any changes in the white precipitate at first formed. This test is given by proteins that contain tyrosine as a constituent amino acid. Repeat the test, using a solution of tyrosine instead of albumin. The reaction is due to the phenol group and is given by phenol itself. Prove this by testing a dilute phenol solution.

[1] **Millon's Reagent.**—By the aid of gentle heat, dissolve 1 part by weight of mercury in 2 parts by weight of HNO_3 (sp. gr., 1.42). Dilute with 2 volumes of distilled water and leave at room temperature several hours. The clear supernatant solution is used as the reagent.

86. The Glyoxylic Acid Test.—To about 5 cc. of albumin solution in a test tube, add 0.5 cc. of glyoxylic acid reagent.[1] This will have been prepared by treatment of oxalic acid with magnesium and contains the magnesium salt of glyoxylic acid. Incline the test tube and pour concentrated H_2SO_4 (2 or 3 cc.) into the tube so that it sinks to the bottom. At the zone of contact between the two layers thus formed, a brilliant color develops after a few minutes or sooner if the tube is gently agitated. Describe. This reaction is given by proteins containing tryptophane as a constituent amino acid. Repeat the test, using a solution of tryptophane instead of albumin.

87. Salting-out of Albumin.—To about 20 cc. of albumin solution in a small beaker, add solid $(NH_4)_2SO_4$ with vigorous stirring until no more will dissolve. The solution is then saturated with the salt, and albumin will be thrown out as a heavy, cloudy precipitate. To show that it is albumin, filter off a little of it, dissolve it in water and make the biuret test, using an excess of KOH and heat to drive off ammonia. All proteins, except peptones, are salted out by saturating their solutions with $(NH_4)_2SO_4$.

Saturate another 20-cc. portion of albumin solution with solid NaCl. Note the absence of precipitation. Now acidify the solution with 2 or 3 drops of 10 per cent acetic acid. The albumin is precipitated. Although some proteins can be salted out by neutral NaCl, albumins are salted out by it only in acid solution.

88. Nitric Acid Precipitation, Heller's Test.—To a few cubic centimeters of concentrated HNO_3 in a test tube, add albumin solution slowly so that it will remain above the acid. Look for a white precipitate at the zone of contact. This is a very delicate test for minute amounts of protein. Repeat using albumin solution diluted 50 times and 100 times. In medical diagnosis this test is used to detect small amounts of albumin in urine.

89. Precipitation of Albumin by Alcohol.—Prepare four test tubes, each containing 10 cc. of 95 per cent alcohol. To the first, add 2 drops of 1 per cent acetic acid; to the second, 2 drops of 10 per cent HCl; to the third, 2 drops of 10 per cent KOH; leave the fourth neutral. Now add 3 or 4 drops of albumin solution to each tube and note results. What conditions determine the precipitation of albumin by alcohol?

90. Heat Coagulation of Albumin.—In each of four test tubes put 5 cc. of albumin solution. To the first, add 1 drop of one per cent acetic

[1] **Glyoxylic Acid Reagent.**—Place 10 grams powdered magnesium in a flask and add enough distilled water to cover the metal. Then, slowly and with gentle shaking, add 250 cc. of a saturated solution of oxalic acid, keeping the flask cooled in running water. Filter to get rid of the precipitated magnesium oxalate, acidify the filtrate with acetic acid and dilute to 1 liter.

acid; to the second, 1 drop of 10 per cent HCl; to the third, 1 drop of 10 per cent KOH; leave the fourth neutral. Now heat each tube to boiling and from a comparison of the results, state what conditions favor heat coagulation.

91. Albumin Precipitation by Metallic Salts.—To 5 cc. of albumin solution in a test tube, add 5 per cent $HgCl_2$ drop by drop and note results. Similarly test the action of 5 per cent $CuSO_4$ solution. Note that the white precipitate, at first formed, goes into a cloudy, bluish solution with excess of $CuSO_4$ Similarly test the action of 5 per cent $Pb(OOCCH_3)_2$ solution. Why are large amounts of egg white given as an antidote for mercury, copper or lead poisoning?

Also test the action of 0.5 per cent $FeCl_2$ solution on albumin solution. Be very careful to add the reagent a drop at a time, with shaking after each addition, and to note all changes. This experiment illustrates the complicated behavior of proteins, forming compounds of differing solubility with varying amounts of the same reagent and with varying hydrogen-ion concentrations of the solution.

92. Albumin Precipitation by Alkaloidal Reagents.—The alkaloids are vegetable, nitrogen-containing bases and include morphine, digitalis and many other drugs. They are precipitated by so-called alkaloidal reagents that also precipitate proteins. As examples of this, try on 5-cc. portions of albumin solution the precipitating action of: (*a*) saturated solution of tannic acid; (*b*) saturated solution of picric acid; (*c*) dilute solution of phosphotungstic acid; and (*d*) 5 per cent solution of trichloracetic acid. How do this and the preceding experiment illustrate the amphoteric character of proteins?

93. Tests on Egg Globulin.—The insolubility of globulin in water was demonstrated by its separation from white of egg on adding water. Globulins are soluble, as one of their distinctive characteristics, in dilute salt solutions. Show this by stirring up the paper bearing the globulin from the egg white in 5 per cent NaCl solution. Filter and test the filtrate by the biuret test. Saturate some of the filtrate with solid NaCl. Again filter and test the filtrate by the biuret test. Is the globulin completely salted out by NaCl in neutral solution? Aside from such physicochemical behavior as solubility and salting-out, globulins give the same reactions, color tests, etc., as the albumins.

94. Elemental Composition of Protein.—Heat a small amount of dry albumin in a dry test tube. Note the charring that proves the presence of carbon. Note the moisture condensing on the upper part of the test tube, indicating the presence of hydrogen and oxygen. Hold a moistened red litmus paper in the fumes coming from the tube. See if the litmus turns blue, indicating ammoniacal fumes, which prove the presence of nitrogen. Hold a piece of filter paper moistened with lead

acetate solution in the fumes from the tube. Look for the formation of a black spot of lead sulphide on the paper, proving the presence of sulphur.

95. Separation of Serum Albumin and Globulin.—Saturate 10 cc. of blood serum with solid NaCl. This precipitates globulin. Filter it off and identify it as a protein by the biuret and Millon's tests. To the filtrate add a few drops of 10 per cent acetic acid. This precipitates albumin. Filter it off and identify it. Try the heat coagulation of a mixture of 2 cc. of blood serum and 8 cc. of water, adding a drop of 1 per cent acetic acid to insure proper coagulation. Try Millon's test on the coagulum.

96. Caseinogen, a Phosphorus-containing Protein.—To 50 cc. of skimmed milk add an equal volume of water and then add 1 per cent HCl carefully, about 1 cc. at a time, stirring and waiting after each addition. When a flocculent precipitate begins to form add the acid a few drops at a time until, after stirring and waiting a minute, heavy flocculent masses are seen with clear liquid between them. Filter off the precipitated caseinogen. Prove that it is a protein by the use of the protein color tests. For this purpose the caseinogen can be used suspended in water. Remove moisture from the rest of the caseinogen as far as possible by pressing it between filter papers. Caseinogen occurs in milk as its calcium salt, Ca-caseinogenate. By freeing it from this combination with calcium, acid causes its precipitation. It will form soluble combinations, however, with either acids or bases. Prove this by suspending some of it in water, adding dilute NH_4OH to one portion and dilute HCl to another. The result should show why caution is necessary in precipitating caseinogen by the above method. Why does sour milk clot?

97. Detection of Phosphorus in Caseinogen.—Place enough caseinogen in a porcelain crucible to make it about one-third full and add a few grams of a mixture of dry K_2CO_3 and KNO_3. Place the crucible on a clay triangle and heat over the Bunsen flame, adjusting it from time to time so as to burn the material in each part of the crucible until all carbon is oxidized. If necessary, a little powdered KNO_3 may be sprinkled over the contents of the crucible. When a white ash is obtained, cool and dissolve in water containing a few drops of HNO_3. Transfer to a test tube, add ammonium molybdate and heat gently. A yellow precipitate of ammonium phosphomolybdate is a test for phosphorus. Caseinogen belongs to the group of so-called phosphoproteins. Why do they have to be ashed before the phosphate test is made?

98. Edestin, a Crystalline Vegetable Globulin.—Place a handful of ground hemp seed in a small beaker and add about 50 cc. of 5 per cent NaCl solution. Put the beaker in a water bath with the flame turned low and stir the mixture at frequent intervals during 20 to 30 min. See that the mixture is kept as nearly as possible at 60°C. throughout this time. A thermometer may be used as a stirring rod. Now filter through

a plaited paper that has been recently wet with hot 5 per cent NaCl solution. Allow the filtrate to collect in a beaker or flask that is surrounded by water at 60°C. The reason for this is that the protein, edestin, dissolved in warm salt solution will go out of solution on cooling but will separate in satisfactorily crystalline form if cooled slowly. On this account allow the filtrate still surrounded by water to come slowly to room temperature. Remove a drop of the mixture containing sedimented crystals by means of a pipette, place on a slide and examine microscopically. Sketch the crystals. Filter them off and identify as protein by trying any two color tests. For this purpose the edestin may be suspended in water.

Pour about 1 cc. of the filtrate from edestin crystals into 25 cc. of distilled water. Note the precipitation of edestin. Compare this precipitation with that of egg globulin. Is this behavior characteristic of all globulins?

On another portion of this filtrate, try the biuret test and the precipitation by HNO_3 (Heller's test); also try precipitation by $HgCl_2$ and by tannic acid. Does this vegetable globulin behave similarly to animal globulins and albumins?

99. Gliadin, a Prolamin, and Other Proteins of Wheat.—Mix about 50 grams of wheat flour with enough water to form a stiff dough. Stir and knead it for 10 or 15 min. to permit the development of tenacity in the dough so that its proteins will not be washed away by the subsequent treatment. Add about 250 cc. of water to the dough and again knead it thoroughly. Note that the water becomes milky because of suspended starch grains. Pour off the water and examine a drop of it on a slide under the microscope to see the starch grains. Add a drop of iodine solution to the slide and note that the grains are stained blue. After the starch grains have settled out of the water, the supernatant liquid can be tested by the biuret test. Are there water-soluble proteins in flour? The dough should now be kneaded with successive portions of water until the latter shows practically no cloudiness from suspended starch grains. The dough remaining is gluten and contains most of the protein of flour. Grind the gluten in a mortar with a little 70 per cent alcohol, shake at frequent intervals during the remainder of the laboratory period and leave standing until the next period. Gliadin dissolves; glutenin, part of the gliadin and traces of other proteins remain undissolved. Filter and reserve both the filtrate and the material on the paper. Evaporate the filtrate to dryness on a water bath. Scrape off the gliadin thus dried on the evaporating dish and apply protein color tests to it.

Glutenin, the chief constituent of that part of the gluten that does not dissolve in 70 per cent alcohol, is soluble in dilute acids or alkalies. Prove this by treating some of the material with 0.5 per cent NaOH, filtering

and applying the biuret test to the filtrate and then treating some more of the material with 0.5 per cent HCl, filtering and applying the biuret test to this filtrate.

100. Crystalline Form of Some Amino Acids.—Examine microscopically crystals of various amino acids on slides that will be supplied for the purpose. Sketch the crystals in each case and compare them with those shown in page 230 of the text.

101. Diffusibility of Proteins.—Soak two small sheets of vegetable parchment-paper with distilled water to make them flexible. Arrange each of them as a small sac by tying on to the end of a glass tube. Test each sac with water to be sure that it does not leak. Pour out the water and half fill one with albumin solution and half fill the other with peptone solution. Place each of them in a small beaker containing about 10 cc. of distilled water. Leave not less than 3 hours and then test the water in the beaker by the biuret test to see if protein has gone through the paper. Explain the results in terms of the size of the colloidal particles of the proteins.

102. Solubility of Fats.—Test the solubility of cottonseed oil in water, cold alcohol, hot alcohol (heated in a test tube in a water bath to avoid catching fire), cold ether, chloroform, carbon tetrachloride and benzine (or gasoline). Use a drop of the oil and about 5 cc. of the solvent for each test. Solubility can usually be detected by the failure of a fat layer to separate out after shaking. In case of doubt some of the solvent can be removed from the test tube by a pipette and evaporated on a dish or watch glass to see if a residue of fat remains. Most of these solvents are inflammable and should be evaporated only well away from the flame, either over a steam radiator or on a hot water bath with flame turned out. Make a list of typical fat solvents based on the results of this experiment and any other information available.

Put a drop of ether-oil solution on a piece of paper and, after the ether has evaporated, determine whether or not the oil can be volatilized by prolonged warming.

103. The Acrolein Test.—To about 10 drops of oil in a test tube, add some crystals of potassium bisulphate ($KHSO_4$) and heat until heavy white fumes come off. Note the odor or rather the irritating effect of the fumes on the nasal membranes. This is due to acrolein formed by the action (dehydration) of the bisulphate on the glycerol part of the fat molecule. To prove this, repeat the test on a few drops of pure glycerol. Write the reaction involved.

104. Separation of Glycerol from Saponified Lard.—Treat about 50 grams of lard with 150 cc. of alcoholic KOH solution[1] in a flask and keep

[1] **Alcoholic KOH.**—Dissolve 30 grams of pure KOH in 1 liter of 95 per cent alcohol.

the mixture warm on a hot water bath until saponification is complete. When this is the case, a drop of the mixture removed from the flask and mixed with a little cold water will completely dissolve and leave no oily film on the surface of the water. Pour the saponified mixture into an evaporating dish, add 200 cc. of water and heat on a water bath until the odor of alcohol is no longer detected. Acidify with HCl to precipitate fatty acids, cool and skim the layer of fatty acid from the surface. Neutralize the remaining solution with Na_2CO_3 and evaporate it to dryness. Extract the residue with alcohol, filter and evaporate the filtrate to obtain a residue of glycerol. Identify it by its sweet taste and by use of the acrolein test. Write equations to represent the reactions that occur in this experiment. Compute the weight of glycerol that could be obtained from 50 grams of triolein, one of the constituents of lard. What can you infer from this experiment as to the solubilities of soaps, fatty acids and glycerol?

105. Properties of Glycerol.—With a specimen of pure glycerol, note its taste, its viscosity and its solubility in water, alcohol and ether. Also try the borax flame test: Dip a loop on the end of a platinum or nichrome wire into powdered borax, then into glycerol and then put it into a colorless Bunsen flame. Describe the resulting flame color, which is due to glycerol borate.

106. Saponification of Bayberry Fat.—To about 10 grams of bayberry wax (chosen because it is nearly all tripalmitin and therefore yields nearly pure palmitic acid) add 50 cc. of 10 per cent KOH in an evaporating dish and boil until saponification is complete, which will be in about 5 min. For test for completeness, see Exp. 104. Write an equation for the reaction occurring in this saponification. The products are glycerol and potassium palmitate, a soft soap. To produce a hard, sodium soap treat about half the saponified mixture (reserving half for the next experiment) with some solid NaCl. Remove the soap thus salted out and squeeze the water from it to form a small cake of soap.

107. Preparation of Palmitic Acid.—To the remainder of the saponified mixture, add enough HCl to acidify it. Note the precipitate of palmitic acid. Write an equation for this reaction. Heat the acidified mixture to boiling so that the fatty acid will separate as an oily layer on the surface. Cool so that it will solidify as a cake. Remove and dissolve it in a little (about 50 cc.) of warm alcohol in a beaker. Filter while still warm and allow the filtered alcoholic solution of palmitic acid to cool slowly for crystallization. Examine some of the crystals under the microscope and sketch them. Filter them off and determine the following points: Is palmitic acid soluble in ether? Does it give the acrolein test?

108. Crystallization of Fat.—Dissolve a small piece of lard in ether in a test tube, add an equal volume of alcohol and let the mixture evapo-

rate spontaneously at room temperature. Examine the resulting crystals under the microscope and sketch them.

109. Emulsification of Fat.—Shake a drop of cottonseed oil with about 5 cc. of water in a test tube. The fat becomes finely divided into droplets, thus forming an emulsion. But note what happens after standing a minute or two.

Shake a drop of the oil with 5 cc. of water containing a little soap. Is this emulsion comparatively permanent? Put a drop of it on a slide and examine under the microscope. What is the explanation of the cleaning value of soap?

Shake a drop of the oil with 5 cc. of water containing a few drops of 0.5 per cent Na_2CO_3. How permanent is this emulsion?

Shake a drop of rancid oil, or oil to which a little oleic acid has been added, with 5 cc. of water containing a few drops of Na_2CO_3. Note and explain the result.

Shake a drop of oil with 5 cc. of a dilute albumin or other protein solution. Is protein an emulsifier?

110. Hardness of Water.—Add a little of a solution of a pure soap to: (*a*) distilled water, (*b*) a dilute solution of a magnesium salt such as the carbonate or the chloride and (*c*) a dilute solution of a soluble calcium salt such as the chloride. Describe the results and compare them with what happens when soap is put into hard water.

111. Preparation of Lecithin from Egg Yolk.—To the mixture of ether and yolk of egg prepared in the first experiment on proteins, add more ether if the latter has largely evaporated. About 50 cc. should be present. Then add 75 cc. of alcohol, shake thoroughly and filter. The filtrate contains ether, alcohol, fat, lecithin, other substances similar to lecithin, cholesterol and traces of proteins and other egg constituents. Evaporate the filtrate to dryness on a water bath. Extract the dried residue, so obtained, with 15 cc. of cold ether and filter. This filtrate now contains only ether-soluble substances. Treat it with about 50 cc. of acetone, which precipitates lecithin together with other phosphorized fats. As lecithin is the most abundant constituent of the precipitate it may be regarded as crude lecithin. Filter it on a small paper and reserve it for the following tests.

112. Emulsification of Lecithin.—Mix a small amount of lecithin with a drop of water on a slide. Put on a cover glass and examine microscopically. How does this mixture differ from a fat emulsion?

113. The Fatty Acids of Lecithin.—To a little lecithin in a test tube, add a few drops of osmic acid solution. Note the result after a few minutes. Repeat the test on palmitic acid and on oleic acid. The latter is a representative of unsaturated fatty acids. What do you conclude as to the character of the fatty acid of lecithin?

114. The Acrolein Test on Lecithin.—With a little lecithin, make the acrolein test as described in Exp. 103. What does the result show with regard to one of the constituent groupings of the lecithin molecule?

115. Test for Phosphorus in Lecithin.—Fuse one-third of your lecithin with a mixture of K_2CO_3 and KNO_3 in a crucible. When burned to a white ash, cool, dissolve in water, add a little HNO_3, treat with ammonium molybdate and warm gently. Do you obtain a test for phosphorus?

116. Test for Nitrogen in Lecithin.—Heat a little lecithin in a test tube with a small piece of metallic sodium. Hold a piece of moistened red litmus paper in the fumes that come off. What is the result and why is it a test for nitrogen? From what constituent atomic grouping of lecithin is it derived? (See text, page 257.)

117. Crystallization of Cholesterol.—Dissolve a little cholesterol in ether, add an equal volume of alcohol and let the solution evaporate slowly for crystallization. Examine the crystals microscopically and sketch them.

118. Sulphuric Acid Test (Salkowski) on Cholesterol.—To some crystals of cholesterol, dissolved in a little chloroform, add an equal volume of concentrated H_2SO_4. Note and describe the various colors and the fluorescence that appear.

119. Solubility of Sugars.—Try the solubility of any one of the common sugars, such as glucose, sucrose or maltose, in water, alcohol, ether, 10 per cent KOH and 10 per cent HCl. To try solubility, shake a small portion (as much as can be held on a knife point) of the sugar with about 5 cc. of the solvent in a test tube and, if in doubt as to the result, filter and evaporate 2 or 3 cc. of the filtrate to see if a residue of sugar is obtained. Only one kind of sugar need be tried, as the solubility of all the sugars is qualitatively the same. What is the significance of the solubility of sugars in the economy of nature?

120. The Phenylhydrazine Reaction to Form Osazones.—Put 1 or 2 grams of a phenylhydrazine mixture (prepared by thoroughly mixing 2 parts of phenylhydrazine hydrochloride and 3 parts of sodium acetate, by weight) into each of six test tubes and then add to each tube 15 cc. of the 5 per cent solution of a sugar. Use the following sugars: (1) glucose, (2) fructose, (3) sucrose, (4) maltose, (5) lactose and (6) the pentose, arabinose. Shake each tube thoroughly and, if a clear solution is not obtained, filter. Label the six tubes containing the clear solutions and place them in a hot water bath. Keep the water boiling 1 hr. and then remove the flame and let the tubes cool slowly in the bath. In this way, the osazones formed during the boiling have a chance to crystallize. With a pipette, take from each tube a drop containing crystals. Place it on a slide with a cover glass, examine microscopically and

sketch the crystals. Compare with the illustrations on page 273 of the text.

Is fructosazone distinguishable from glucosazone? Explain in terms of the molecular structure of these sugars. What sugars can be positively identified by the use of the phenylhydrazine reaction in qualitative analysis? Explain the result of this reaction with sucrose.

121. Copper Reduction (Trommer's) Test for Sugars.—To 5 cc. of 5 per cent KOH in a test tube, add 5 drops of 5 per cent $CuSO_4$ and shake the mixture. Explain the changes observed. Now add 10 drops of dilute (about 1 per cent) glucose solution. Heat gently and note all changes that occur. As a control test, heat a similarly prepared mixture of KOH and $CuSO_4$ without sugar. Explain and write equations to represent the changes in the copper compounds in both tests. What is reduced in this test? What is oxidized? What is the nature of the oxidation products formed?

Similarly test, in turn, each of the three sugars: sucrose, maltose and lactose. What are the results? These sugars all belong to the same group of carbohydrates. Which one? They are all represented by the same empirical formula. What is it? In spite of this similarity, they do not respond alike in this test. Explain in terms of their molecular structure.

122. Benedict's Test.—To 5 cc. of Benedict's solution[1] add 8 drops of a 1 per cent glucose solution and boil 1 or 2 min. Note results. Set the tube aside and after it has cooled and completely sedimented observe again. Repeat this test using 1 per cent solutions of other carbohydrates, *e.g.*, fructose, arabinose, lactose, maltose, sucrose and soluble starch. Explain the results.

Benedict's test is widely used in testing for reducing sugars. What advantages does it afford as compared with other similar tests, *e.g.*, Trommer's?

123. Fermentation Test.—Try this test on each of the four sugars: glucose, sucrose, maltose and lactose. In each case rub up a small piece of a compressed yeast cake with about 20 cc. of a 1 per cent solution of the sugar. Transfer each mixture to a fermentation tube, filling the closed arm completely. Set aside for 12 hr. or longer. If the sugar is fermentable, alcoholic fermentation will occur and CO_2 will collect in the closed arm of the tube. Which of these sugars are fermentable? After fermentation is completed, introduce a little 10 per cent KOH solution into the closed arm of the tube by means of a bent pipette. Place the

[1] Benedict's solution for qualitative tests is prepared as follows: Dissolve 173 grams of crystalline sodium citrate and 100 grams of anhydrous sodium carbonate in about 800 cc. of water. Filter and add 17.3 grams of copper sulphate dissolved in 100 cc. of water. Add water to make up to 1 l.

thumb tightly over the opening of the tube and invert it. Explain the result.

124. Formation of Caramel.—Gently heat a small amount of powdered glucose in a test tube. After the sugar has melted and turned brown, cool the tube, add water and warm. The coloring matter produced is caramel. Is it soluble? What are some of the compounds contained in it? (See text, page 274.) What property of sugars permits the formation of these compounds?

125. Preparation of Potato Starch.—Pare a raw potato, pulp it with a fine grater, mix the pulp with 300 cc. of water, stirring vigorously, and strain through cloth to remove the coarser particles. From the strained material, starch grains settle out rapidly. When sedimentation appears to be complete, decant the water. To wash the starch, stir it up again with water, sediment it and decant the water. This may be repeated until the starch is perfectly white. Drain the starch and spread it out on a glass plate to dry in the air. This preparation may be used in some of the following experiments.

126. The Forms of Starch Grains.—With the high power of the microscope make an examination of potato starch grains suspended in a little water. Sketch them. Note their stained appearance after running some iodine solution under the cover glass. Similarly examine, sketch and stain the grains of other kinds of starch, *e.g.*, corn, wheat, arrowroot and rice.

127. The Iodine Test on Starch Paste.—To make starch paste, grind 2 grams of dry starch with about 10 cc. of water in a mortar. Meanwhile bring 150 cc. of water to the boiling point and slowly pour the suspension of starch grains into the boiling water while stirring vigorously. Continue boiling 2 min. and cool the paste. To a little paste in a test tube, add dilute iodine solution, heat the tube and then cool it under running water. Note results and explain them. To some blue starch-iodine paste in a test tube, add some alcohol and, to another lot of starch-iodine paste, add 10 per cent KOH solution. What conditions must be avoided in making the iodine test for starch?

128. Hydrolysis of Starch.—Place 50 cc. of starch paste in a small beaker, add 1 cc. of concentrated HCl and boil. At the end of each minute of boiling, remove a few drops of the solution with a pipette to a test tablet or tube and make the iodine test. From time to time, add a little more water to the contents of the beaker to prevent too great a concentration. As the testing proceeds, the iodine reaction shows certain modifications. Describe and explain them. When the starch is sufficiently hydrolyzed, no color is obtained with iodine. This is the so-called achromic point. At this stage, cool the solution, neutralize it with KOH and try Benedict's test on it. On some more of the solution,

try the phenylhydrazine test. What sugar is produced by hydrolysis of starch?

129. Preparation of Glycogen.—Grind a few scallops in a mortar with clean sand. This breaks up the tissue cells. Oysters or the liver of a recently killed animal that has been fed on a carbohydrate-rich diet are also sources of abundant glycogen, indeed, nearly all animal tissues contain some glycogen. But the scallop (using the bivalve muscle of the shellfish, *Pecten irradian*) is chosen because it always yields comparatively large amounts of glycogen. Transfer the ground up material to a porcelain dish, add enough water to nearly fill the dish and boil 10 min. While boiling the mixture, faintly acidify it by adding 0.5 per cent acetic acid until acid to litmus. What object is attained by acidifying? The opalescence of the solution is characteristic of glycogen. Filter and use the filtrate in the following experiments.

130. The Iodine Test on Glycogen.—To 5 cc. of glycogen solution in a test tube, add iodine solution, drop by drop, until the maximum color change is produced. Now add the same amount of iodine solution to 5 cc. of water. Compare the colors of the two solutions by reflected and transmitted light. The color of glycogen with iodine may be described as port-wine red. If you have difficulty in differentiating between the iodine-glycogen color and that of iodine solution itself, proceed as follows: Make some glycogen solution acid with HCl and divide into two parts. Boil one portion to hydrolyze the glycogen. Add equal amounts of iodine to both portions and compare the colors.

131. Reduction Test on Glycogen.—Try Benedict's test on glycogen solution. The muscle proteins and phosphates present interfere somewhat with this test, so that precipitates other than red cuprous oxide should be disregarded.

132. Hydrolysis of Glycogen.—To 20 cc. of glycogen solution add 1 cc. of concentrated HCl and boil 10 min. Cool, neutralize with KOH and try Benedict's test. What is the result? If a reducing sugar is found, how may its identity be established?

133. Precipitation of Glycogen.—To some glycogen solution, add two volumes of 95 per cent alcohol. Allow the glycogen precipitate to settle, decant the supernatant fluid and filter the remainder. Test some solid glycogen in a porcelain dish or against a white background with iodine solution. Compare the color of the stained particles with that obtained in Exp. 130.

134. Molisch Test on Various Carbohydrates.—To 5 cc. of glucose solution in a test tube add a few drops of an alcoholic solution of alpha-naphthol (Molisch reagent). Into the inclined test tube, pour concentrated H_2SO_4 so that it sinks to the bottom. Look for a red ring at the zone of contact between the two layers.

Repeat this test on a solution of another sugar, *e.g.*, one of the disaccharides. Also try the test on starch paste and glycogen solution.

This reaction is due to the liberation of furfural from carbohydrates by the action of concentrated H_2SO_4. Try the test on a dilute solution of furfural.

Pentoses and pentosans yield furfural under the action of HCl instead of H_2SO_4.

135. A Special Test (Seliwanoff) for Fructose.—To about 5 cc, of Seliwanoff's reagent (0.05 g. of resorcinol in 100 cc. of 12 per cent HCl) in a test tube, add a few drops of 5 per cent fructose solution and boil. Look for the appearance of a red color. A precipitate that forms when sufficient fructose is used can be filtered off and dissolved in alcohol to give a brilliant red solution. Although maltose and some other sugars may give this test upon prolonged heating, only fructose gives it readily.

136. The Mucic Acid Test for Galactose.—To 20 cc. of 1 per cent galactose solution in an evaporating dish, add 5 cc. of concentrated HNO_3 and evaporate on a water bath to about 5 cc. Transfer to a test tube and cool. A white precipitate of mucic acid settles out.

Repeat, using a lactose instead of a galactose solution. Explain why lactose gives this test.

137. Relative Oxidizability of Monosaccharides and Disaccharides.—To 5 cc. of Barfoed's solution[1] in a test tube, add 1 cc. of 1 per cent glucose solution and boil 1 min. Set the tube aside for sedimentation of cuprous oxide. Repeat this test on the sugars: fructose, maltose and lactose. In the case of the disaccharides, try heating during a second period of 1 or 2 min. Does this alter the result? Which of these sugars are more susceptible to oxidation?

138. Precipitation of Polysaccharides by Alcohol.—Find how much 95 per cent alcohol is required to precipitate the starch from 10 cc. of a 2 per cent starch paste. Similarly, test the precipitability by alcohol of a 2 per cent solution of soluble starch and a 2 per cent solution of dextrine. What other polysaccharide have you precipitated by alcohol?

139. Polariscopic Observation of Sugars.—Observe and describe the mutarotation of a freshly prepared glucose solution as demonstrated for you with the polariscope.

On a sucrose solution, of which the exact strength will be given you, make readings with the polariscope to determine the angle of rotation of the solution. Make several determinations on the same solution and average your results. Compute the specific rotation of sucrose (see text, page 273).

[1] Barfoed's solution is prepared as follows: Dissolve 13.3 grams of crystalline cupric acetate in 200 cc. of distilled water, filter and add 5 cc. of 38 per cent acetic acid.

CHAPTER VII

THE BIOLOGICAL SIGNIFICANCE OF WATER AND OTHER ELECTROLYTES

140. Electrical Conductivity of Solutions and Biological Fluids.—In preparation for this work read the discussion in the text on pages 305 to 307, inclusive. This work will be covered by demonstration but students with sufficient experience may make the measurements for themselves. The apparatus for the measurement of electrical conductivity will be assembled and connected. Examine the various pieces of apparatus and determine the purpose of each. Become familiar with the manner in which the electrical connections are made and learn how the circuits work (see Fig. 91 in the text). Make a diagram of the electrical connections and apparatus employed.

The solutions to be used should be kept at a chosen constant temperature in a constant-temperature water bath. The conductivity cell is kept immersed in the water bath, and the measurements are all made at the one temperature. Obtain the value for the cell constant from the instructor or determine its value, employing a $N/50$ KCl solution accurately prepared, preferably with conductivity water. After the value for the cell constant has been obtained, measure the electrical conductivity of KCl solutions having the following concentrations: $N/8$, $N/16$, $N/32$, $N/64$, $N/128$, $N/256$, $N/512$ and $N/1024$. The conductivity cell should be rinsed first with a sample of the solution to be used. Employ the edge of a clean piece of filter paper to remove any solution remaining on the vessel or electrodes after rinsing. Touch only the edges and not the surfaces of the electrodes with the filter paper.

The specific conductivity of an unknown solution is obtained by means of the equation

$$\kappa = \frac{1}{R}K$$

where κ = specific conductivity.
R = resistance in ohms.
K = cell constant.

Plot in graphical form the specific conductivity of these solutions against their normalities. What is the form of the curve? Explain.

The specific conductivity of urine may be measured after the person has been on a restricted water intake for 24 hr. and again after there has been an abundant water intake for the same length of time. Measurements may also be made on blood serum and on fresh milk.

141. Balanced Isotonic Salt Solutions.—Before doing these experiments read in the text the discussion on pages 309 to 312, inclusive. Prepare a kymograph for graphic recording and on a support in front of it mount a light heart lever and a time marker. The lever should be counterpoised and provided with a cellophane point. Below the lever and time marker attach to the support a heart holder consisting of a cork disk provided with a bent glass rod for mounting in a clamp. The holder is illustrated in Fig. 16. Adjust the level at which the heart holder is placed on the support so that the cork disk may be placed near the bottom of a 600-cc. beaker that is allowed to rest on a small wooden stand. Temporarily set the beaker and wooden stand aside.

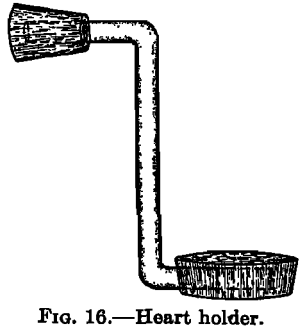


FIG. 16.—Heart holder.

The heart of a large frog or turtle should be employed. If a turtle heart is to be used, it may be exposed as follows. With a hack saw quickly sever the head from the body of the animal and cut through the narrow part of the plastron at both sides. With a knife cut the skin and muscle close to the plastron so that the plastron may be removed. With scissors open the pericardium. If a frog heart is selected for use, the animal should be pithed.

Excise the frog or turtle heart by cutting the large veins and arteries near it, leaving the heart uninjured and still attached to the stumps of the blood vessels. Pin the latter to the cork disk on the heart holder and moisten with Ringer's solution. Tie a thread to an S-shaped pin and pass this hook through the apex of the ventricle. Attach the other end of the thread to the heart lever and adjust the lever so it may record smoothly on the smoked paper. Place Ringer's solution, at room temperature, in the 600-cc. beaker and put the beaker in position so that the heart is immersed in the solution. Put the wooden stand under the beaker. Bubble oxygen or air through each solution used.

Record the contractions of the heart with the drum revolving at medium speed and with the time marker in operation. What is the ionic composition of Ringer's solution? This is a balanced isotonic salt solution.

Remove the Ringer's solution from the beaker and in its place put 0.7 per cent NaCl solution. Use this first sample of NaCl solution to rinse and then replace with fresh NaCl solution. Record the contractions at intervals until the heart shows a less vigorous beat.

Replace the NaCl solution with a 0.9 per cent KCl solution after one rinsing with the latter solution. Record the beats at intervals until the heart almost comes to a complete standstill (potassium inhibition). Is the heart in a relaxed or in a contracted condition?

Replace the KCl solution with a 1.0 per cent $CaCl_2$ solution after one rinsing and take tracings at intervals until the heart shows improvement. If the heart does not recover from the effects of the potassium take a fresh heart. What effect does calcium have on the heart beat?

Restore the heart to Ringer's solution and record at intervals. Does the heart assume a normal or nearly normal beat? Label and shellac the records. What is the practical use of this solution? What are the main features of Ringer's solution that make it useful?

CHAPTER VIII

THE PHYSIOLOGICAL SIGNIFICANCE OF HYDROGEN AND HYDROXYL IONS

142. Comparison between Titratable Acidity and Actual Acidity.—Before doing this experiment read the discussion on pages 323 to 325, inclusive, in the text. The titration acidity is a measure of the alkali-combining power of the solution whereas the actual acidity is the hydrogen-ion activity of the solution. The hydrogen-ion activity is usually expressed by means of the pH scale.

a. A 0.1 *N* acetic acid solution and a 0.0014 *N* HCl solution will be furnished. Both solutions have practically the same pH. Using a volumetric pipette, place exactly 10 cc. of the 0.1 *N* acetic acid solution into a clean beaker. Employing phenolphthalein as an indicator, titrate this against 0.1 *N* NaOH solution delivered from a burette. Repeat to check and record the readings.

Similarly titrate 10 cc. of the 0.0014 *N* HCl solution against the 0.1 *N* NaOH solution. Repeat and record the readings. Note the differences between the acids.

b. Add 5 cc. of 1.0 *N* NaOH solution to 10 cc. of 1.0 *N* acetic acid solution. Take 2 cc. of this mixture and add them to 18 cc. of distilled water. This gives a solution that is ten times more dilute than the first solution. However, the pH is practically the same for both solutions. Titrate 10 cc. of the first solution against 0.1 *N* NaOH, using phenolphthalein as indicator and record the quantity of alkali used. Repeat using the more dilute solution. What do you conclude from this series of experiments?

143. The Colorimetric Method of Measuring pH.—Before doing this work read the discussion in the text on pages 329 to 332, inclusive.

Examine the various sets of permanent color standards contained in the sealed tubes as supplied by LaMotte Chemical Products Co. Arrange these sets on the table in order of increasing pH to cover as much of the pH scale as possible. Make a record of the useful pH range of each set of color standards and of the color at each end of the range of each series. What pH change must occur in the solutions to give a detectable change in color?

Proceed as follows to make a colorimetric measurement of the pH of a solution: Obtain three test tubes of clear glass and of the same

diameter as the standards. It is convenient to use tubes with 10-cc. marks. Fill one tube to the 10-cc. mark with tap water that is at room temperature and add 0.5 cc. of bromthymol blue indicator solution (0.04 per cent) and mix well. The use of the thumb on top of the test tube in mixing is to be avoided. Chemical purity and cleanliness are very important in this type of work. The bromthymol blue has a range of pH 6.0 to 7.6, and hence it covers the neutral point of pH 7.0. The test with this indicator will tell therefore whether the pH of the unknown falls within the range of this indicator or on the acid or alkaline side of it. This aids in selecting the proper indicator. Continue the tests until the proper indicator is found. *The final color of the solution must be inter-*

FIG. 17.—The block comparator. (*LaMotte Chemical Products Co.*)

mediate between the colors of the standards at opposite ends of the range of the indicator employed. Never match the color of the unknown against that of the last standard at one end of the range of an indicator. Employ a block comparator in making the color comparisons.

Use the block comparator in the following manner: Note that at the middle of the block comparator there are two rows of holes, each row consisting of three holes. These are shown in Fig. 17. For clear solutions use the front row of holes, placing the unknown with indicator added in the middle hole and a color standard on each side. For turbid solutions use the same arrangement, but in addition use the back row of holes, placing a tube of distilled water in the middle hole directly behind the unknown solution to which the indicator has been added. On each side of the distilled water place a test tube containing the unknown solution without indicator. The two color standards should differ from each other by only 0.2 pH, *e.g.*, pH 7.4 and pH 7.6 may be used. Hold the block comparator so that the frosted glass at the back is towards the source of light and on the side of the block that is away from the eye. While holding the comparator, arranged as indicated above, to a good source of light, examine the tubes and, if necessary,

change color standards, so that the color of the unknown solution is matched by a color standard or is intermediate between the two standards. After the match is made, the pH of the unknown is obtained by reading the pH marked on the standard with which the color match is obtained. If the color of the unknown is intermediate between the colors of the standards on opposite sides, then the pH is considered to be the average of the two standards. By way of illustration, if the color of the unknown lies between the colors of the standards having pH values of 7.4 and 7.6 the pH of the sample is considered to be 7.5.

Find the pH of tap water and of distilled water. Five buffered solutions of different pH values will be furnished. Using the method that has been outlined, find the pH of each solution. These solutions have been standardized in advance and you should ask the instructor for the correct pH values after you have made the tests. Note that it is not necessary to use bromthymol blue indicator in the preliminary test if a range-finding indicator is available. It is convenient to use the LaMotte Range-finding Indicator or the Hellige Wide-range Indicator in order to get the approximate pH before selecting the indicator for the final measurement. The manner in which the color changes with pH is indicated below for each of these indicators:

LaMotte Range-finding Indicator[1]

pH	Color
3.0	Red
4.0	Orange-red
5.0	Orange
6.0	Yellow
7.0	Yellow-green
8.0	Green
9.0	Green-blue
10.0	Violet
11.0	Red-violet

[1] Made by LaMotte Chemical Products Co., 418 Light St., Baltimore, Md.

Hellige Wide-range Indicator[1]

pH	Color
1.0	Deep red
2.0	Red
3.0	Salmon
4.0	Orange
5.0	Yellow
6.0	Lemon
7.0	Moss green
8.0	Green
9.0	Greenish blue
10.0	Blue
11.0	Violet

[1] Distributed by Empire Laboratory Supply Co., Inc., 507–559 West 132nd St., New York.

144. The Use of the H-ion Bicolorimeter.—In preparation for this work read in the text pages 334 and 335.

By means of the H-ion bicolorimeter, pH measurements may be made with a rather high degree of accuracy, when the proper values for the dissociation constants of the indicators are known. A detailed description and special instructions[1] for use will be furnished at the time the instrument is used. The use of the instrument will be demonstrated, and measurements made on various solutions, under supervision.

145. The Use of the Quinhydrone Electrode (Youden).—By means of the quinhydrone electrode, measure the pH values of the five buffer

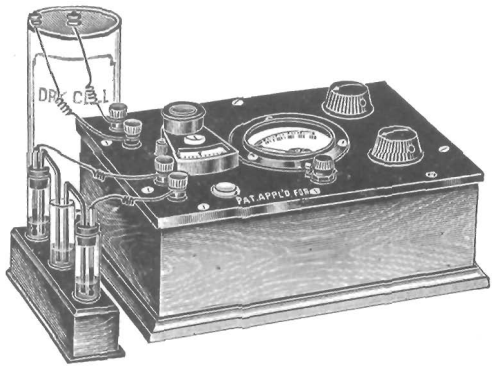


FIG. 18.—Youden quinhydrone-electrode apparatus. (*W. M. Welch Manufacturing Co.*)

solutions already tested colorimetrically in Exps. 143 and 144. Compare the results obtained with the two methods. Use in this work the Youden[2] pH outfit or other quinhydrone-electrode assembly that is available. The directions for the use of the Youden pH outfit given below are mainly selected passages from instructions furnished by and printed in part with the permission of the W. M. Welch Manufacturing Co.

Examine the apparatus and connect it as shown in Fig. 18. A 1.5-v. dry battery is used as a source of current and should be attached to the binding posts marked "batt." Care should be taken to connect the positive pole of the battery to the binding post marked +. The salt bridges (or U-tubes that connect the electrode vessels with a common cup) are filled with a mixture made by heating together on a water bath 50 cc. of saturated potassium chloride, 50 cc. of water and about 3 grams of agar. The U-tubes may be conveniently filled by attaching a piece of rubber tubing and, after warming the glass, sucking up the hot mixture into the bridge. The mixture is held in place by pinching the rubber

[1] A special booklet entitled, "Use and Care of the Hydrogen-ion Colorimeter," may be obtained from the Bausch and Lomb Optical Co., Rochester, N. Y.

[2] Manufactured by W. M. Welch Manufacturing Co., 1515 Sedgwick St., Chicago, Ill.

tubing and holding the U-tube in a stream of cold water until the material solidifies. The agar jelly should extend to the tips of the bridges. The bridges will keep indefinitely if they are placed with their ends down in a saturated solution of potassium chloride. A convenient receptacle is a Mason or other form of screw-top jar wide enough for the bridges. The performance of old bridges may be greatly improved by breaking off about a centimeter of each arm. (Bridges will be furnished to you ready to use.)

No treatment of the electrodes is necessary, except that the platinum anodes should be kept clean, being washed with a strong acid occasionally, to dissolve the brown film of oxidized quinhydrone, and then thoroughly rinsed in distilled water. Do not lay them down when not in position but keep them in a beaker of distilled water.

A standard 0.05 *M* solution of potassium acid phthalate is required. This solution contains 10.21 grams per liter, and the salt used for the preparation must be of the best quality. The solution at 25°C. has a pH of 3.98. If at all possible, the salt should be taken from a supply that is known to give this pH under the conditions stated. The 0.05 *M* solution does not keep so well as a stronger solution. A 0.2 *M* solution keeps satisfactorily, and it is recommended that this solution be prepared, and the 0.05 *M* solution made up by dilution from it at intervals of about 2 weeks. This solution is excellently buffered, and consequently the dilution may be effected by pipetting out 25 cc. of the 0.2 *M* solution and diluting to 100 cc. in a 100-cc. volumetric flask. After dilution the solution should be thoroughly mixed.

The electrode assembly as shown in the cut is essentially correct, one outside vial containing the standard 0.05 *M* solution of potassium acid phthalate and the other the solution whose pH is being measured. The manufacturers, however, advise eliminating the stoppers in the outside vials and putting a stopper in the center vial containing a saturated solution of potassium chloride, this stopper to have a little V-groove cut in the side so that when it is inserted in the vial the air pressure will be relieved, so as not to force the agar out of the bridges. This center vial with the two bridges becomes a unit in itself and the manufacturers advise the plan of having an extra block or other form of container handy with two vials in the outside holes containing potassium chloride solution. It is also very convenient to have the jar in which the extra bridges are kept big enough to hold this center vial and bridges (bridges pulled around together). In this connection, care should be taken, whenever they are put back into new solutions, to wipe off the excess potassium chloride of the two free bridge ends with a bit of absorbent cotton so as to avoid contaminating the solutions with excess potassium chloride. The spring clips on the electrodes are designed to slip over

the edge of the vial so as to support the platinum electrodes about ⅛ to 1/16 in. above the bottom of the vial. When changing solutions, the bridge unit can be lifted out and a bit of cotton rubbed over the bridge end that has been in the unknown liquid so that, when the new sample is put in and the bridge inserted, contamination is avoided. The vial that contains the test sample should be rinsed out with distilled water before a new sample is introduced. The standard solution of potassium acid phthalate should not be used for more than about 2 hr. without being changed for a fresh solution. Powdered quinhydrone must be added to the standard solution of potassium acid phthalate and to the test sample in the vials. Excessive amounts of quinhydrone need not be used. After adding quinhydrone, shake each vial for about a minute and make sure that a few particles remain undissolved in the solutions. This ensures complete saturation.

After a 1.5-volt dry cell is connected to the potentiometer box, closure of the switch should cause the millivoltmeter to show a reading that may be varied at will by operating the rheostats. Adjust the rheostats so that a reading under 50 mv. is obtained. Release the catch that locks the galvanometer movement. Connect the two terminals marked "elect" by a copper wire and momentarily depress the galvanometer button. A sharp deflection of the galvanometer needle should be observed. If the needle does not move, go over all the wires and make sure that the ends are bright and that good contacts are present at all points. If this fails to change matters, the outfit is in need of repairs. Do not leave the switch on the potentiometer box in the "on" position when the apparatus is not in use. If a dry cell will not give the full deflection of 300 mv. by throwing out the 50-ohm rheostat a new battery should be used.

Method of Making Determinations: Rinse out one of the electrode vessels (it is a rather good plan to keep the same electrode at least for the phthalate solution, labeling it in some way) and pour in about 2 cc. of the 0.05 *M* potassium acid phthalate solution. Add a small pinch of quinhydrone powder, carefully shake the vial for about a minute, insert the electrode and, removing the copper wire from the binding posts, connect the electrode terminal tentatively to the positive binding post. Insert the bridge. This electrode is known as the reference electrode. Similarly, place some of the unknown solution in the other vessel and repeat these operations. Connect the electrode for this vessel to the negative binding post. Close the switch and depress the push button for an instant. If the galvanometer needle fails to move, make sure that the bridges reach the solution and that there are no air pockets. Alter the reading of the millivoltmeter by turning one of the rheostats and observe whether or not the galvanometer needle moves more or less

violently. Acting on this indication, adjust the rheostats until there is no movement of the galvanometer needle when the button is depressed. The reading of the millivoltmeter is then recorded and the pH obtained from Table A. If it is found impossible to obtain a point of balance, or, in other words, any setting of the galvanometer rheostats that will give no deflection of the needle, reverse the wires leading to the electrodes. It will then be found possible to obtain a point of balance, and the millivoltmeter reading is recorded, and the pH read from Table B.

Cautions: The quinhydrone electrode does not function properly in solutions more alkaline than about pH 8.5. Make sure that the bridges are in good condition and that the solutions are saturated with quinhydrone. Place the switch on the potentiometer box in the "off" position when finished making measurements and disconnect the dry cell.

TABLES OF VALUES FOR pH USING THE QUINHYDRONE ELECTRODE AND 0.05 *M* POTASSIUM ACID PHTHALATE AS REFERENCE

TABLE A

To be used when the phthalate half-cell is connected to positive electrode terminal

E (mv.)	0	1	2	3	4	5	6	7	8	9
0	3.98	3.99	4.01	4.03	4.05	4.06	4.08	4.10	4.11	4.13
10	4.15	4.16	4.18	4.20	4.21	4.23	4.25	4.27	4.28	4.30
20	4.32	4.33	4.35	4.37	4.38	4.40	4.42	4.43	4.45	4.47
30	4.49	4.50	4.52	4.54	4.55	4.57	4.59	4.60	4.62	4.64
40	4.65	4.67	4.69	4.71	4.72	4.74	4.76	4.77	4.79	4.81
50	4.82	4.84	4.86	4.87	4.89	4.91	4.93	4.94	4.96	4.98
60	4.99	5.01	5.03	5.04	5.06	5.08	5.09	5.11	5.13	5.15
70	5.16	5.18	5.20	5.21	5.23	5.25	5.26	5.28	5.30	5.31
80	5.33	5.35	5.37	5.38	5.40	5.42	5.43	5.45	5.47	5.48
90	5.50	5.52	5.53	5.55	5.57	5.59	5.60	5.62	5.64	5.65
100	5.67	5.69	5.70	5.72	5.74	5.75	5.77	5.79	5.81	5.82
110	5.84	5.86	5.87	5.89	5.91	5.92	5.94	5.96	5.97	5.99
120	6.01	6.03	6.04	6.06	6.08	6.09	6.11	6.13	6.14	6.16
130	6.18	6.19	6.21	6.23	6.24	6.26	6.28	6.30	6.31	6.33
140	6.35	6.36	6.38	6.40	6.41	6.43	6.45	6.46	6.48	6.50
150	6.52	6.53	6.55	6.57	6.58	6.60	6.62	6.63	6.65	6.67
160	6.68	6.70	6.72	6.74	6.75	6.77	6.79	6.80	6.82	6.84
170	6.85	6.87	6.89	6.90	6.92	6.94	6.96	6.97	6.99	7.01
180	7.02	7.04	7.06	7.07	7.09	7.11	7.12	7.14	7.16	7.18
190	7.19	7.21	7.23	7.24	7.26	7.28	7.29	7.31	7.33	7.34
200	7.36	7.38	7.40	7.41	7.43	7.45	7.46	7.48	7.50	7.51
210	7.53	7.55	7.56	7.58	7.60	7.62	7.63	7.65	7.67	7.68
220	7.70	7.72	7.73	7.75	7.77	7.78	7.80	7.82	7.84	7.85
230	7.87	7.89	7.90	7.92	7.94	7.95	7.97	7.99	8.00	8.02
240	8.04	8.06	8.07	8.09	8.11	8.12	8.14	8.16	8.17	8.19
250	8.21	8.22	8.24	8.26	8.28	8.29	8.31	8.33	8.34	8.36

TABLE B

To be used when phthalate half-cell is connected to the negative electrode terminal

E (mv.)	0	1	2	3	4	5	6	7	8	9
0	3.98	3.96	3.94	3.93	3.91	3.89	3.88	3.86	3.84	3.83
10	3.81	3.79	3.77	3.76	3.74	3.72	3.71	3.69	3.67	3.66
20	3.64	3.62	3.61	3.59	3.57	3.56	3.54	3.52	3.50	3.49
30	3.47	3.45	3.44	3.42	3.40	3.39	3.37	3.35	3.34	3.32
40	3.30	3.28	3.27	3.25	3.23	3.22	3.20	3.18	3.17	3.15
50	3.13	3.12	3.10	3.08	3.06	3.05	3.03	3.01	3.00	2.98
60	2.96	2.95	2.93	2.91	2.90	2.88	2.86	2.84	2.83	2.81
70	2.79	2.78	2.76	2.74	2.73	2.71	2.69	2.68	2.66	2.64
80	2.62	2.61	2.59	2.57	2.56	2.54	2.52	2.51	2.49	2.47
90	2.46	2.44	2.42	2.40	2.39	2.37	2.35	2.34	2.32	2.30
100	2.29	2.27	2.25	2.24	2.22	2.20	2.18	2.17	2.15	2.13
110	2.12	2.10	2.08	2.07	2.05	2.03	2.02	2.00	1.98	1.96
120	1.95	1.93	1.91	1.90	1.88	1.86	1.85	1.83	1.81	1.80
130	1.78	1.76	1.74	1.73	1.71	1.69	1.68	1.66	1.64	1 63
140	1.61	1.59	1.58	1.56	1.54	1.53	1.51	1.49	1.47	1.46
150	1.44	1.42	1.41	1.39	1.37	1.36	1.34	1.32	1.31	1.29

146. The Use of the Hydrogen Electrode.—In preparation for this work read the discussion in the text on pages 336 to 345, inclusive.

The hydrogen-electrode assembly for the measurement of hydrogen-ion activity will be used or demonstrated. Students who are qualified may make measurements under supervision. Make a diagram of the essential parts of the apparatus, showing the electrical connections of the circuits. Label fully.

Why does the electrode have colloidal platinum upon every part of the conducting surface exposed to the liquid under examination? Why is only pure hydrogen gas permissible for use? Why is a saturated solution of KCl chosen for making contact with the unknown solution? The voltage read on the potentiometer represents a difference of potential between what two points? Why is it necessary to control temperature when accurate measurements are being made?

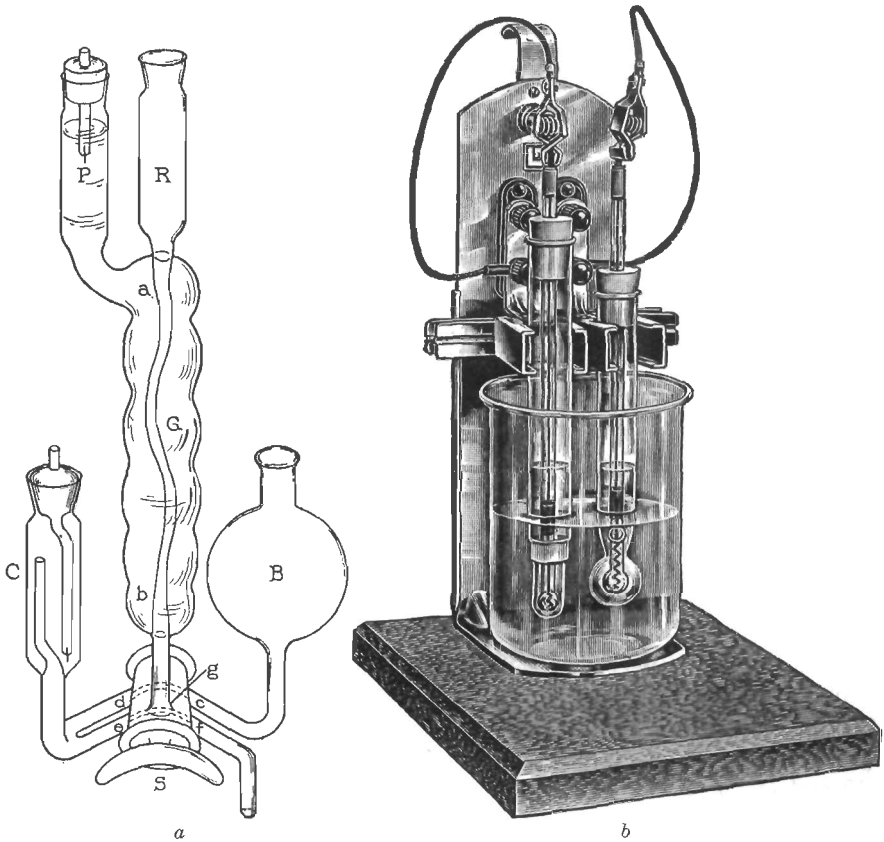
Measure the pH values of four or five buffer solutions that will be furnished or that you may prepare for this purpose. Convert the voltage readings that are obtained into pH units, using the equations given in the text. Check these results by making the conversion with the Leeds and Northrup hydrogen-ion calculator.

147. The Use of the Glass Electrode.—In preparation for this work read the discussion in the text on pages 345 and 346.

The glass-electrode assembly will be demonstrated by the instructor or used by students with sufficient experience. The principles that are

involved in the use of the various pieces of apparatus will be explained by the instructor. Various types of glass electrodes and vessels for them have been designed by several investigators. Two convenient types of glass electrodes are illustrated in Fig. 19.

In measuring the potentials across the thin glass membrane one may employ a thermionic vacuum-tube potentiometer or electrometer



a *b*

FIG. 19.—Two types of glass electrodes. (*a*) Glass electrode designed by MacInnes and Belcher.[1] (*b*) Glass electrode. (*Leeds and Northrup Company.*)

in connection with an ordinary potentiometer with accessory equipment. In doing highly accurate work it is essential to control the temperature of the glass electrode and the reference electrode to within a fraction of a degree. Make a complete wiring diagram of the particular glass-electrode assembly that is available.

[1] MACINNES, D. A. and LONGSWORTH, L. G., *Trans. Electrochem. Soc.*, Vol. 71, p. 73, 1937.

Using successively three standardized buffer solutions of known pH in the glass electrode, measure the e.m.f's. and calculate the value for the cell constant[1] by means of the following equation:

$$e = (0.000198322T \times \text{pH}) - E \tag{1}$$

where e = cell constant.
T = absolute temperature.
E = measured e.m.f.

Employ the value of e obtained to convert the e.m.f. readings (found for four or five buffer solutions that will be furnished) into pH values.

$$\text{pH} = \frac{E + e}{0.000198322T} \tag{2}$$

where the symbols have the same meanings as in Eq. (1).

THE BUFFER EFFECT

148. Buffer Action of a Salt of a Strong Base in Solution with a Weak Acid.—In preparation for this work read pages 332 to 334, inclusive, in the text.

To 10 cc. of distilled water in a clean beaker add 10 cc. of 0.1 *N* NaOH solution measured with a burette or volumetric pipette. Using phenolphthalein as indicator (4 drops) titrate with 0.1 *N* HCl solution until the solution just turns from red to colorless. The solution now has a pH value close to 8.3, and it is mainly a NaCl solution. Place 5 drops of methyl-orange indicator solution into the solution that has just been titrated and again add 0.1 *N* HCl solution drop by drop until the point is reached where the color changes to an orange-red. The solution now has a pH value close to 3.1. From the burette readings determine how many cubic centimeters of 0.1 *N* HCl solution were used in changing the pH from 8.3 to 3.1.

Now, using phenolphthalein as the indicator, titrate 10 cc. of 0.1 *N* NaOH solution plus 10 cc. of distilled water against 0.1 *N* acetic acid solution. When the acetic acid is added to the sodium hydroxide solution, sodium acetate is formed. As before, add methyl orange as indicator and titrate using 0.1 *N* HCl solution. In this case how many cubic centimeters of 0.1 *N* HCl solution were required? Compare with the corresponding titration above. Explain.

149. The Buffer Action of Sodium Bicarbonate.—To 20 cc. of 0.25 per cent $NaHCO_3$ solution in a clean beaker, add 5 drops of methyl-orange indicator solution. From a burette add 0.1 *N* HCl solution until the color change is obtained. It will be observed that CO_2 is evolved.

[1] This is conveniently obtained by the method described by W. C. STADIE, H. O'BRIEN, and E. P. LAUG, *Jour. Biol. Chem.*, Vol. 91, p. 243, 1931.

Now repeat the titration using 20 cc. of distilled water instead of the sodium bicarbonate solution. Compare the amounts of acid used in both cases.

Place 20 cc. of 0.25 per cent NaCl solution and 20 cc. of 0.25 per cent $NaHCO_3$ solution separately in two 50-cc. flasks. To each solution add 10 drops of phenol-red indicator solution. Place the end of a pipette into the NaCl solution and blow expired air through it into the solution. The solution changes in color readily. Now repeat using the $NaHCO_3$ solution. Does the color change? To aerate, shake the flask containing the NaCl solution. Does the solution become red again due to a pH change? Explain.

Sodium bicarbonate acts as an important buffer in the blood against the acids produced during metabolism. Blood pH is kept at a constant level by the buffers of the blood, the pH variations being quite small.

150. The Buffer Action of Acetic Acid Plus Its Salt, Sodium Acetate.—When a solution contains a weak acid and one of its salts, the pH is mainly dependent upon the relative proportions of these two substances with respect to each other, and it depends only slightly upon the actual concentrations of these substances. Accordingly, if one dilutes such a solution with distilled water, the pH changes very slightly. The explanation is that the weak acid tends to dissociate to a slight degree, and the dissociation is further depressed by the presence of its salt, which furnishes an ion common to both acid and salt.

Obtain a 0.1 *N* CH_3COONa solution that has been made by dissolving 1.36 grams of crystallized sodium acetate per 100 cc. of distilled water. Also obtain a 0.1 *N* CH_3COOH solution that has been standardized against 0.1 *N* NaOH solution with phenolphthalein as indicator. Arrange eight clean test tubes on a rack and mix the two solutions to make four combinations in the following manner, placing each mixture in a separate test tube:

Solution	Mixture No.			
	1	2	3	4
No. of cc. 0.1 *N* CH_3COONa	5	7	8.5	9
No. of cc. 0.1 *N* CH_3COOH	5	3	1.5	1

Prepare a second series of mixtures by adding 8 cc. of distilled water to each of four other test tubes and by transferring 1.0 cc. from each tube of the first series to a corresponding tube of the second series. To each test tube of both series add 2 drops of methyl-red solution.[1] Com-

[1] Methyl-red solution is prepared by dissolving 0.1 gram of the powder in 50 per cent alcohol (500 cc.).

pare the colors of the solutions in the two series. Note that the solutions of the first series correspond in color and pH to those of the second series, although the first series is ten times more concentrated than the second. The pH depends rather closely upon the relative amounts of free acetic acid and sodium acetate present in the solution. The hydrogen-ion concentration of the mixture is given by the equation

$$[H^+] = K_a \frac{[\text{acid}]}{[\text{salt}]} \tag{1}$$

where K_a = dissociation constant of acid.

[ ] = molar concentration.

Examples of dissociation-constant values at 25°C. are as follows:

Acetic acid, 1.86×10^{-5}.

Boric acid, 6.4×10^{-10}.

Uric acid, 1.5×10^{-6}.

Equation (1) may be written,

$$\text{pH} = \text{p}K_a + \log \frac{[\text{salt}]}{[\text{acid}]} \tag{2}$$

These equations represent only fairly close approximations, since the hydrogen-ion concentration is actually a little higher than indicated by them.

151. Buffering Action of a Solution of Acetic Acid and Sodium Acetate.—Add 1.0 cc. of 1.0 *N* sodium acetate solution to 10 cc. of 1.0 *N* acetic acid solution in a test tube and shake to mix. The pH of this solution is about 3.7. Place 2 drops of methyl-orange indicator solution[1] in the mixture and note the color. Put 10 cc. of 0.9 per cent NaCl solution in another test tube and add 2 drops of methyl-orange solution. Then, drop by drop, add 0.01 *N* HCl solution until the NaCl solution assumes the same color as that of the mixture of acetic acid and sodium acetate. Introduce 1.0 cc. of a 1.0 per cent gelatin solution into each test tube and mix. Note that the solution containing NaCl now becomes yellow, whereas no color change occurs in the other solution. The gelatin tends to combine with hydrogen ions and is able to change the pH of a poorly buffered solution. Although both solutions had the same initial hydrogen-ion concentration, they differed as regards buffering capacity.

152. Buffering Effect of a Mixture of Primary and Secondary Phosphates.—Obtain *M*/15 KH_2PO_4 (primary phosphate) solution and a *M*/15 Na_2HPO_4 (secondary phosphate) solution and measure the pH of each, using the colorimetric method. Add 20.0 cc. of the primary

[1] Methyl-orange solution is prepared by dissolving 0.1 gram of methyl orange in 100 cc. of distilled water.

solution to 30.0 cc. of the secondary phosphate solution in a clean flask and mix. With a volumetric pipette, place 10 cc. of the mixture in each of two 400-cc. beakers and measure the pH of the remaining portion of the mixture.

To the 10-cc. sample of the mixture in one beaker add a suitable quantity of the indicator used in obtaining the pH of the primary phosphate solution. Using $M/15$ HCl solution delivered from a burette, titrate the mixture until the same pH is obtained as found for the primary phosphate solution. To the other 10-cc. sample of the mixture add a suitable amount of the indicator employed in obtaining the pH of the secondary phosphate solution. Using $M/15$ NaOH solution, titrate the mixture until the same pH is reached as found for the secondary phosphate solution. How much acid and base were used in the titrations? Explain.

THE ISOELECTRIC POINT

153. The Isoelectric Point of Gelatin.—Make up buffer solutions by mixing potassium acid phthalate and NaOH solutions to give pH values of 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2 and 5.4. The directions for preparing these solutions are given in the Appendix (page 141) of this manual.

Place 10 cc. of each buffer solution in a separate clean test tube and add to each tube 1.0 gram of powdered gelatin and 1.0 cc. of $M/128$ potassium ferrocyanide solution. Allow the gelatin to remain in contact with the solutions for an hour and a half, decant off the supernatant solution and wash several times with very cold water to remove any free salt. Dissolve the gelatin by adding warm distilled water to each tube and keep the tubes in a water bath at 40°C. until all the gelatin has dissolved. Make the volume of each solution up to 40 cc. and remove in each case enough solution to fill a clean test tube. Stopper each test tube and seal with paraffin. Allow the tubes to stand for 3 days. In the solutions in which the pH is less than 4.7 the color is blue because the gelatin forms the cation of a salt in which $Fe(CN)_6$ is the anion. A blue ferric salt forms on standing. The solutions having pH values greater than 4.7 remain colorless. We may infer that the isoelectric point is close to pH 4.7 (see the text, pages 352 and 353).

CHAPTER IX

SURFACE ACTION

154. Surface-tension Phenomena.—The discussion on pages 359 to 365, inclusive, in the text should be read before these experiments are started.

Fill a large beaker with water and place a thin film of oil on the surface of a needle by rubbing it with the fingers. Hold the needle in a horizontal position and gently drop it onto the surface of the water in such a manner that the needle will remain on the surface. What supports the needle? Wash the needle in a Na_2CO_3 solution and repeat the test handling the needle with clean forceps. Does the needle now remain on the surface? Explain.

A soap solution[1] and a circular wire frame[2] with a loop of silk thread attached will be furnished. Dip the wire circle into the soap solution in a Petri dish and remove it so as to form a film that holds the loop of thread. This is really a double film. Touch the inside of the loop with the point of a hot dissecting needle. Explain the result.

Form a soap film on the circular wire frame. The film will be flat if the wire frame is flat. Pull the circular frame out to form a long ellipse and curve it at right angles to the radii of the ellipse. Examine a soap film placed on this curved loop. What is its form? May the shape of the film be explained according to the principle of least or minimal surfaces?

Using a clay pipe, blow a soap bubble and watch it decrease in size on standing. Why does this occur? Connect two clay pipes together by means of a Y-tube and blow two bubbles of unequal sizes allowing them to remain on the pipes. Close the end of the Y-tube to prevent loss of air. Which bubble increases in size while the other decreases? The curved soap bubble presses inward and the degree of pressure is inversely proportional to the radius of the bubble. Why is a soap bubble spherical?

155. Amoeboidlike Movements Due to Surface-tension Changes.—Put a globule of clean mercury about half an inch in diameter in a watch glass of medium size. Add some 2 per cent HNO_3 solution to

[1] A suitable soap solution may be made by dissolving 1 part (by weight) of shavings of pure castile or palm-olive soap in 8 parts of distilled water and then adding 4 parts of glycerin. The solution should then be well mixed and allowed to stand. Siphon off and keep for use the clear portion at the bottom of the solution.

[2] The wire frame should be shaped as indicated in Fig. 105 in the text.

cover the mercury. Does any change occur? Now drop a crystal of potassium bichromate into the solution near the mercury. What happens? Describe the movements and explain why they occur.

Mercury must not be thrown in the sink, but, after use, it is to be put in the vessel that is provided for this purpose.

156. Ostwald's Physical "Heart."—Place a globule of clean mercury about 1 in. in diameter in a medium-sized watch glass and cover the mercury with 15 per cent H_2SO_4 solution. Add a small amount of 0.1 *N* potassium bichromate solution until the mixture has a pale-yellow color. Push the blunt end of a clean needle into a cork and mount the cork in a clamp on a stand so that the point of the needle just touches the margin of the mercury globule. The needle should be set at an angle of about 45 deg. with respect to the surface of the table. Adjust the position of the needle until the globule of mercury pulsates rhythmically. Note what happens to the diameter of the globule when the mercury makes contact with the needle and when the contact is broken.

The needle becomes negatively charged, and the mercury assumes a positive electric charge. The negative charge of the needle is transferred to the mercury when contact is made, and this causes an increase of surface tension, which makes the globule become more spherical. The contact is broken as the diameter decreases, and the mercury becomes positively charged again with an accompanying decrease in surface tension and an increase in diameter that permits contact with the needle to be reestablished. The cycle is then repeated. This accounts for the rhythmical pulsations.

157. Electrical Alteration of Surface Tension.—Place a globule of clean mercury about ½ in. in diameter in a watch glass and cover the mercury with 10 per cent HNO_3 solution. Connect three dry cells in series and attach wires to the free positive terminal and free negative terminal. Dip the ends of the wires into the solution on opposite sides of the mercury globule. How does the mercury move relative to these wire electrodes? Place the wires a few millimeters apart near the margin of the globule. Also place them in various positions in the solution and notice to which pole the particles on the surface of the mercury move.

Touch the mercury for a second or two with the wire connected to the negative terminal while the other wire is in the solution. Then place the tip of the wire attached to the negative pole close to the globule, which will pulsate rhythmically as long as the wire is held in position. Explain.

158. Measurement of Surface Tension with a Stalagmometer.—Before making these measurements read in the text the discussion on pages 361 to 363, inclusive.

Pass the upper end of a stalagmometer through the hole of a rubber stopper and attach a piece of rubber tubing to this end of the stalagmometer. Mount the stalagmometer in a vertical position on a stand placing the clamp around the rubber stopper. Attach a Hofmann clamp or a two-way stopcock to the free end of the rubber tubing. The apparatus should be used in a room in which the temperature will remain rather constant. Air drafts are to be avoided. The stalagmometer and all glassware to be used must be chemically clean. A trace of grease on the dropping surface, a rapid flow of liquid from the stalagmometer and shaking of the instrument will cause errors. All solutions used should be at room temperature.

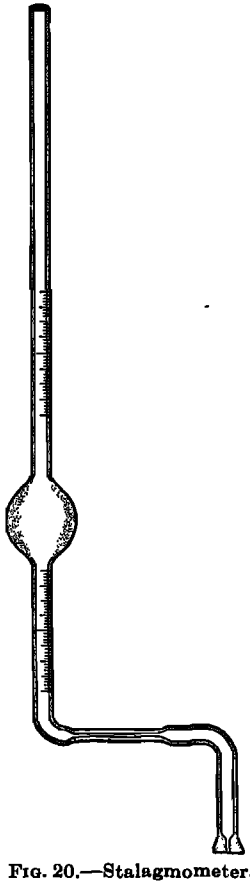


FIG. 20.—Stalagmometer.

The stalagmometer must be calibrated before it is used unless the calibration is already known. This is accomplished in the following manner: Note the two graduated scales on the instrument. Suck distilled water into the stalagmometer until the water is just above the upper scale. Allow the water to flow out and when the meniscus is on the top part of the upper scale note the exact position of the meniscus when a drop leaves the dropping surface. Note the exact position of the meniscus when the next drop falls. The difference between the two readings, *i.e.*, number of divisions, gives the range on the scale covered by 1 drop. Make the measurements three times and average them to obtain the number of finest divisions on the scale covered by 1 drop. What fraction of a drop is represented by one division? If 20 small divisions are covered by 1 drop, then each division represents $\frac{1}{20}$ drop.

Similarly, find for the lower scale what fraction of a drop one small division represents. Check the temperature of the water at intervals.

In calibrating the stalagmometer and in making measurements, the rate of outflow should be so regulated by the Hofmann clamp or stopcock on the rubber tubing that 15 to 18 drops per minute will fall. The rate should not be greater than 20 per minute.

Note that there is a main mark on the upper and lower scales. Each main mark runs completely around the tube. Next find the number of drops of water that fall when the meniscus passes from the main mark on the upper scale down to the main mark on the lower scale.

The count is made to a fraction of a drop, employing the values previously obtained when the fraction of a drop represented by one scale division was found for both scales. The drop count is begun when the meniscus is near the main mark on the upper scale, being either above or below it, and at the instant that a drop falls. Do not count this drop but note the position of the meniscus at the moment it leaves the dropping surface. The count of the number of drops begins with the next drop that falls. Continue the count until the meniscus is close to the main mark on the lower scale and note the exact position of the meniscus on the scale when a drop falls. This drop, the last one to be noted, is included in the count. Since the object is to determine the number of drops, to a fraction of a drop, between the two main marks correct the drop count by subtracting fractions of a drop that occur above the upper main mark or add fractions of a drop that occur below the same main mark as the case might be. However, fractions of a drop above the lower main mark must be added and fractions below it must be subtracted from the number of drops counted. As an example the following case may be considered: Suppose that each small scale division has been found to be equal to $\frac{1}{20}$ drop. Also suppose that 19 drops were counted between the second scale division above the upper main mark and the fifth scale division above the lower main mark. Then the true drop count between the two main marks is $19 - \frac{2}{20} + \frac{5}{20} = 19\frac{3}{20}$ drops.

Calibrate the stalagmometer, using the method described above and repeating the measurements to check. Obtain the average value.

To measure the surface tension of a liquid proceed in the following manner. Suck the liquid up into the stalagmometer and let it flow out to rinse the instrument. Repeat. Making sure that the dropping surface and other parts are chemically clean, determine the drop count for the liquid in the same manner as for water. This should be repeated until the readings check closely.

The relative surface tension of the liquid may be obtained by means of the formula

$$T = \frac{N_w \cdot D}{N}$$

where T = relative surface tension.
N_w = drop count for water.
N = drop count for the liquid.
D = specific gravity of the liquid.

To obtain the surface tension of the liquid in dynes per centimeter, multiply the relative surface tension of the liquid by the surface tension of water at the temperature employed.

The following are the values of the surface tension of water at the temperatures indicated:

Temp., °C.	Surface Tension, Dynes per Centimeter (Water)
10	74.22
15	73.49
20	72.75
25	71.97
30	71.18
35	70.37
40	69.56
45	68.73
50	67.91

Determine the relative surface tensions and also the surface tensions in dynes per centimeter of the following aqueous solutions of ethyl alcohol:

5 per cent ethyl alcohol, specific gravity 0.9928.
10 per cent ethyl alcohol, specific gravity 0.9866.
15 per cent ethyl alcohol, specific gravity 0.9811.
(Percentage is by volume)

The temperature at which the measurements are made should be noted and taken into consideration in making the calculations.

159. Adsorption.—Before doing the following experiments dealing with adsorption read pages 367 to 370, inclusive, in the text.

Into a clean test tube, put about 10 cc. of a solution of thymol blue in 0.1 *N* HCl. Cover the mouth of the tube with the thumb and shake as rapidly as possible for a few seconds. Notice the appearance of the froth and the increased concentration of the thymol blue just below the froth after most of the froth has disappeared. Explain. Repeat the shaking several times and each time, with a pipette, transfer froth to another test tube. After enough froth has been collected to give about 3 or 4 cc. of solution, compare the colors of the solutions in the two test tubes. What do you observe? Repeat the procedure using a dilute solution of methylene blue. Does the methylene blue become adsorbed on the films of the bubbles as in the case of thymol blue? Now introduce a sufficient amount of the methylene-blue solution into the thymol-blue solution to produce a violet color. Shake this mixture in a test tube and remove and collect the froth as before, being careful not to transfer any of the solution. What is the color of the solution obtained from the froth?

160. Adsorption by Charcoal.—Place 2 or 3 grams of cane sugar in a casserole or beaker and heat to caramelize. When the brown color has been obtained, add about 100 cc. of distilled water and dissolve.

Put 5 grams of animal charcoal into the above solution, boil for about 5 min. and then filter. A colorless filtrate should be obtained. Explain.

Add 1 gram of animal charcoal to 10 cc. of a dilute solution of crystal violet in a test tube and shake well. Then filter and observe that the filtrate is colorless. Transfer the charcoal that is on the filter paper to a clean test tube and add 10 cc. of distilled water. Shake the tube well and note that the water is only slightly colored. Now add 5 or 6 cc. of a mixture of equal parts of methyl and ethyl alcohol and shake. Does the dye leave the charcoal and go into solution? Explain.

To 10 cc. of 0.01 *N* HCl solution in a test tube add a few drops of methyl-red indicator solution and observe the color. Add 1 gram of animal charcoal to 10 cc. of 0.01 *N* HCl solution in a clean test tube and shake. Filter and test the filtrate with methyl-red indicator solution. Compare the colors of the solutions in the two test tubes. The HCl is readily adsorbed by the charcoal. Methyl-red indicator solution has a pH range of 4.4 (red) to 6.0 (yellow).

Place about 25 cc. of a slightly colored aqueous solution of Congo red in a flask and add about 1 gram of animal charcoal. Shake for a few minutes and then filter. The solution becomes decolorized. Explain.

CHAPTER X

THE COLLOIDAL STATE

161. Test of Diffusibility of Colloids through Membranes.—Prepare a collodion dialyzing sac in the following manner. Place about 15 cc. of collodion into a clean dry Erlenmeyer flask having a capacity of 125 cc. Tilt the flask and slowly rotate it so that a thin film of collodion forms over the entire inner surface of the flask. Bubbles of air are liable to form if the procedure is not carried on slowly. Drain out any excess solution and place the flask in an inverted position on a ring stand for 10 to 15 min. while the collodion dries. Then with a knife blade loosen the edges of the film at the mouth of the flask and put water between the film and flask to aid in removing the sac. Keep the sac immersed in water until ready to use. Fill the sac about half full with a colloidal solution of Prussian blue,[1] tie the mouth of the sac closed and fasten a glass rod at the mouth of the sac. Lay the glass rod across the top of a beaker containing distilled water and allow the sac to remain immersed in the water.

Using the same procedure place a 5 per cent solution of $CuSO_4$ in another collodion sac and dialyze. Allow both sacs to stand for 24 hr. and then examine. Which substance has passed through the membrane? Explain.

162. Action of Electrolytes on Arsenious Sulphide Suspensoid.—Add 100 cc. of distilled water to 100 cc. of an arsenious sulphide suspensoid,[2] which will be furnished, and mix well. This colloid bears a negative charge. Place 9 cc. of the diluted suspensoid in each of five test tubes. Add to each tube, respectively, 1.0 cc. of 1.0 *M*, 0.8 *M*, 0.6 *M*, 0.4 *M* and 0.2 *M* NaCl solutions. Place 9 cc. of the diluted suspensoid in a sixth tube and add 1.0 cc. of distilled water as a control. A 1.0 *M* NaCl solution will be furnished, and the above solutions should be prepared from it. To make a 0.6 *M* solution, for example, from this,

[1] A colloidal solution of Prussian blue may be prepared by placing 0.02*N* $K_4Fe(CN)_6$ solution in a beaker and mixing it with an equal volume of 0.02 *N* $FeCl_3$ solution. This colloid is negatively charged.

[2] The arsenious sulphide suspensoid may be prepared by adding a saturated solution of H_2S to an equal volume of a 1.0 per cent solution of arsenious oxide (As_2O_3) made by boiling the oxide solution. Air should be bubbled through the mixture to remove the excess H_2S, and the suspensoid should be dialyzed against distilled water for about 48 hr. before using.

you would take 6 cc. of the 1.0 *M* NaCl solution and add distilled water to the 10-cc. mark. After adding the NaCl solutions to the suspensoid mix well. Centrifuge each mixture and in this way determine the smallest amount of electrolyte that will cause precipitation of the colloid.

Repeat using 0.01 *M* barium chloride solution and then 0.001 *M* aluminum chloride solution diluting them as you did the NaCl solution to make two series. Calculate, in millimols per liter, the concentration necessary to cause precipitation. Explain (see the Schultz-Hardy rule on pages 402 and 415 in the text). Also read the discussion on pages 402 to 404, inclusive, in the text.

163. Mutual Precipitation of Suspensoids of Opposite Charge.—Into each of five clean test tubes put 5 cc. of a colloidal solution of arsenious sulphide. Add to each of the five test tubes 3, 4, 5, 6 and 7 cc., respectively, of a colloidal solution of ferric hydroxide.[1] This colloid is positively charged. At a certain relative proportion, depending upon the concentration of the colloids, there will be a complete precipitation of both. Centrifuge the mixtures to determine whether or not precipitation occurs. Explain.

164. Protective Action of Emulsoids.—With a graduated pipette add 0.05 *N* sodium sulphate solution, drop by drop, to 10 cc. of a colloidal solution of ferric hydroxide in a clean test tube until precipitation just occurs. Note the amount of sodium sulphate solution required. To 25 cc. of the colloidal ferric hydroxide solution add 1.0 cc. of a 1.0 per cent fresh gelatin solution. After mixing well put 10 cc. of the mixture in a test tube. Add 0.05 *N* sodium sulphate solution, drop by drop as before, to determine how much is now required to just cause precipitation. Explain.

165. Adsorptive Stratification (Liesegang Phenomenon).—A Petri dish will be supplied to you containing a thin layer of gelatin to which potassium dichromate has been added. This mixture was prepared by dissolving 4 grams of commercial gelatin in 100 cc. of warm distilled water and adding, with stirring, 2 cc. of a saturated solution of potassium dichromate. The mixture was poured out while warm in thin layers in Petri dishes and allowed to set.

Place 2 large drops of 40 per cent $AgNO_3$ solution some distance apart on the film of gelatin. Put the top on the dish and place in a horizontal position in the dark until the next laboratory period. What do you find? Explain (see page 412 in text).

166. The Swelling of Gels.—From a sheet of gelatin cut out 10 rectangles each 1.0 by 4.0 cm. Arrange a series of 10 Petri dishes so

[1] To prepare a colloidal solution of ferric hydroxide, bring 200 cc. of distilled water to a boil and add 1.0 cc. of a 33 per cent $FeCl_3$ solution. Before using, dialyze against distilled water for about 48 hr.

that each will contain 50 cc. of one of the following solutions, all of which should be used:

(*a*) distilled water.

(*b*) 0.2 *N*, 0.1 *N*, 0.05 *N* HCl.

(*c*) 0.2 *N*, 0.1 *N*, 0.05 *N* NaOH.

(*d*) 0.2 *N* NaCl.

(*e*) 0.05 *N* HCl plus 0.1 *N* NaCl (equal parts), 0.05 *N* NaOH plus 0.1 *N* NaCl (equal parts).

Label the dishes and into each solution place one of the gelatin rectangles. Measure the length of the rectangles at the end of every hour

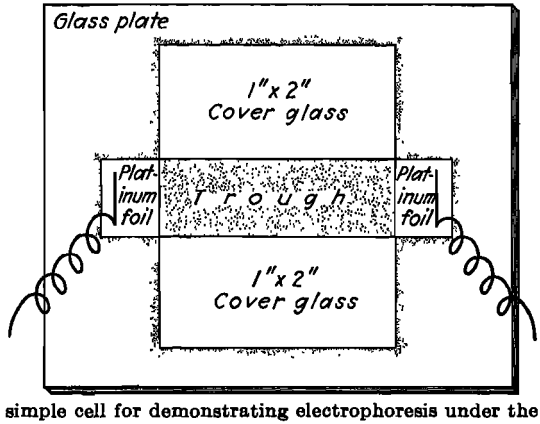


FIG. 21.—A simple cell for demonstrating electrophoresis under the microscope.

during a period of 3 hr. How does the degree of swelling vary with the degree of acidity or alkalinity? Does NaCl modify the swelling? (See Fig. 103 and pages 412 and 413 in the text.)

167. Electrophoresis of Particles and Cells.—Read in the text the discussion on pages 391 and 392 in preparation for this work.

A simple electrophoresis cell[1] of the type shown in Fig. 21 will be furnished.

Mount the cell on a microscope stage and connect the electrodes to a 45-volt B battery through a reversing switch as shown in Fig. 22.

Prepare a light suspension of powdered charcoal in tap water and fill the trough of the electrophoresis cell with some of it, making sure that the suspension touches both electrodes. Cover the trough with a long cover glass and wait about 3 min. for the suspension to stop moving. Observe the migration of the charcoal particles under the microscope

[1] This cell is readily constructed by sealing two long cover glasses (24 by 50 mm.) and two pieces of platinum foil to a glass plate (such as a 3¼- by 4-in. plain, lantern-slide glass) with Canada balsam. A small insulated copper wire should be soldered to each piece of platinum foil before the foil is cemented to the glass. The trough between the two cover glasses should be about 1.0 cm. wide.

with the electric current flowing first in one direction and then in the other. The use of an ocular micrometer is recommended. To which pole do the particles travel? Repeat using 90 volts. How is the velocity of migration changed?

Repeat using a very dilute suspension of red blood cells in physiological salt solution. What is the sign of charge on the charcoal particles and on the red blood cells?

168. The Relative Viscosity of Water and of Aqueous Solutions of Alcohol.—In preparation, read in the text pages 392 to 396, inclusive.

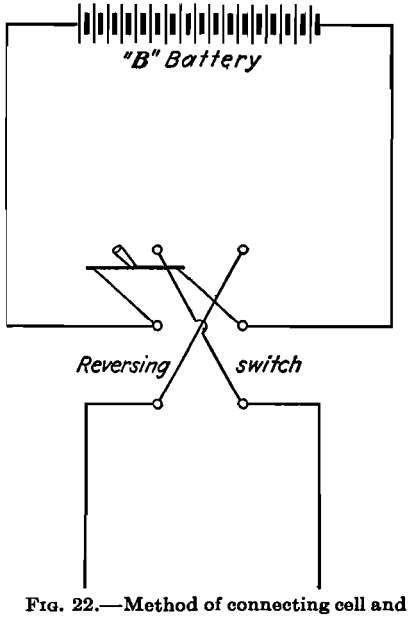


FIG. 22.—Method of connecting cell and battery by means of a reversing switch.

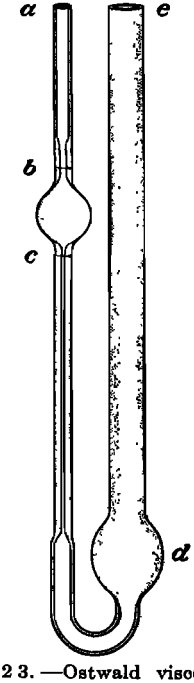


FIG. 23.—Ostwald viscosity tube.

An Ostwald viscosity tube (see Fig. 23) having a time of outflow for water at room temperature of 60 to 100 sec. will be used. The tube must be very clean before using, and it is usually sufficient to cleanse with distilled water, alcohol and ether in succession. Air is sucked through the tube to dry it. Mount the tube in a vertical position on a stand by attaching to the larger arm of the tube a clamp with rubber-covered jaws. The temperature of the liquids to be used should be noted and controlled as well as possible. For work of the highest accuracy the instrument should be partly immersed in a transparent constant-temperature water

bath. Attach a piece of rubber tubing about a foot in length to the end *a* of the smaller arm. Introduce distilled water into the viscosity tube at *e* and carefully measure the volume necessary to just bring the water level to the top of the bulb *d*. Always use the same volume of liquid when employing the same tube. Ordinarily 5 cc. of liquid are required. Suck the distilled water up into the smaller arm until the meniscus is just above the upper mark *b*. Allow the water to flow back through the capillary and by means of a stop watch measure the time required for the meniscus to pass from the upper mark *b* to the lower mark *c*. Make at least three measurements of the time necessary for the meniscus to move from the upper to the lower mark and find the average value. This gives the time of outflow for water, and this value is employed in obtaining the viscosity of other liquids or solutions relative to water.

Using the technique described above, measure the relative viscosities of 25, 50, 75 and 100 per cent aqueous solutions of ethyl alcohol. The viscosity tube should be rinsed with the solution to be used before the measurement is made. If the outflow time for water is 70 sec. and for the liquid 175 sec., then the relative viscosity of the liquid is 175 divided by 70, *i.e.*, 2.5. The effect of differences in density may be ignored here, since the error involved is less than 1.0 per cent. What is the effect of dilution on the viscosity of alcohol? Explain. Plot your results in the form of a graph.

169. Imbibition.—Obtain a thin sheet of rubber about 4 by 1 in. and cut it almost its entire length into two strips of equal width. Fill a large test tube with benzene, immerse one strip in it and allow the other strip to hang on the outside. At the end of 15 min. remove the rubber strip from the test tube and measure its length. Compare with the control strip. Rubber has the power of imbibing certain liquids.

170. Heat of Imbibition.—For this experiment 50 grams of dry commercial starch powder at room temperature will be furnished. This has been dried by heating at 105 to 108°C. for 15 to 20 min., placed in a desiccator and allowed to cool to room temperature. Put the starch in a dry thermos bottle, insert the stopper through which a thermometer should pass and note the temperature of the starch. Then add 40 cc. of distilled water at the same temperature as the starch and again place the end of the thermometer in the starch. Read the thermometer at intervals during a period of 10 min. Explain why an increase in temperature occurs.

171. The Amphoteric Behavior of Gelatin.—Place 5 grams of granulated gelatin in 100 cc. of 0.01 *N* HNO_3 solution and an equal amount of the gelatin in 100 cc. of 0.01 *N* NaOH solution. Stir thoroughly and then place each mixture *in the cold* for 1 hr. Arrange to have on hand 500 cc. of cold distilled water. Remove the gelatin solutions from the refrigerator when the hour has elapsed and drain off the solution from

the gelatin. By decantation, wash the gelatin with the cold distilled water.

Divide each sample of gelatin into two equal portions and add to one of these portions from each of the two samples a dilute solution of methylene blue. Similarly add a dilute solution of acid fuchsin to an acid-treated portion and also a base-treated portion of gelatin. Place the four mixtures obtained in this way in the refrigerator for half an hour and then wash the gelatin as before with cold water.

Methylene blue (basic dye) enters into combination with anions, whereas acid fuchsin (acid dye) combines with cations. Explain the results in terms of the dissociation of and charges upon the gelatin.

172. The Effect of Electrolytes on the Viscosity of Gelatin.—Using the technique employed in the previous experiment (168) determine the relative viscosities of gelatin solutions under the following conditions. Obtain a freshly prepared 1.0 per cent solution of gelatin, make the dilutions indicated below and measure the relative viscosity of each. The mixture in each case should be made only immediately before the measurement, in order to control the time factor. Colloids change with age.

To 10 cc. of the 1.0 per cent gelatin add 5 cc. of one of the following solutions and mix well:

(*a*) distilled water.
(*b*) 0.02 *N* HCl.
(*c*) 0.2 *N* HCl.
(*d*) 0.02 *N* NaOH.
(*e*) 0.2 *N* NaOH.
(*f*) 1.0 *N* Na_2SO_4.
(*g*) 1.0 *N* NaCl.

Discuss your results (see text, Fig. 103 and pages 397 and 398).

173. Effect of Acid on Imbibition.—Tie a weight (about 200 grams) to the end of a catgut string (violin string) and allow the weight to rest on the bottom of a 250-cc. graduate cylinder. Attach the free end of the catgut to a counterpoised heart lever arranged to write on a smoked drum. Allow the drum to revolve quite slowly and record. Now almost fill the cylinder with distilled water and record the changes. Add enough HCl to make the solution contain about 2 per cent HCl. Record the changes.

174. Reversal of Phases in an Emulsion.—Place 5 cc. of 0.1 *N* NaOH solution in a test tube and add to it 5 cc. of olive oil stained red with Sudan III. Close the tube with the thumb, shake vigorously and then examine the emulsion. Place a drop or two of the emulsion under the microscope and examine. Is the oil suspended in water or the water in the oil? To the emulsion in the test tube now add 1.0 cc. of 0.1 *M* $BaCl_2$ solution. Shake well and examine as before. What change do you observe? Explain (see pages 406 and 407 in the text).

CHAPTER XI

THE PHYSICOCHEMICAL STRUCTURE OF LIVING MATTER

175. Experimental Alteration of Form.—Arrange a dry cell and a key in series with the primary of an inductorium so that faradic stimulation may be obtained. To the secondary terminals, connect two insulated wires of sufficient length to reach to a glass slide placed on the stage of a microscope. Place a few filaments of Spirogyra or Ulothrix lengthwise on the glass slide and cover the filaments with water from their natural environment. Examine under the microscope and sketch a normal cell showing the main features.

Place the bare ends of the wires from the inductorium near opposite ends of a filament and stimulate briefly while watching through the microscope. What is observed? After changes in form are produced in this way make a sketch to show the changes. Could alterations in surface tension or viscosity account for what occurs?

Take some fresh filaments of Spirogyra or Ulothrix and mount them as before on a glass slide. Place the glass slide on a small inverted dish resting on the microscope stage. Examine the cells to see that they are normal. With a microburner heat one end of the slide slowly until changes in the appearance of the cells are observed. Describe what occurs.

Repeat using chloroform as the agent instead of heat.

176. Streaming Movements (Cyclosis).—Place on a glass slide on the stage of a microscope part of an Elodea plant that has been in strong sunlight for at least an hour. Examine a leaf under the microscope and note the structure of the cells. Study within a cell the movement of the cytoplasm and chloroplasts. What is the effect of mild electrical stimulation on the movements?

177. Changes Induced in Amoeba.—Place a drop of culture medium containing amoebae on a glass slide under the microscope. Arrange a few small broken pieces of a cover slip to support a cover slip over the drop. Using low and high powers of the microscope, examine the amoebae and note as much of the detail of the protoplasmic structure as possible. It will be necessary to adjust the mirror and the condenser of the microscope to get optimum lighting conditions.

Focus on an amoeba and with a medicine dropper drawn out to a fine bore place a drop of 0.1 *N* HCl solution at one side of the drop con-

taining the amoeba. Watch the amoeba and describe what happens. Apply 2 drops of 0.1 *N* NaOH at the same place. What happens?

On a glass slide equipped with metal electrodes cemented in position, place some culture medium containing an amoeba. Connect these electrodes to the secondary terminals of an inductorium and include a dry cell and simple key in series with the primary circuit. Adjust the inductorium to deliver a weak faradic current. Mount a cover slip over the medium containing the amoeba and make sure that the medium touches both electrodes. Focus on the amoeba and watch it for a short time. Apply faradic stimulation while watching the animal. If nothing happens, increase the intensity of the current until the amoeba responds but do not apply the current for more than 2 or 3 sec. at a time. What is observed? Now adjust the inductorium to give its strongest current and stimulate for several seconds. What happens?

CHAPTER XII

DIFFUSION AND OSMOSIS, OSMOTIC PRESSURE

178. Osmotic Pressure of a Sugar Solution.—Read in the text the discussion on pages 436 to 440, inclusive, before doing this experiment.

On each side of a Cenco-Troxel osmometer (see Fig. 24) clamp in position a membrane of cellophane or parchment. Attach to the vertical tube at the top of the osmometer a piece of glass tubing of small bore and about 4 ft. in length. Fill the osmometer with 1.0 *M* sucrose solution, introducing the solution through the larger tube set at an angle at the top of the osmometer. Close this tube tightly with a rubber stopper. Stand the osmometer on the bottom of a large beaker, arrange a support for the glass tube and fill the beaker with distilled water. Record the height, in millimeters, of the solution in the glass tube every 15 min. over a period of about 3 hr. Plot the results to show how the height of the column in the tube varies with time. Explain.

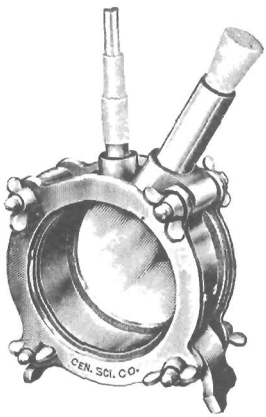


FIG 24.—Cenco-Troxel osmometer. (*Courtesy of Central Scientific Co.*)

If an osmometer of the type mentioned above is not available, a suitable osmometer may be prepared in the following manner: Pour upon the surface of clean mercury in a Petri dish some collodion solution to give an even layer about 1.0 mm. thick. Watch the collodion, testing it from time to time by gently lifting it at the edge with forceps. Find the stage at which enough alcohol and ether have evaporated to leave a membrane of dense, flexible texture but not dry or brittle. Now lift the membrane from the mercury, lay it over the wide mouth of a thistle tube, tie it in place with thread and seal it onto the glass by carefully applying a few drops of collodion solution to the edges of the membrane. Rotate the glass during the drying of the collodion in such a way that it will be evenly distributed and completely seal the membrane. When the collodion is quite dry, immerse the closed end of the tube in water to see that the membrane does not leak. If the osmometer is thus shown to be satisfactory, fasten it open end upward by a clamp to a support. By means of a fine-tipped funnel, introduce enough 1.0 *M* sucrose solution into the tube to fill its bulbous part. Attach a piece of glass tubing of small bore and about 2 ft. in length

to the small end of the thistle tube. Lower this osmometer into a beaker full of distilled water until the level of the solution inside is the same as the water outside. Fasten the tube in this position and record the increase in height of the solution in the osmometer as directed above.

179. Electroosmosis.—In preparation for this experiment read pages 440 to 442 in the text.

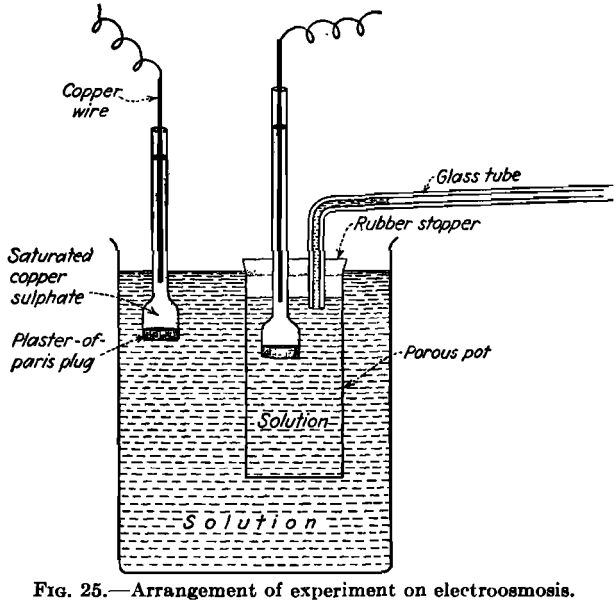


FIG. 25.—Arrangement of experiment on electroosmosis.

An unglazed-porcelain porous cup[1] equipped with a rubber stopper bearing a glass tube of small bore and a nonpolarizable electrode[2] will be furnished. Completely fill the porous cup with 0.05 per cent K_2SO_4 solution and insert the rubber stopper, leaving no air bubbles inside the cup. Immerse the porous cup in a 0.05 per cent K_2SO_4 solution contained in a large beaker and arrange a nonpolarizable electrode to dip into the solution. The arrangement is shown in Fig. 25.

Connect the two nonpolarizable electrodes through a reversing switch (see Fig. 22) to a 45-volt B battery. Measure the rate of movement of the meniscus along the glass tube, which should be approxi-

[1] The cup should be allowed to soak for a few hours in 0.05 per cent K_2SO_4 solution before using.

[2] The glass parts for the inner and outer electrodes may be made readily from a discarded volumetric pipette by cutting through the bulbous part. To some plaster of Paris some 0.9 per cent NaCl solution is added to make a thick paste about 6 mm. deep in a beaker. The wide ends of the glass parts of the electrodes are pushed into the paste and allowed to remain until the plaster hardens. The electrodes are then cleaned and filled with saturated copper sulphate solution into which a bare copper wire dips in each case.

mately horizontal, having the inner electrode act as the cathode. In what direction does the solution move? Reverse the current and again measure the rate. Is the direction of movement now reversed?

Repeat using 22½ volts. Explain the phenomenon observed. What is the sign of electrical charge on the pores of the porous cup?

180. Isotonic Salt Solutions.—Arrange a series of small dishes containing, respectively, 2.0 cc. of the following strengths of NaCl solutions: 2 *M*, 1 *M*, *M*/2, *M*/6, *M*/20; in another dish place 2.0 cc. of distilled water. Add a drop of fresh frog's blood to each. Stir all the suspensions, transfer a drop of each to a slide and examine under the microscope. What differences do you find at the end of 10 or 15 min.? Which of these salt solutions is isotonic with the red blood corpuscles?

Place a drop of frog's blood in 2.0 cc. of 0.3 *M* urea solution and mix. Put a drop of the suspension under the microscope and examine. Hemolysis occurs quickly.

Place a few drops of Ringer's solution in a test tube. Add a drop of ether and a drop of frog's blood. Place some of the suspension under the microscope and examine. What is the effect of the ether? What conclusions may be drawn from this series of experiments?

181. Plasmolysis of Cells.—Before doing this work read in the text pages 443 to 446, inclusive.

Observe some filaments of Spirogyra or Ulothrix in the pond water in which they were collected. Use a small glass dish under low power of the microscope. Select filaments that are in good condition and place a few of them in each of a series of dishes containing, respectively, *M*/2, *M*/3, *M*/4, *M*/5, *M*/6 and *M*/7 sucrose solution. Avoid dilution of the solutions with the pond water. Observe under the microscope during a period of about half an hour. In which solutions does plasmolysis occur? See pages 444 and 445 in the text. What strength of sugar solution has approximately the same osmotic pressure as these cells?

Similarly, place filaments of Spirogyra or Ulothrix in *M*/3, *M*/4, *M*/5, *M*/6, *M*/7, *M*/8 and *M*/9 KNO_3 solutions. Determine in which solutions plasmolysis occurs. What strength of KNO_3 solution has approximately the same osmotic pressure as the cell contents? How do the sugar and KNO_3 solutions that are isotonic with the cells compare as regards their concentrations?

182. Passage of Water into Muscle in Hypotonic Medium.—Pith a frog and dissect out both gastrocnemius muscles or two other muscles of equal size. With filter paper, dry the surfaces of the muscles and then weigh them. Place one muscle in 0.7 per cent NaCl solution (isotonic) and the other in 0.1 per cent NaCl solution (hypotonic). At the end of 2 hr. dry the muscles as before and weigh them. Has any change in weight occurred? Explain.

CHAPTER XIII

THE PERMEABILITY OF MEMBRANES AND LIVING CELLS

183. Permeability to Alkalies.—In preparation read the discussion in the text on pages 455 to 457 and 464 to 467, inclusive.

Lightly color some pond water with neutral red and in it immerse some Spirogyra filaments that are in good condition. This indicator is red in acid medium and yellow in basic medium. Allow the cells to remain in this solution for 20 to 30 min. to permit the interiors to become stained.

Place 0.025 *N* NaOH, 0.025 *N* $Ba(OH)_2$ and 0.025 *N* NH_4OH solutions in separate watch glasses. When the cells have been adequately stained, place some of the Spirogyra filaments in each of the above solutions and record the time. Avoid dilution with pond water. Examine the cells under the microscope and determine the time necessary for color change to occur in each case. When the filaments show color change, transfer them to watch glasses containing only pond water and examine at intervals to see whether or not they assume the color which they had when first stained. The cells in the NH_4OH solution become yellow quickly and when this occurs place some of them in a 0.025 *N* NaOH solution. Do they become red again? Explain.

Repeat the above procedures but just before placing the cells in the three alkaline solutions put them in pond water saturated with chloroform for 5 min. to kill them. Then make the transfers and tests. Has any change in permeability occurred? Is the differential effect still noticeable?

184. Effect of NaCl and of $CaCl_2$ on Cell Permeability.—Stain Spirogyra cells with neutral red in pond water as in the previous experiment (183). Place filaments that are in good condition in watch glasses containing, respectively, the following solutions:

(*a*) 0.025 *M* NaOH.

(*b*) 0.025 *M* NaOH plus an equal volume of 0.125 *M* NaCl.

(*c*) 0.025 *M* NaOH plus an equal volume of a mixture of 5 parts (by volume) of 0.125 *M* NaCl and 95 parts (by volume) of 0.125 *M* $CaCl_2$. Avoid diluting the solutions with pond water in introducing the filaments. Note the time of immersion in each case and determine the time necessary for the penetration of the NaOH. What are the effects of the Na^+ and Ca^{++} ions on the permeability to the alkali? Is there any indication of an antagonistic effect between these two ions?

185. Penetration of CO_2 through a Living Skin of a Frog.—Dissect out the skin of a frog to secure as large a surface area as possible. Stretch the skin over the mouth of a flared glass tube and fasten it with a rubber band taking care not to injure the skin. Now fill the glass tube with distilled water to which has been added some phenol-red solution and a trace of $NaHCO_3$ solution to make the mixture only very slightly alkaline. By means of a clamp and stand suspend the tube so that it will be partly immersed in a 0.01 *N* H_2SO_4 solution colored with 2 drops of phenol-red solution. Allow this to stand for 5 min. and note that no color change inside the tube occurs. Now add to the outside solution enough saturated $NaHCO_3$ solution to make it quite basic. How long does it take for the internal solution to become acid? Explain.

186. Permeability of a Lipoid Solvent to CO_2. Intracellular Acidity with Extracellular Alkaline Medium.—Obtain four "shell vials" (about 10 by 40 mm.) and fill each to within about 4 mm. of the top with a solution prepared by adding to distilled water a little phenol red as indicator and a trace of $NaHCO_3$ to produce a very slight alkaline reaction. Fill the vials to the top with paraffin oil or xylol. Dip the mouth of a test tube into a fairly thick collodion solution, withdraw the tube and press the film on its mouth over the top of a vial to seal it. Apply membranes to the openings of the other vials in a similar manner. The collodion membranes allow the passage of certain ions and molecules but prevent the loss of the contents of these "artificial cells" when immersed in water.

Into a series of four stoppered test tubes, place, respectively, the following solutions to almost fill the test tubes:

(*a*) Distilled water.

(*b*) Distilled water saturated with CO_2.

(*c*) 0.5 *M* $NaHCO_3$.

(*d*) 0.5 *M* $NaHCO_3$ saturated with CO_2.

Place an "artificial cell" in each test tube, stopper tightly and examine about 2 hr. later. What happens? Explain.

187. Membrane Potentials Across an Apple Skin.—Read the discussion in the text on pages 460 and 461.

A potentiometric assembly and two saturated calomel electrodes will be furnished. For this experiment the potentiometer employed in the Youden pH outfit (see Exp. 145) has been found to be very satisfactory. Test the calomel electrodes to see that they are isoelectric by dipping the tips of the contact side arms in a saturated KCl solution and measuring any difference in potential.

Select an uninjured apple and mount it on a glass ring resting on the bottom of a shallow dish so that the depression at the base of the apple stem is at the side. Place a small ring of vaseline on top of the apple

taking care to get no vaseline on the skin of the apple within the ring. Place 3 or 4 drops of 0.1 *N* KCl solution on the apple within the ring. Connect the outside surface of the apple to a calomel electrode through this KCl solution. Add 0.1 *N* KCl solution to the dish until the apple is immersed about a quarter of an inch. Dip the other calomel electrode in this solution and connect the calomel electrodes to the potentiometer. Test for the existence of a potential.

Keeping the same solution as before on top of the apple, measure the potential when the apple is partly immersed in 0.02 *N* KCl solution, then in 0.004 *N* KCl solution and finally in 0.0008 *N* KCl solution. What do you find? This is known as the concentration effect. Record the potentials in millivolts.

Cut off a clean slice from the top of the apple as it rests in the dish to leave a region about an inch across without any skin. Make a small depression in the center of this region and put 3 or 4 drops of 0.004 *N* KCl in it. Connect one calomel electrode with this solution. In the dish place 0.004 *N* KCl solution so that the apple is partly immersed as before. Dip the other calomel electrode into this solution and measure the potential across the apple skin. What is the significance of such a potential. Which side of the membrane is negative?

CHAPTER XIV

CATALYSIS AND ENZYMES

188. Starch Hydrolysis by Salivary Amylase.—Chapter XVII (Catalysis and Enzymes) in the text should be read before this work is undertaken.

Chew a small piece of soft paraffin to stimulate salivary secretion. Collect the saliva in a beaker and filter through plaited filter paper wet with distilled water. Add 5 drops of the filtered saliva to 5 cc. of 1.0 per cent starch paste.[1] Shake thoroughly. With a pipette, remove a few drops of the mixture and test it with a very dilute solution of iodine in a test tablet. Repeat this test at 1-min. intervals and note the colors obtained. If at the end of 5 min. the iodine reaction is still blue indicating the presence of starch, add 5 drops more of filtered saliva. When no iodine reaction is obtained (the achromic point) test for sugar by Benedict's test. This is carried out as described in Exp. 122 (page 64 of this manual). If sugar is present the solution will turn to a reddish-yellow color. What is the action of the amylase on the starch? What do the successive iodine tests show the various stages to be in the process of hydrolysis? (See pages 277 and 521 in the text.)

189. The Effect of Temperature on Salivary Amylase Action.—Place 5 cc. of starch paste in each of four test tubes. Chill one to about 5°C. in a beaker of ice water. Warm two of them to 40°C. in a water bath and allow one to remain at room temperature. After they have reached the desired temperatures, add 5 drops of filtered saliva to each of three of them and to one of those at 40°C. add 5 drops of some saliva that has been boiled. Shake each tube thoroughly. Follow the progress of hydrolysis by use of the iodine test as in the preceding experiment (188) in order to find the time required to reach the achromic point. What do you conclude as to favorable temperature conditions for enzyme action? What is the result in the case of boiled saliva? Explain.

190. The Effect of Hydrogen-ion Activity on Salivary Amylase Action.—Phosphate buffer solutions having the following pH values will be furnished: 5.0, 6.0, 7.0, 8.0. In each of four clean test tubes, place 8 cc. of one of these solutions, using a different solution in each tube. Label each tube according to the pH of its solution. To each add exactly 0.5 cc. of a mixture of 1 part of filtered saliva and 4 parts of

[1] To prepare, add a little distilled water to 2 grams of powdered starch in a mortar and grind. Heat 200 cc. of distilled water to the boiling point and put the starch in it while stirring. Again heat to the boiling point and permit it to cool.

distilled water. Shake all tubes thoroughly. Now add 2 cc. of starch paste to each tube and again mix thoroughly. Keep all the tubes at 40°C. in a water bath and, by use of the iodine test, follow the progress of digestion in each case. Remember that alkaline solutions must first be neutralized before applying the iodine test. What do you find to be the optimum pH and how does this compare with that of your saliva?

191. The Effect of Amylase on Raw Starch Grains.—Shake some starch grains with 10 cc. of distilled water in a test tube and add 1.0 cc. of filtered saliva. Put the test tube in a water bath at 40°C. and observe the progress of digestion by means of the iodine reaction. Shake the tube before each test. After about 30 min., filter some of the mixture and apply Benedict's test to the filtrate. Do you find any evidence of starch hydrolysis? What is the significance of this experiment as regards the comparative digestibility of raw and cooked starch?

192. The Effect of Dilution on Enzyme Action.—Take a series of test tubes each containing 9 cc. of distilled water. Add 1.0 cc. of filtered saliva to tube 1 and shake thoroughly. Remove 1.0 cc. of the contents of tube 1 and transfer it to tube 2. After mixing well, transfer 1.0 cc. from tube 2 to tube 3. Continue in this manner until you have six tubes of saliva solutions of regularly decreasing strength. Now add 2.0 cc. of starch paste to each tube, mix thoroughly and place in a water bath at 40°C. After 10 and after 20 min., try the iodine test and Benedict's test on samples from each tube. In how great a dilution does your saliva show amylolytic action?

To each tube add 2 or 3 drops of toluene for antisepsis and allow the tubes to stand until the next laboratory period. Again test each one by the iodine and by Benedict's test. Do you obtain any evidence tending to prove that an enzyme is a catalyst?

193. Action of Sucrase, Invertase of Yeast.—Grind one-fourth of a yeast cake with about 10 cc. of water in a mortar with a little clean sand to break the yeast cells. Filter and add 1.0 cc. of the filtrate to 5.0 cc. of 2 per cent sucrose solution in a test tube. Add 1.0 cc. of boiled filtrate to another 5.0-cc. portion of the sugar solution. Keep both tubes at 40°C. for about an hour. Then try Benedict's test on both. Note and explain the results. Sucrose will not reduce Benedict's solution but glucose or fructose will. Sucrose hydrolyzes into glucose and fructose.

194. Action of a Protein Precipitant on Enzyme Activity.—Into each of three test tubes, place 5.0 cc. of 1 per cent starch paste. To one tube add 1 cc. of dilute malt extract containing the enzyme, malt diastase. To another tube add 1 cc. of the malt extract and, in addition, some $HgNO_3$ crystals (poison). To the third tube add 1 cc. of the malt extract after bringing it to the boiling point and then cooling. Place the three

tubes in a water bath at 40°C. Test for hydrolysis every 10 min., employing the iodine test. What is the result?

195. Action of Pepsin.—Read pages 530 and 531 in the text.

Place 5 cc. of each of the following mixtures separately in test tubes:

(*a*) 5 cc. of a 0.5 per cent solution of commercial pepsin plus 5 cc. of 0.6 per cent HCl solution.

(*b*) 5 cc. of a 0.5 per cent solution of commercial pepsin plus 5 cc. of distilled water.

(*c*) 5 cc. of a 0.5 per cent solution of commercial pepsin that has been boiled plus 5 cc. of 0.6 per cent HCl solution.

(*d*) 5 cc. of a 0.5 per cent solution of commercial pepsin plus 5 cc. of a 0.5 per cent NaOH solution.

(*e*) 5 cc. of distilled water plus 5 cc. of 0.6 per cent HCl solution.

Shake each tube well and add to each a piece of fibrin as small as can be clearly observed, selecting pieces of uniform size. Place all five tubes in a water bath at 40°C. and shake occasionally. Note that the fibrin swells at first and in certain of the solutions it is digested (hydrolyzed). In which mixtures does digestion occur? Explain.

196. Action of Gastric Rennin on Milk.—Arrange a series of five test tubes to contain the following:

(*a*) 5 cc. of fresh milk plus 0.2 per cent HCl solution (add slowly from a pipette until precipitation occurs).

(*b*) 5 cc. of fresh milk plus 1.0 cc. of 1 per cent rennin solution.

(*c*) 5 cc. of fresh milk plus 10 drops of 0.5 per cent Na_2CO_3 solution.

(*d*) 5 cc. of fresh milk plus 10 drops of a saturated solution of ammonium oxalate.

(*e*) 5 cc. of fresh milk plus 10 drops of 0.1 per cent HCl solution.

Place 1.0 cc. of 1 per cent rennin solution in tubes (*c*), (*d*) and (*e*). Thoroughly mix the contents of each tube. Place the five test tubes in a water bath at 40°C. and after 15 min. examine each tube. Explain the results in each case. To tube (*d*) add an excess of calcium chloride. Explain what happens (see page 531 in the text).

197. Action of Pancreatic Lipase.—This enzyme hydrolyzes fats into fatty acids and glycerol.

Place 5 cc. of a 1.0 per cent solution of commercial steapsin[1] in each of two test tubes. Boil the contents of one tube. In a clean test tube put 5 cc. of olive oil and add 5 cc. of distilled water. Shake well while adding 2 drops of 0.01 *N* NaOH solution to form an emulsion. Put into the emulsion 5 drops of litmus solution and again shake. Add 5 cc. of this colored emulsion to each of the steapsin solutions. Place the tubes in a water bath at 40°C. and examine at intervals for change in color of the indicator. Shake occasionally. Explain what happens.

[1] Powdered steapsin (lipase) may be obtained from the Fisher Scientific Company, 709–719 Forbes Street, Pittsburgh, Pa.

CHAPTER XV

DIGESTION

198. Movements of the Alimentary Canal.—Read in the text pages 528 and 529 and also pages 542 to 544, inclusive.

This experiment will be demonstrated with the assistance of the members of the class. Obtain a motility tube made from a glass tube about 5 in. long and 1 in. in diameter. This tube should be compressed at one end to form an opening about 1⅛ in. long and ⅜ in. wide. This opening should be slightly flared outward and a small hole should be provided through the wall of the tube at each of the two narrow ends of the oval opening. Threads will later be passed through these small holes. A clamp on a stand should be prepared to hold the tube in place when ready. Arrange a heart lever to write on a smoked drum on a kymograph. Arrange a time marker to record 5-sec. intervals. Put a rabbit under urethane anesthesia (see the Appendix in this manual for the dosage) and secure it to an animal board. Supplement with ether anesthesia if necessary. Make a median incision through the linea alba in such a manner as to open the abdominal cavity as far from the diaphragm as possible. Use hemostats to check any bleeding that might occur. Expose the small intestine and observe the movements. With forceps pinch a motionless loop of the small intestine, noting where contraction and relaxation occur. Pinch the ascending colon in the same way. Result?

Apply a long piece of thread (about 18 in.) to the jejunum by passing it through the superficial layer of the intestine with a needle and tying. Support the motility tube directly over this stitch on the intestine with the oval end downward and pass the thread upward through the tube. Arrange the tube so that the point of attachment on the intestine is directly in the center of the opening at the lower end. Directly under each small hole near the margin of the opening of the tube, stitch a thread to the superficial layer of the intestine and fasten the intestine to the tube by passing the threads through the holes and tying. A line drawn through these two stitches should lie along the longitudinal axis of the intestine. Lower the motility tube into the abdominal cavity, arrange the abdominal wall so that it fits closely around the tube and hold it in place by means of two hemostats placed on opposite sides of the tube. Adjust the position of the motility tube to exert a slight out-

ward pull upon the abdominal wall and connect to the heart lever the first thread that was attached. Introduce mammalian Ringer's solution, warmed to 38°C., into the abdominal cavity by way of the motility tube and place absorbent cotton soaked with the same solution on the abdominal wall around the lower end of the motility tube. Throughout the entire procedure keep the intestine moist and avoid traumata as much as possible. Record the intestinal movements.

Remove the Ringer's solution from the motility tube and introduce in its place several drops of 0.0031 *M* $BaCl_2$ solution warmed to 38°C. Record the movements. A marked increase in peristalsis should occur. Now apply some 0.0031 *M* $CaCl_2$ solution warmed to 38°C. to inhibit the movements and record. Again apply Ringer's solution to wash out the previous solutions and record.

With the same arrangement as above and using a fresh part of the intestine if necessary, apply a few drops of 0.1 per cent physostigmine solution and record the movements. Now apply a few drops of 0.1 per cent atropine solution and record. This neutralizes the effect of the physostigmine. Wash the intestine with Ringer's as before.

Connect the apparatus to a fresh part of the intestine and while taking the record inject into the femoral vein 0.5 cc. of 1:20,000 adrenalin solution. Result?

Repeat these tests using a loop of the ascending colon.

199. Influence of Adrenalin on the Intestine.—From the rabbit used in the preceding experiment (198), cut out a section of the duodenum about 4 in. long. The rabbit may now be killed with ether. Place the piece of duodenum at once in Ringer's solution at about 10°C. and after 10 min. cut off a section from it about 1 in. in length. Put this short piece in cool Ringer's solution in a beaker set over a Bunsen burner without a flame. Over the beaker mount a counterpoised heart lever arranged to record on a smoked kymograph drum. Fasten a piece of thread to one end of the short piece of duodenum and pin the other end of the tissue to the cork disk on a heart holder (see Fig. 16), which should then be immersed in the solution in the beaker. Attach the thread to the heart lever, allow the drum to rotate slowly and record while gradually heating the solution with a low flame. Raise the temperature of the Ringer's solution to about 32°C. After records of the movements are obtained add 0.5 cc. of adrenalin solution (1:10,000) for every 300 cc. of Ringer's solution. Observe and record the effect upon the length and upon the contractions of the piece of intestine (see pages 48 and 801 in the text).

200. Absorption of Water.—Decapitate a turtle that has had no food for at least 4 or 5 days and expose the stomach and intestines. At the cardiac end of the stomach, tie a ligature around the sphincter and above

the ligature make a transverse cut across the esophagus. Also section the duodenum a short distance below the pyloric sphincter. Wash the interior of the stomach out with water and drain. Fill the stomach with water, measuring the quantity used, and tie a ligature around the pyloric sphincter. In the same way fill a section of excised small intestine with a known volume of water. Place the stomach and piece of intestine in a closed vessel, the air of which is saturated with water vapor. At the end of 2 hr., measure the amount of water present in the lumen of the stomach and of the small intestine. Calculate the percentage of water absorbed in each case and compare. Which tissue absorbs the greater amount of water?

201. Absorption and Secretion.—In each of 10 test tubes put approximately 5 cc. of a mixture of 5 parts (by volume) of a thin starch paste and 2 parts of concentrated HNO_3. Swallow a gelatin capsule containing 0.6 gram of potassium iodide. Note the time and wash out the mouth with water. Put some of this wash water in one of the prepared test tubes to see that it contains no potassium iodide. Chew a piece of clean paraffin to promote the flow of saliva. At 2-min. intervals after the capsule is swallowed put some saliva in one of the test tubes and mark the time on the tube. Use a different test tube for each test and rinse the mouth out with water each time. Repeat until a blue color is obtained in a test tube indicating the presence of iodine. How long does it take for the potassium iodide to appear in the saliva? Why does the time vary in different individuals? It also varies in the same individual under different conditions. Explain.

CHAPTER XVI

THE CHEMISTRY OF THE BLOOD AND LYMPH

202. The Structure of Blood.—In a drop of blood freshly drawn from a finger prick, note with the high power of the microscope the red corpuscles (erythrocytes) and the white corpuscles (leucocytes). Sketch them. Can any blood platelets be detected? What physiological function is furthered by their rapid disintegration in shed blood?

203. The Hemolysis of Blood.—Look through a thin layer of defibrinated blood on a glass slide at some printing. Is the blood transparent? To some of the same blood in a test tube add about five volumes of water. Is it now transparent? Examine microscopically some of the diluted blood. In this condition it is called laked or hemolyzed blood. What change has occurred?

Shake thoroughly 2 cc. of blood with 5 cc. of ether in a test tube. Examine a drop of the mixture microscopically. Explain the result.

204. Osmotic Pressure Effects on Blood Corpuscles.—Place a few cubic centimeters of blood in each of three test tubes. Lake the blood in the first tube with water, add an equal volume of isotonic (0.9 per cent) NaCl solution to the second tube and an equal volume of 10 per cent NaCl solution to the third one. Mix thoroughly and after a few moments examine, under the microscope, a drop from each of the three tubes. What are the results and how are they explained from the standpoint of osmotic pressure?

205. Detection of Sugar in Blood Serum.—Place 10 cc. of freshly prepared blood serum in a small evaporating dish, add 5 cc. of water, heat to boiling and acidify slightly with acetic acid. What is the coagulum? Get rid of it by filtration and test a part of the filtrate (reserve the remainder) for sugar by Benedict's test. If the blood serum is fresh, reduction will occur. To what sugar is it due? Why does this sugar disappear within a short time after the blood is removed from the circulation?

206. Detection of Chlorides.—Test the remainder of the filtrate from the preceding experiment with nitric acid and silver nitrate. What do you conclude with regard to the relative abundance of chlorides in blood?

207. Counting of Blood Corpuscles with the Hemacytometer.—The instrument used in blood counting consists of a microscope slide con-

structed of heavy glass and provided with a central counting cell. This cell, with the cover glass in position, is exactly 0.1 mm. deep. The floor of the cell is divided by fine lines into squares each of which is $\frac{1}{400}$ of a square millimeter in area. The volume of blood, therefore, between any particular square and the cover glass above must be $\frac{1}{4000}$ cu. mm. Accompanying each instrument are two capillary pipettes, each constructed with a mixing bulb in its upper portion. The bulb is provided with an enclosed glass bead, which is of assistance in mixing the contents of the chamber. The stem of each pipette is graduated in tenths from the tip to the bulb. The final graduation at the upper end of the bulb is 101 on the pipette used for the erythrocyte count and 11 on the pipette used for the leucocyte count. In making blood counts with the hemacytometer it is necessary to use some diluting fluid. Two satisfactory forms of fluid for this purpose are Toison's and Sherrington's solutions.[1] When either of these solutions is used as the diluting fluid, it is possible to make a satisfactory count of both erythrocytes and leucocytes in the same preparation, since the leucocytes are stained by the methyl or methylene blue.

Procedure.—Thoroughly cleanse the tip of the finger or lobe of the ear of the subject by the use of soap and water, alcohol and ether, applied in the sequence given. Puncture the skin by means of a sterile needle or lance and allow the blood drop to form without pressure. Place the tip of the pipette in contact with the blood drop, being careful to avoid touching the skin, and draw the blood into the pipette up to the point marked 0.5 or 1, according to whether a dilution of 200 or 100, respectively, is desired. Diluting 200 times affords easier and more accurate counting if the blood contains a normal number or an excess of erythrocytes.

Quickly remove excess of blood from the exterior of the tip of the pipette by touching it to absorbent paper and immediately draw up the diluting solution into the pipette exactly to the 101 mark. Now thoroughly mix the blood and diluting fluid within the chamber by tapping the pipette gently against the finger or by shaking it while holding it securely with the thumb at one end and the middle finger at the other. After the two fluids have been completely mixed, the diluting fluid contained in the capillary tube below the mixing bulb should be discarded in order to insure the collection of a drop of the thoroughly mixed blood and solution for examination. Transfer a drop from the pipette to the ruled floor of the counting chamber and place the cover glass firmly in

[1] Toison's solution contains: methyl violet, 0.025 gram; NaCl, 1 gram; Na_2SO_4, 8 grams; glycerol 30 grams; and distilled water, 160 grams.

Sherrington's solution contains: methylene blue, 0.1 gram; NaCl, 1.2 grams; neutral potassium oxalate, 1.2 grams; and distilled water, 300 grams.

position. Some hemacytometers can be filled from the side by capillarity after the cover glass is in position.

Using the high power of the microscope, count the erythrocytes in each of a number of squares, counting the corpuscles that are in contact with the upper and the right-hand boundaries of any square as belonging to that square only. Take the squares in some definite sequence in order that recounting of the same corpuscles may be avoided. The greater the number of squares examined, the more accurate the counting tends to be. One should judge when a satisfactorily accurate count has been obtained, according to the evenness of distribution of cells over the counting area. For the highest accuracy, the counting chamber should be filled a second time and the counting repeated.

To calculate the number of erythrocytes, divide the total count by the number of squares observed in order to obtain the average number per square, multiply this number by 4000 to obtain the number per cubic millimeter of diluted blood and multiply this product by 100 or 200, according to the dilution, to obtain the number of erythrocytes per cubic millimeter of undiluted blood.

Proper cleaning of the diluting pipette is very important. Immediately after using, it should be rinsed many times with water, twice with alcohol and once with ether. Finally, dry air should be drawn through the capillary, and a horse hair inserted to prevent the entrance of dust particles.

Procedure for Leucocyte Count.—If the diluting fluid used with the blood is one of those described above, the leucocytes may be counted in the same preparation used for erythrocytes. For this purpose, however, one observes with the low power of the microscope and counts in the large squares formed by the lines so ruled upon the floor of the counting chamber as to make squares 25 times the area of the small ones. The blue-stained leucocytes are easily detected and may be counted over the entire ruled area of the slide.

Another procedure is to dilute the blood 10 times, in a pipette especially designed for this purpose, using 0.5 per cent acetic acid which hemolyzes the erythrocytes and tends to fix the leucocytes.

To calculate the number of leucocytes per cubic millimeter, multiply the average per large square by 160 and by the dilution factor.

208. The Formation of Hemoglobin from Oxyhemoglobin.—To a little laked blood in a test tube add ammonium ferrotartrate. Make the latter fresh just before using, by adding 5 per cent ammonium hydroxide, drop by drop, to a few cubic centimeters of ferrous tartrate solution[1] until the latter is a pale-green color, and the precipitate, at first

[1] Ferrous tartrate may be prepared by dissolving 2 grams of ferrous sulphate in cold water and adding 3 grams of tartaric acid which has been previously dissolved

formed, is redissolved. Ammonium ferrotartrate should be added to the blood until its bright, arterial-red color is changed to a dark, venous red. The reducing action of the ammonium ferrotartrate has taken oxygen from the oxyhemoglobin and left hemoglobin in solution. Expose it to oxygen of the air by pouring from one tube to another repeatedly until no further change occurs. Explain the result. What methods other than the use of reducing agents may be employed to remove oxygen from oxyhemoglobin?

209. Absorption Spectra of Hemoglobin and Oxyhemoglobin.—Dilute 1 cc. of blood with 80 cc. of water. Observe the absorption spectrum with either a direct-vision or an angular-vision spectroscope, holding the diluted blood in a flat-walled container of 1 cm. inside diameter. What wave lengths of light are absorbed? Describe and sketch the spectrum.

Reduce the oxyhemoglobin with ammonium ferrotartrate as in the preceding experiment and again observe and sketch the absorption spectrum. Reoxygenate the reduced blood. Does the spectrum of oxyhemoglobin return?

210. Detection of Iron in Hemoglobin.—Lake 10 cc. of defibrinated blood with 10 cc. of water and add 60 cc. of 95 per cent alcohol. Filter off the precipitate of blood proteins. Press it dry between filter papers. Burn it in a crucible to a nearly white ash. Dissolve the ash in 10 cc. of water containing a few drops of HNO_3 and test the solution with NH_4SCN. What is the result? To get an approximate idea of the amount of iron present, compare your test with similar ones made upon solutions of $FeCl_3$ containing 0.1, 0.05 and 0.01 per cent of $FeCl_3$.

211. The Benzidine Test.—Dilute defibrinated blood with 100 parts of water. To 3 cc. of this mixture, add 2 cc. of a saturated solution of benzidine in glacial acetic acid. Then add 3 per cent hydrogen peroxide with shaking until no further change occurs. Describe the result. Try the test on blood diluted with 200 parts of water. Does this test detect small amounts of blood? The color reaction is due to the oxidation of benzidine by oxygen liberated catalytically from the peroxide. It is thus not specific for blood but is obtained with pus and some other organic substances.

212. Separation of Serum Albumin and Globulin.—Saturate 10 cc. of blood serum with solid $MgSO_4$. A large amount of the salt will be required. This salts out serum globulin. Filter it off (reserve the filtrate) and show by the biuret test and Millon's test that it is a protein. Dissolve some of it in water. The salt clinging to it will make it soluble. Then add a few drops of this solution to 10 cc. of water. Does the result

in water. Mix and dilute to 100 cc. When this has been treated with ammonia to make ammonium ferrotartrate, it is known as Stokes' reagent.

prove this protein to be a globulin? To the filtrate from serum globulin add a few drops of acetic acid. What is the result? Prove your conclusion by tests.

213. The Hemin Test.—Evaporate a drop of diluted blood on a microscope slide. Add a small crystal of NaCl and crush it into the dried stain. Lay a cover glass over this preparation and then run a drop or two of glacial acetic acid under the cover glass. Gently heat over a low flame until the acid is seen to boil. Keep it warm until the acid has nearly all boiled away. Avoid rapid boiling which would drive the material out from under the cover glass. When the preparation has cooled for a few minutes, examine it under the microscope for the chocolate-colored, rhomboidal prisms of hemin crystals. Sketch them. Write a description of the chemical reactions involved in this test. Repeat it on an extract of a blood stain on cloth. This is a specific test for hemoglobin and certain of its decomposition products and can be used to detect blood.

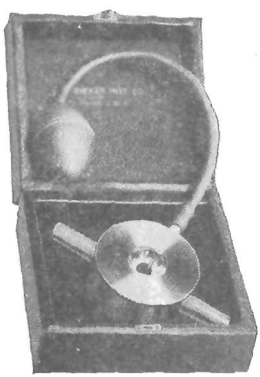


FIG. 26.—Rieker coagulometer. (*Brodie-Russel-Boggs pattern.*)

A modification of this test may be used. To the blood dried on the microscope slide add two drops of Nippe's solution.[1] Put the cover glass in position, heat gently, cool and examine microscopically for hemin crystals. Compare the usefulness of the hemin test and the Nippe modification of it.

214. Measurement of Coagulation Time.—Measure the coagulation time of your blood using the Brodie-Russel-Boggs coagulometer,[2] which is illustrated in Fig. 26. Obtain the blood from the finger or ear by puncture with a sterilized lancet. Before taking the blood, clean the spot to be punctured with alcohol. The following directions, which should be employed in making the measurements, represent passages from directions kindly furnished by the Rieker Instrument Co., and they are presented here with the permission of this company.

The principle of the Brodie-Russel-Boggs method of determining the coagulation time of blood is very simple. A drop of blood placed in a moist chamber can be set in motion by a very light draught of air tangentially directed. When coagulation has occurred, movement ceases. The process is watched under the low power of the microscope.

Coagulation time is defined as follows: It is the period, measured

[1] Nippe's solution contains 0.1 gram each of KCl, KI and KBr in 100 cc. of glacial acetic acid.

[2] Manufactured by the Rieker Instrument Co., 1919–1921 Fairmount Ave., Philadelphia, Pa.

by the coagulometer, that elapses between the exit of the blood from the blood vessel (vein, etc.) and occurrence of coagulation. The time should be counted from the first appearance of the drop to be used, and not from the moment when it is picked off. This beginning point is a constant for all records, which is not the case in any other method of estimation, as the speed of the flow of drops is variable. Coagulation proceeds while the drop is forming; and the added period of skin contact has a marked effect in hastening it. The longer a drop is in contact with the skin surface, the more rapidly it clots when transferred to the instrument. With a freely flowing drop the effect is minimal, and the beginning point is shortened by only a few seconds. Each second of skin contact added shortens the coagulation time by minutes. Cleanliness of the skin and the instruments is of prime importance.

The technique of examination is as follows: The cover with the glass cone is removed, and the flat surface at the apex of the cone cleansed with water, alcohol or ether. The microscope is to have a low-power objective.

The beginning point should be the first appearance of the blood from which a drop is to be taken for observation. The blood is allowed to well out of a deep puncture in the form of a small globule, and the flat surface of the apex of the cone is touched lightly to it. The flat surface of the cone must be touched to the drop and care taken not to smear the edges with it. It is very simple to get a drop the exact size of the cone surface. When the glass is approached close enough, the blood seems to spring up and flow just to the edges of the disk. However, this will not occur upon a dirty cone surface. If the sides of the cone are smeared, or the drop fails to completely cover the cone surface, the latter must be carefully cleaned and dried.

With the drop on the cone, the latter is fitted quickly into the moist chamber. The apparatus is placed under the low power of the microscope and the motion of the blood caused by blowing with the rubber bulb is watched. Very light blowing is all that is required at 30-sec. intervals. Only the periphery of the drop need be watched. Here it is thinnest and clotting begins and ends first. Because of its thinness the process can be better seen and the changes are more definite.

The changes in the movement of the red blood corpuscles set up by blowing, may, for the convenience of description, be divided into four stages:

1. Rotation of the drop as a whole or free flowing of the corpuscles individually.

2. Cohesion of corpuscles and flowing movement in clumps and groups.

3. Marked elasticity, flowing having ceased, a vibratory, elastic, circular motion.

4. Elastic, radial motion and indentation of the edge, of which the first appearance is the end point.

Circular elasticity still persists, but the two are easily distinguishable. When struck by the stream of air, the corpuscles, owing to their radial elasticity, move as a gelatinous mass a little way towards the center of the drop and quickly spring back to their old places. There may be at the same time a small indentation at the point where the corpuscles recede, which immediately fills in on the rebound. The first appearance of this movement marks the end point of the determination. The time is taken at once, the cone of the instrument immediately removed, and an attempt made to pick off a clot from the cone surface with a cloth, a confirmation that coagulation has occurred.

The following table was constructed from a series of 251 records, all taken with a Boggs instrument having a cone surface 4 mm. in diameter. Each record tabulated was confirmed by the presence of a microscopic clot.

TIME OF COAGULATION, MINUTES

Time of day	−3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11+	Total
A.M.; 8–10....	..	1	3	3	4	4	4	7	5	15	46
10–12....	1	5	7	2	6	4	1	3	1	7	37
P.M.; 12– 2....	..	1	3	..	4	1	2	1	..	3	15
2– 4....	2	2	4	6	2	2	3	2	..	4	27
4– 6....	8	15	14	18	12	13	8	6	5	6	105
6+.....	..	1	2	6	2	5	2	..	1	2	21
Total.....	11	25	33	35	30	29	20	19	12	37	251

It is fair to conclude that records below 7 to 8 min. are normal, whereas coagulation periods passing this limit are proportionally delayed.

CHAPTER XVII

THE CIRCULATION OF THE BLOOD

215. The Heart Beat of the Turtle.—Mount a counterpoised heart lever and a time marker below it on a support. Arrange so that they may record on a smoked drum. Decapitate a turtle and saw through the plastron on both sides with a hack saw. With a scalpel cut through the muscles attached to the plastron, so as to free it from the rest of the body, and remove it. Identify the various organs exposed. Note the heart beating within the pericardial sac. With forceps pull the pericardium slightly away from the heart and make an incision across the pericardium with scissors. Note the frenum, which is the connective tissue at the tip of the apex. Find the ventricle with its conus and aortas, the right and left ventricles, the sinus venosus and the venae cavae.

Place a small wooden stand with V-shaped cuts in the legs in an inverted position on the table and rest the turtle ventral side upwards within the V-shaped openings. If a stand is not available tie the turtle to a turtle board. Securely fasten a silk thread to the frenum and cut the frenum at its end farthest from the heart. Attach the thread to the heart lever, start the time marker and record the contractions. The lever should be counterpoised so that the apex of the heart is pulled upward somewhat by the weight of the lever. Use a slow drum and then a fast one.

Tie a piece of thread to a bent S-shaped pin and pass this hook through the tip of the right auricle. Connect this thread to another counterpoised heart lever and record the simultaneous contractions of the auricle and ventricle. The point of one lever should be directly under that of the other. Record on a slow and then on a fast drum. Which chamber contracts first? Note carefully the sequence of the contractions. Which chamber beats with the greater force? What is the rate per minute? (See pages 586 to 588 in the text.)

216. Vagus Inhibition of the Heart.—Leave the apparatus set up as in the preceding experiment (215) but support the levers so that their weight is removed temporarily from the heart. Find the vagus nerve on the right side and on the left side near the base of the neck. Pass a silk thread under each nerve and bring the ends together without tying a knot. The threads will be used later to tie ligatures. Connect

platinum electrodes to the secondary terminals of an inductorium and, in series with the primary circuit, include two dry cells and a signal magnet mounted to record on the drum below the heart-lever points. Adjust the inductorium to deliver moderate tetanizing currents. Stimulate each of the two vagus nerves in turn for a second to make sure that they are the correct ones. Sometimes the left vagus will have no noticeable effect on the rate of heart beat of the turtle. Adjust the heart levers and prepare to take a record. Apply Ringer's solution to the heart and exposed nerves as necessary.

Record the normal contractions of the auricle and ventricle and while doing so apply the electrodes to the right vagus and stimulate for 3 sec. Allow the heart to return to normal and then stimulate for 6 sec. How long is the latent period?

Repeat the above procedures using the left vagus instead of the right. Is there a difference?

Using a fast drum, record the effects of stimulating the right vagus for 2 sec. Record also the return to the normal condition.

Using a very slow drum, stimulate the right vagus until the heart shows escape from the vagus. Also record the recovery. Repeat using the left vagus. How does vagus stimulation affect the rate, amplitude and the *A V* interval?

Place the platinum electrodes on the sinoauricular part of the heart. Record a few normal contractions and then stimulate for a few seconds. Result? Is the effect the same as stimulation of the vagus? (See pages 600–602 in the text.)

217. Action of Nicotine on the Heart.—If the heart used in the preceding experiment (216) is still in good condition, use the same heart, otherwise take a fresh turtle. Use the same arrangement of apparatus, etc., and stimulate the right vagus to be certain that it still will inhibit the heart. With a medicine dropper, apply to the surface of the heart about 10 drops of a 0.2 per cent solution of nicotine. Support the levers to remove their weight from the heart. At the end of 5 min. arrange the levers to record and stimulate the right vagus. The heart should not respond now to the vagus but if it does apply more nicotine and after an interval repeat the test.

Now stimulate the sinoauricular region. The heart should be inhibited. Explain. Nicotine paralyzes the endings at the synapses between the preganglionic and postganglionic nerve fibers.

218. Action of Pilocarpine and of Atropine.—Keep the same arrangement of apparatus and wash the heart used in the preceding experiment (217) well with Ringer's solution to remove the nicotine. Using a slow drum, record several contractions and then apply 10 drops of a 1.0 per cent solution of pilocarpine to the heart with a medicine dropper. Record

at once and for several minutes the contractions of the heart. Note the effect on the rate and tone of the cardiac tissue.

With Ringer's solution wash the heart well to remove the drug. Record several contractions and then apply to the surface of the heart 10 drops of a 0.5 per cent solution of atropine. At the end of 5 min. record the contractions and while doing so stimulate the right vagus nerve. Result? Stimulate the nerve plexus in the region of the sinoauricular groove (at the sinoauricular junction). The heart now fails to be inhibited. Atropine paralyzes the distal terminations of the postganglionic nerve fibers. In this experiment difficulty may be experienced in abolishing the effect of the previous treatment with nicotine by washing, and accordingly it may be necessary to employ a fresh turtle.

FIG. 27. Gaskell clamp. (*Harvard Apparatus Co.*)

219. Partial and Complete Heart Block.—Read in the text pages 592 to 594.

Mount two heart levers on a support so as to record on a smoked kymograph drum. Include a time marker. Below the levers mount a Gaskell clamp with the edges of the jaws in a vertical plane. The accompanying view shows the nature of the Gaskell clamp.

Decapitate a turtle, expose the heart and remove it from the body by cutting the blood vessels at a distance from the heart. Place the heart between the jaws of the Gaskell clamp so that the jaws when brought close together will lie at the *AV* junction. Avoid including the large blood vessels between the jaws. With threads provided with S-shaped pins, connect separately the tip of the right auricle and the apex of the ventricle to the heart levers. Make sure that all apparatus is working properly and then take a tracing of the contractions on a drum fast enough to permit measurement of the *AV* interval. Then slowly compress the *AV* node by means of the Gaskell clamp until a change appears in the beats. Make a tracing and then increase the degree of compression slightly and record. Continue in this way applying varying degrees of heart block but be careful not to cause complete block. Record the recovery from the block that was produced. Explain what is observed.

Now with the Gaskell clamp apply enough compression to bring the ventricle to a complete standstill. Only enough pressure to do this should be used. Is the ventricle in a relaxed or contracted state? Explain the cause of the block.

220. The "All or Nothing" Law.—Using the same heart and arrangement as in the previous experiment (219), disconnect the auricle from its lever and arrange an inductorium to give simple make and break

shocks through the platinum electrodes. Using only break shocks, find the threshold stimulus when the electrodes are applied to the ventricle. Waiting 20 sec. between shocks, to permit recovery, stimulate the ventricle with break shocks of gradually increasing strength above the threshold value. Record the height of contraction for each stimulation using a stationary drum that is moved a little after each contraction. Do the contractions progressively increase in height with increasing intensity of stimulation as in the case of skeletal muscle or is the first response resulting from the threshold stimulus as great as when maximal stimulation is employed? Explain (see page 585 in the text).

221. The Refractory Period of the Heart.—Decapitate a turtle and expose the heart by removing the plastron. Make sure that the heart is beating strongly and then inject a few drops of a 0.5 per cent solution of atropine solution into the pericardial sac to remove control by the vagus. Arrange a heart lever for recording on a smoked kymograph drum, connect a signal magnet, a key and two dry cells in series in the primary circuit of an inductorium and attach platinum electrodes to the secondary terminals. Carefully adjust the point of the signal magnet so that it is exactly under that of the lever. Adjust the inductorium to give shocks of maximal strength.

After an interval of at least 5 min. following the application of the atropine, stimulate the right vagus nerve briefly with faradic stimulation to make sure that it no longer controls the heart. Open up the pericardium and connect the ventricle to the heart lever with the lever counterpoised so that the heart will be raised out of the pericardial sac. Clamp the platinum electrodes so that the points just touch the surface of the ventricle at its side. Adjust the inductorium to give make and break shocks. Start the drum at high speed, close the key of the primary circuit and $\frac{3}{5}$ sec. later open it again. Repeat allowing an interval of $\frac{3}{5}$ sec. between the closing and the opening of the key and then use an interval of $\frac{4}{5}$ sec. Continue in this manner until the heart gives contractions to both shocks in a pair. By reference to the signal line, find what part of the heart beat is covered by the refractory period. Examine the records for the appearance of compensatory pauses and extra systoles. Proceed at once to the next experiment.

222. Effect of a Tetanizing Current.—Employ the same heart and arrangement as in the preceding experiment (221). Adjust the inductorium to deliver a tetanizing current that is just sufficient to produce an extra systole. Record the contractions of the ventricle on a slow drum and stimulate the ventricle for 10 sec. Does the heart show a continuous contraction during stimulation? Repeat using a current of greater intensity. Interpret the records (see page 596 in the text).

223. Reflex Cardiac Inhibition.—Place a large frog under an inverted beaker covering some cotton to which ether has been added. As soon as the animal is under anesthesia put it on its back on a frog board and through the ventral body wall carefully make a hole, with sharp-pointed scissors, just large enough to permit the counting of the heart rate. Determine the rate of beat per minute. Now tap lightly on the ventral part of the abdomen with the flat handle of a scalpel. Continue to tap regularly until the heart slows down. Count the rate of beat. Explain. If you were to section the vagus nerves and then repeat the experiment there would no longer be a reflex cardiac inhibition. Proceed to the next experiment at once.

224. Stimulation of the Vagus and of the Accelerator Nerve Fibers to the Frog's Heart.—Apply water to the skin of the frog used in the preceding experiment (223) and allow it to recover from the effects of the ether. Pith the animal and place it on its back on a frog board. Ask the instructor to show you the position of the right vagus and of the sympathetic nerve to the heart if you do not know how to locate them. Attach the apex of the ventricle to a heart lever arranged to record on smoked paper on a kymograph drum. Connect a signal magnet, two dry cells and a key in series with the primary circuit of an inductorium and attach platinum electrodes to the secondary terminals. Arrange the inductorium to give moderate faradic stimulation. Record the normal beats of the ventricle and while doing so stimulate the right vagus nerve. Repeat but stimulate the accelerator nerve fibers to the heart until the heart shows a marked increase in rate. Compare the duration of the latent periods in the two cases.

225. The Effect of Temperature on the Heart.—Arrange a heart lever and time marker for recording on a smoked paper on a kymograph drum. Below the lever clamp a heart holder (see Fig. 16) to a support so that the cork disk is about 6 in. above the table and obtain a beaker large enough to accommodate the heart holder. Arrange for a stand or support to hold the beaker in position with the heart holder within it. Decapitate a turtle or pith a frog and remove the heart by cutting the large blood vessels several millimeters from the heart. Fasten the heart down to the top of the cork disk on the heart holder by pinning through the large attached blood vessels. Attach a piece of thread to a bent S-shaped pin, pass this hook through the apex of the ventricle and fasten the thread to the heart lever, which should be counterpoised so that the apex of the heart is pulled upwards.

Place some Ringer's solution cooled to 5°C. in the beaker, which should be placed so that the heart will be immersed in the Ringer's solution. Record the contractions and determine the rate of beat. Replace the

solution in the beaker with Ringer's solution at 10°C. and repeat. Continue in this way until the heart is killed increasing the temperature to which the heart is exposed by 5°C. after each recording and measurement of the rate. What is the temperature coefficient for an increase of 10°C.? Is the coefficient the same throughout the temperature range used?

226. Effects of a Direct Current on the Heart.—Pith a frog, expose its heart and open up the pericardial sac. Connect two dry cells in series and bring the end of the wire from the negative pole into the animal's mouth to make a contact. The end of the wire from the positive pole is touched to the surface of the ventricle. Notice that, when the ventricle contracts, the region where the anode touches remains relaxed and flushed. Now remove the wire from the heart and observe that contraction occurs at the localized point, although the rest of the heart may be in diastole. The anode depresses on the make but stimulates on the break. Now reverse the wires so that the cathode is applied to the heart. Repeat the tests. The cathode excites on the make and depresses on the break.

227. The Electrocardiogram.—Read in the text pages 589 to 592 inclusive, in preparation for this work.

The construction of the electrocardiograph will be explained by the instructor. The method of operating the instrument will be demonstrated. Let the subject lie on a couch near the electrocardiograph for at least 5 min., at the end of which time the electrocardiogram will be recorded using leads 1, 2 and 3. For the three leads the connections are made to the string of the galvanometer as follows:

Lead 1: right arm and left arm.

Lead 2: right arm and left leg.

Lead 3: left arm and left leg.

The records will be developed and allowed to dry. Examine the records and identify the *P*, *Q*, *R*, *S* and *T* waves. What was the rate of beat? Were the cardiac cycles evenly spaced? What was the duration of the *P-R* interval?

"Elements of Electrocardiographic Interpretation" by L. N. Katz and V. Johnson will be found to be very useful in connection with the examination of the records.

228. Rate of Heart Beat.—By means of a stethoscope listen to and identify the heart sounds and then count the rate of heart beat per minute under the following conditions:

(*a*) Sitting down.

(*b*) Standing up.

(*c*) Standing up after running up and down the stairs.

229. The Capillary Circulation.—Anesthetize a frog by injecting into the dorsal lymph sac 0.03 cc. of 10 per cent urethane per gram of frog. At the end of an hour place the frog on its back on a sheet of cork having a hole about ½ in. in diameter at one end. Place the web of one hind foot over the hole and by means of pins fasten it in a stretched position, being careful not to stretch too much. Place this foot of the animal under low power of the microscope, keeping the frog moist with a wet cloth. Observe the blood vessels of different sizes and note that the velocity of flow is different in the various vessels. In which is the velocity the greatest? Focus on a capillary and notice how the red cells move through it. Is the flow steady or intermittent? What causes the variations in velocity? Repeat the observations, using high power. Temporarily cut off the circulation to the foot by means of a ligature. Now what is the appearance of the blood vessels? Remove the ligature and watch the return of the circulation. Apply to the web the point of a pin that has been dipped in strong acetic acid. Observe this spot under the microscope. Do the white corpuscles collect there? What is the nature of the flow of blood there in the vessels?

Expose the intestine and pull out a loop of it. Arrange the intestine on a glass slide over the hole in the cork sheet so that the mesentery is stretched out over the glass. Keep the mesentery moist with Ringer's solution. Cover the mesentery with a cover glass and observe the capillaries under low and high powers.

Place the frog on its ventral side and put its head close to the hole in the cork sheet. Stretch the tongue over the hole and pin it in place. Examine the capillaries in the tongue under low and high powers. How does the flow in the larger vessels compare with that in the smaller ones? Apply a drop of 0.1 per cent adrenalin to the frog's tongue, using a fine medicine dropper, and note the effect.

230. Arterial Pulse in Man.—Apply a Dudgeon sphygmograph to the wrist so that the spring will rest over the radial artery. The clockwork mechanism should be directed towards the elbow. Adjust the knob at the side of the instrument so that the writing lever shows proper movement. Take a smoked sheet of kymograph paper and cut it transversely into several strips of proper width to fit the sphygmograph. Take a tracing on a strip of the smoked paper and repeat two or three times. Run up and down stairs and take additional tracings. Compare (see pages 597 to 599 in the text).

231. Inhibition of the Vagus Impulses.—Sit down after obtaining a drinking glass full of water. Have your partner count your heart rate by feeling the pulse at the wrist. Now sip the water a little at a time. After half of the water has been sipped have your partner count your

heart rate again. Has the swallowing had any effect on the heart rate? Explain.

232. Blood Pressure in Man.—Read in the text pages 580 to 584, inclusive.

Take the sphygmomanometer and stethoscope to a quiet room and sit, completely relaxed, with the bare left arm resting on a table or arm of a chair at about the level of the heart. Have your partner place the rubber bag in the sleeve of the sphygmomanometer over the brachial artery above the elbow and wrap the rest of the sleeve around the arm tucking the end into a fold. The pressure gauge is then connected to the upper rubber tube leading from the rubber bag in the sleeve, and the rubber inflating bulb is connected to the lower rubber tube. Support the pressure gauge by hooking it onto a fold in the sleeve. Make sure that the arm is completely relaxed and at rest. Your partner should then put the bell of the stethoscope on the inner surface of the arm over the spot where the radial and ulnar arteries join below the bend of the elbow. The rubber bag is now inflated to about 140 mm. pressure or to a value above this at which no sound is heard in the stethoscope. Your partner listens in the stethoscope and gradually releases the pressure by means of the release valve at the rubber bulb. A point will be reached at which a sound will be heard for each heart beat. This signifies that blood is flowing through the artery again. Now increase the pressure again and find the highest pressure at which a sound can be heard. This gives the systolic pressure.

To get the diastolic pressure, the pressure must be reduced gradually. As the pressure in the bag drops the sound grows louder but suddenly is reduced and alters. Take the pressure reading at the point where the loud tone changes to a much lower one. This gives the diastolic pressure. Measurements of the systolic and diastolic pressures should be made several times and the average values found.

Lie down for 10 min. and at the end of this time have your blood pressure measured while in this position. Compare with the previous measurements.

Run up and down stairs and again have your blood pressure measured. Find out how long it takes for the blood pressure to return to the level at rest.

233. Plethysmography.—For a diagram of the apparatus used see the text on page 603 (Fig. 172).

Connect the arm plethysmograph by means of a piece of heavy-walled rubber tubing to a sensitive tambour with a cellophane point. Arrange for the tambour to record on a smoked paper on a kymograph drum. A short piece of rubber tubing should be connected to each of the side tubes leading from the plethysmograph and two Hofmann clamps

should be available for the purpose of clamping these pieces of rubber tubing later on. Bare the arm to the elbow and apply some talcum powder to the arm. Insert the arm through the rubber cuff. The tips of the fingers should be able to touch the end of the chamber when extended. Have your partner clamp shut the two pieces of rubber tubing attached to the side tubes. Allow the arm to be relaxed inside the plethysmograph, which should rest on the table. Record the waves due to the volume changes of the arm and examine the records for changes due to the heart beats, respirations and vasomotor reactions. While the record is being taken, multiply mentally two numbers mentioned by your partner, for example 46 by 87, but do not watch the record while doing so. Is there any change in the appearance of the record?

234. Working Model of the Circulatory System.—The circulation scheme furnished by the Harvard Apparatus Co. may be used to demonstrate some of the outstanding phenomena exhibited by the vertebrate circulatory system. Examine the apparatus, noting the elastic tubes and the piece of bamboo that acts as a peripheral resistance. One-way valves permit the flow of the fluid in one direction only. The pump acts as the heart (left ventricle) and the tubes between the pump and bamboo represent the arteries. The veins are represented by the tubes on the side of the resistance that is opposite to the arteries. The rim of the eccentric of the pump is built to reproduce the intraventricular pressure curve in the dog. Mercury manometers are provided on the arterial and venous sides to show the changes in pressure accompanying the heart beat.

Open the side branch shunting the bamboo resistance and operate the pump at the rate of 20 r.p.m. Note the pressure on the arterial side and on the venous side. Is the outflow on the venous side continuous and uniform? Increase the rate to 60 r.p.m. and note any changes.

Close the side branch shunting the bamboo resistance (peripheral resistance) and operate the pump at the rate of 60 r.p.m. Note the arterial and venous pressures and the outflow and feel the elastic tubes representing the arteries. Compare with the circulatory system.

CHAPTER XVIII

RESPIRATION

235. Respiratory Movements.—Prepare a kymograph with smoked paper on the drum and mount a Marey tambour on a support so that it may record on the paper. Figure 28 below shows a view of a tambour that is satisfactory for this purpose. Sit close to the kymograph but facing away from it so that you will not see the record as it is being taken. Have your partner place a pneumograph around your chest and fasten it. The pneumograph should be connected to the tambour by means of a piece of rubber tubing having a T-tube in the line. See that a short piece of rubber tubing is connected to the free branch of the T-tube and that a Hofmann clamp is available to clamp it when ready. The tambour should be provided with a cellophane point and adjusted to bear lightly on the paper. Add a time marker and adjust the drum to rotate at a moderate speed.

FIG. 28.—Marey tambour. (*Harvard Apparatus Co.*)

Your partner may now apply the Hofmann clamp to the rubber tubing on the free branch of the T-tube and when this is done recording of the respiratory movements will commence. Record a series of 15 to 20 normal respirations. Examine the record and label. Which stroke signifies inspiration and which expiration? Does a pause occur at any time in the rhythm?

236. Influence of Forced Respiration.—With the same arrangement as before (Exp. 235), record a few normal respirations and then hold your breath as long as possible recording meanwhile. Also record the recovery after this. When the effect has passed away, breathe very rapidly and quickly (forced respiration) as long as you find it easy. Now again hold your breath until forced to breath again. Examine and label. Explain the difference noted.

237. Influence of Restricted Supply of Air.—With the same arrangement as before (Exp. 235), record a few normal respirations and then breathe through a pipette with a narrow opening so as to obtain a restricted air supply. How does this modify the record? Explain. Label the record.

238. Modified Respiration.—With the same arrangement as before (Exp. 235), take records of a sigh, a yawn, a sneeze, a cough, of laughing and of reading aloud. Allow the drum to rotate at a faster speed than in the preceding experiments and have each record preceded by a record of a few normal respirations.

239. Effect of Exercise.—Make a record of a few normal respirations using the same arrangement as before. Open the side branch on the

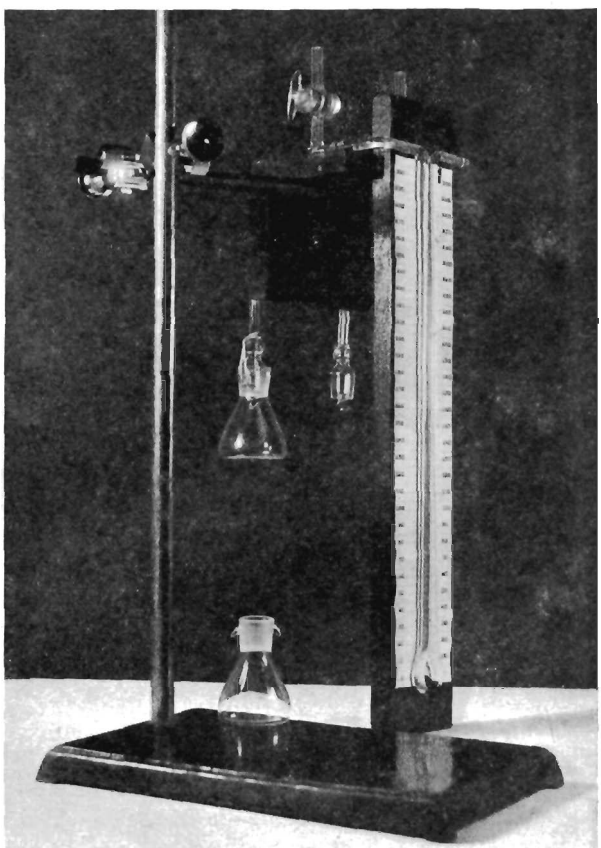


FIG. 29.—Differential manometer.

T-tube and disconnect the rubber tubing from the tambour. Run up and down stairs and immediately make a record of the respirations. What is the underlying cause of the differences in the records?

240. The Gaseous Exchange of a Small Animal.—A Krogh differential manometer, a constant-temperature water bath and a small animal such as a pupa will be furnished. Special directions concerning the use

of the manometer will be given. Measure the rate of oxygen consumption over a period of an hour taking readings every 15 min. Then measure the rate of carbon dioxide production for the same length of time. Again measure the rate of oxygen consumption. From the value of the manometer constant, which will be furnished, and the manometer readings, calculate the rate of oxygen consumption per gram of animal per hour. Also calculate the rate of carbon dioxide production, employing the same units. Find the value of the respiratory quotient (see pages 674 to 675 in the text). What is the significance of the R. Q.?

CHAPTER XIX

PHYSIOLOGICAL OXIDATIONS

241. Oxidase System of Potato.—Pare a potato, cut off a thin slice and place it on a clean glass surface. Examine the piece of potato at intervals during the laboratory period and note the color changes. A brown color will appear due to the oxidation of paroxyphenyl substances such as tyrosine in the cells and juice of the potato (see page 653 in the text). Two oxidases, tyrosinase and laccase, are capable of causing these oxidations.

Scrape a potato with the edge of a piece of glass, extract the scrapings with water, strain through cheesecloth and then filter. Place 5 cc. of the filtered extract in each of five test tubes and add the following to establish a series:

(*a*) 5 drops of ether (control).

(*b*) 5 drops of 1.0 per cent phenol solution.

(*c*) 5 drops of 1.0 per cent phenol solution after bringing the extract to a boil and cooling.

(*d*) 10 drops of guaiac solution.[1]

(*e*) 10 drops of alpha-naphthol solution.[2]

Shake each tube well and examine at intervals. Keep the solutions until the next laboratory period and examine for color effects. In tube (*d*) the color is due to the oxidation of guaiaconic acid to guaiac blue. In tube (*e*) the color depends upon the oxidation of alpha-naphthol to dinaphthol. It is believed that in the living cell oxidase may favor the utilization of oxygen in a manner comparable to the way in which potato oxidase promotes the oxidation of chromogens in the tests described above.

242. Oxidase System of Muscle.—Place some frog muscle on a clean glass plate and macerate it into small pieces with the edges of two glass microscope slides. Into each of three test tubes put a small quantity of the chopped muscle. To one tube add 4 or 5 drops of gum guaiac in alcohol, to another tube add guaiac as before and, in addition, 4 or 5 drops of 1.0 per cent hydrogen peroxide and to the third tube add guaiac as before and, in addition, 4 or 5 drops of old turpentine. The guaiac gives a blue color when active oxygen is present.

[1] The guaiac solution is prepared by dissolving 0.5 gram of guaiac resin in 30 cc. of 95 per cent alcohol.

[2] The alpha-naphthol solution is a 5 per cent alcoholic solution.

Place some frog muscle in a small amount of water and bring to a boil. Macerate the tissue as before and repeat the tests. Results? (Read pages 648 to 651, inclusive, in the text.)

243. Action of a Hydrogen Acceptor.—Place 5 cc. of distilled water into each of four test tubes and to each add one drop of methylene-blue solution to give a light blue color. To one tube add some freshly chopped muscle taken from a frog that has just been killed. To another tube add an equal amount of the chopped muscle that has been boiled in a few drops of water. To the third tube add the same amount of chopped muscle that has been washed in several fresh portions of distilled water. The fourth tube serves as a color control. After several minutes compare the colors of the various solutions with that of the control.

The loss of the blue color by the methylene blue indicates that it has been reduced by acting as an hydrogen acceptor. The loss of hydrogen from a substance is a form of oxidation.

244. Action of Catalase.—Animal and plant tissues contain an enzyme, catalase, which has the ability to decompose hydrogen peroxide with the evolution of molecular oxygen.

Fill a 500-cc. graduate cylinder completely with water, cover it with the palm of the hand and invert it, placing the mouth of the cylinder in water in a large open vessel. Clamp the cylinder in a vertical position with its mouth about an inch from the bottom of the vessel containing water. Fit a low broad-mouthed bottle with a stopper and delivery tube leading to the mouth of the graduate cylinder. Kill a frog, remove the liver and grind it up with a mortar and pestle using clean sand. Put the macerated tissue in a small piece of filter paper to form a bag. Place 20 cc. of neutral 2 per cent hydrogen peroxide in the bottle, drop the bag with tissue into the bottle and apply the stopper immediately. In the cylinder, collect the oxygen that is liberated. Note the amount of oxygen that is liberated, making sure to shake the bottle occasionally.

Repeat, using the same amount of leg muscle from the frog. Is there any difference in the rate or degree of liberation of oxygen? It has been suggested that catalase protects the tissues from the toxic action of hydrogen peroxide by decomposing it.

CHAPTER XX

EXCRETION AND PROTEIN METABOLISM

URINE

245. Specific Gravity.—With urine specific-gravity spindles (urinometers), test as many samples of urine as are available. Note that the reading of the spindle, which is graduated at 15°C., must be corrected for temperature. For each 3° that the urine temperature is lower than 15, one in the third place of decimals must be subtracted from the reading. For every 3° that the urine temperature exceeds 15, one in the third place of decimals must be added. What is the normal range of urine specific gravities? What are extreme variations in health? What pathological significance may high or low values have?

246. General Characteristics.—Note color, reaction to litmus and appearance (clear, cloudy or sedimented) of available samples of urine. What causes the normal color and its variations? What may cause abnormal colors? Cloudiness or sediment is caused in normal urines by: (1) mucous material, recognized by microscopic detection of epithelial cells from the urinary passages and by the very slight and nebulous appearance of the sediment; (2) phosphates that separate from alkaline urine, increase in amount visible when the urine is heated but disappear when the urine is acidified with acetic acid; (3) ureates that form in acid urines, tend to dissolve when heated and reappear when acidified with acetic acid. What sediments can you detect in the specimens of normal urine?

247. Urea. *a. Solubility.*—With small amounts of pure urea crystals, try solubility in water, alcohol and ether (save the water solution for the next tests).

b. Crystals.—Place a drop of urea solution in water on a slide, let it slowly evaporate and examine and sketch the microscopic crystals.

c. Urea Nitrate.—Treat a drop of urea solution on a slide with a drop of concentrated HNO_3. Evaporate. Examine and sketch the microscopic crystals.

d. Sodium Hypobromite Reaction.—Add a drop of bromine to 3 cc. of 5 per cent NaOH. Warm gently to form sodium hypobromite (NaOBr) and then add a crystal of pure urea. Note the evolution of N_2. Write the reaction involved.

Similarly, prepare enough NaOBr to fill a ureometer, introduce a measured quantity of urine by means of the measuring device accompany-

ing the apparatus and, after the evolution of N_2 is finished estimate the concentration of urea in the urine.

e. The Urease Reaction.—To 5 cc. of 2 per cent urea solution in a test tube, add 1 cc. of urease solution prepared from the soy bean. After 10 or 15 min., heat to boiling and test the vapor for NH_3 by red litmus paper. Note the odor of NH_3. Explain this reaction. Practical use is made of it in the quantitative determination of urea.

248. Uric Acid. *a. Preparation from Urine.*—Acidify 200 cc. of filtered urine with 5 cc. of 25 per cent HCl and keep the mixture at room temperature 24 hr. or more for the formation of uric acid crystals. Examine them microscopically and sketch. Their red color is due to urine pigments; pure uric acid is colorless.

b. Solubility.—Try the solubility of pure uric acid in water, alcohol, dilute H_2SO_4, pure concentrated H_2SO_4 and dilute NaOH. The effect of NaOH is due to the formation of soluble ureates.

c. Crystallization of Pure Uric Acid.—To the solution in NaOH, add enough HCl to acidify. Let it stand until crystals form. Examine them microscopically and sketch. Under what conditions can uric acid crystals form in the urinary passages and kidney tubules? Discuss the relation of uric acid crystallization to gout and arthritis.

249. Quantitative Determination of Uric Acid.—The principle of the method (Benedict and Franke) is to measure the intensity of the blue color formed by the action of arsenophosphotungstic acid upon uric acid in the presence of NaCN. The intensity of the blue color is proportional to the amount of uric acid present and can be measured in a colorimeter by comparison with a standard solution of pure uric acid in which the same blue color has been developed by the same reagents.

In order to calculate the daily output of uric acid, make the determination on a 24-hr collection of urine. A small amount of urine is used for the actual analysis because the color reaction is an intense one. The urine is so diluted that 10 cc. will contain 0.15 to 0.30 mg. of uric acid. This is attained in most urines by diluting twenty times. Thus if the urine has a specific gravity within the normal range, transfer 5 cc. with an accurate pipette to a 100-cc. volumetric flask and make up to volume. Put 10 cc. of this dilution (equivalent to 0.5 cc. of urine) into a 50-cc. volumetric flask, add 5 cc. of the sodium cyanide reagent,[1] mix and then add 1 cc. of Benedict's uric acid reagent.[2] Again mix the contents of the flask.

[1] This reagent is a 5 per cent solution of NaCN to which has been added 2 cc. of NH_4OH (conc.) per liter. It should be added from a burette to minimize the danger of poisoning.

[2] This reagent is prepared as follows: Put 100 grams of pure sodium tungstate in a liter pyrex flask and dissolve in about 600 cc. of water. Add 50 grams of pure arsenic acid (As_2O_5) and 25 cc. of 85 per cent HCl. Boil the mixture for about 20 min., cool, and make up the volume of 1 l.

Similarly treat 10 cc. of a standard uric acid solution,[1] containing 0.2 mg. of uric acid, in a 50-cc. volumetric flask, with the cyanide and the Benedict reagents. After both flasks have stood 5 min. for development of the color reaction, dilute to 50 cc. and compare in a colorimeter. Use 15 mm. depth of the standard and match the urine-containing solution to it. The depth of the urine preparation at the matching point can be read from the colorimeter scale and will be inversely proportional to the amounts of uric acid. Match the unknown five or six times and use the average result in computation. Compute according to the proportionality:

$$15 \text{ (depth of standard)} : y \text{ (depth of urine solution)} = x : 0.2$$

where x is the milligrams of uric acid in the amount of urine actually used (usually 0.5 cc.) for analysis. Calculate the uric acid for the 24-hr. collection.

250. Quantitative Determination of Creatinine.—An important reaction of creatinine is that with picric acid in alkaline solution, giving an orange color of intensity proportional to the amount of creatinine. To 10 cc. of urine in a 500-cc. volumetric flask, add 15 cc. of saturated picric acid solution and 5 cc. of 10 per cent NaOH. Similarly, treat 10 cc. of a standard pure creatinine solution containing 10 mg. of creatinine.[2] After mixing and waiting 5 min. for development of the color reaction, dilute both mixtures to 500 cc. and compare them in a colorimeter. Use 8 mm. as the depth of the standard and match the unknown to it repeatedly until satisfactorily reproducible results are obtained.

[1] For this reagent, a stock solution is prepared as follows: Dissolve 9 grams of Na_2HPO_4 and one gram NaH_2PO_4 (pure crystalline salts) in about 250 cc. of hot water. Filter if the solution is not perfectly clear. Dilute the clear solution to 500 cc. with hot water and pour it upon exactly 200 mg. of pure uric acid suspended in a few cubic centimeters of water in a liter volumetric flask. Mix until the uric acid is completely dissolved. Cool, add exactly 1.4 cc. of glacial acetic acid, dilute to 1 l. and mix. Add 5 cc. of chloroform to prevent the growth of bacteria and molds. This stock solution, which contains uric acid largely in the form of its monosodium salt, is so buffered by the phosphates as to resist oxidative destruction of the uric acid.

The working standard for use in the determination of uric acid in urine is prepared fresh at intervals of about 10 days by treating 50 cc. of the stock solution in a 500-cc. volumetric flask with 350 cc. of water and 25 cc. of 3.6 per cent HCl and making up the volume to 500 cc.

[2] Instead of a solution of pure creatinine, treated similarly to the urine, 0.5 N potassium bichromate may be used as the standard for comparison because it has almost exactly the same quality and intensity of color as that furnished by 10 mg. of creatinine in the form of the alkaline picrate diluted to 500 cc. But if potassium bichromate is used, set its depth in the standard cup of the colorimeter at 8 mm. and compute as though it were 8.1 mm. in depth and contained 10 mg. of creatinine.

The standard creatinine solution is prepared by dissolving 1 gram of pure creatinine (or 1.61 grams of creatinine zinc chloride) in a liter of 0.1 N HCl.

Computation is made by applying the same principle used in computing uric acid. Calculate the creatinine for the 24-hr. collection of urine.

251. Test for Indican.—Of the several ethereal sulphates in urine, indican is most readily detected because it is easily oxidized to indigo blue. To about 20 cc. of urine in a test tube, add an equal volume of Obermayer's reagent (HCl containing a little $FeCl_3$). Shake thoroughly. Add 5 cc. of chloroform, shake again and let the chloroform settle. As it dissolves indigo, it will be colored blue if the urine contained indican. Compare the formulas for indican and indigo blue. What does the presence of indican and other ethereal sulphates in urine show with regard to conditions in the intestine? The intensity of the blue color obtained in this test is a rough index of the concentration of indican in the urine. Occasionally, with a low concentration of indican, a reddish or purple color is obtained.

CHAPTER XXI

DIETETICS. VITAMINS

252. To Demonstrate the Need of Vitamin B.—For this experiment pigeons should be selected so that they will be of about the same age and weight. Place the pigeons in suitable cages with labels and keep the controls separate from the others. Feed one group of animals on polished rice and water but give the other group (the controls) whole (brown) rice and water. Use newspaper on the bottom of the cages. Make a record of each bird's weight taken at weekly intervals. Note the appearance and behavior of the animals as the experiment proceeds. What are the symptoms of polyneuritis? After the symptoms have become apparent, add brown rice to the diet of those that had been fed polished rice. What is the result? (See the text on pages 552 to 553.)

253. To Demonstrate the Need of Vitamin C.—In this experiment use guinea pigs of about the same age and weight and, preferably, of the same litter. Why are guinea pigs used for this experiment? Keep the animals in suitable cages so that the control group is separate from the other animals. Feed hay, oats and water *ad lib.* to all of them. Give the control animals, in addition, plenty of fresh raw carrots. Weigh each animal at weekly intervals and note the appearance and behavior of all of them. After two weeks, signs of scurvy should be looked for daily. When the animals show signs of scurvy, characterized by lying on the side, squealing when their joints are pressed and marked loss of weight, add carrots to the diet. Note whether or not the signs of scurvy disappear and the weight increases.

254. The Bio-assay of Vitamin D.—The principle of the method is to compare the degree of healing of rickets produced in control rats by feeding standard cod-liver oil with that produced in experimental rats by feeding the substance to be assayed. A group of rats are fed a ricketogenic diet until their bones are suitably depleted of Ca.

Rats for depletion are satisfactory if, at 30 days of age, their weights exceed 44 grams and do not exceed 60 grams. Throughout the depletion, rats are fed one of the following ricketogenic diets and distilled water *ad lib.:*

RICKETOGENIC DIET NO. 1 (MCCOLLUM)

	Per Cent		Per Cent
Whole yellow maize, ground	33	Gelatin	15
Whole wheat, ground	33	Calcium carbonate ($CaCO_3$)	3
Ground gluten	15	Sodium chloride (NaCl)	1

RICKETOGENIC DIET NO. 2 (STEENBOCK)

	Per Cent
Whole yellow maize, ground	76
Ground gluten	20
Calcium carbonate ($CaCO_3$)	3
Sodium chloride (NaCl)	1

Depletion is considered satisfactory for the assay period if it takes more than 18 days and not more than 25 days to produce evidence of rickets. This may be established by the line test tried upon the bones of a few (three or four) of the rats, or by the X ray.

After rickets is produced, each rat continues to receive the depletion diet and the distilled water *ad lib.* The negative controls receive this diet only. The reference control group receives, in addition to depletion diet and water *ad lib.* for 8 days, a daily supplement of reference oil sufficient to produce a 2 plus degree of healing. The reference oil commonly used is a standard cod-liver oil that has been compared with the International vitamin D_2 standard. A total dose of 35 mg. of this oil per rat will usually produce the desired result. After 8 days on this supplement, the rats are kept 2 days more on the depletion diet and water only. The assay groups receive, in addition to depletion diet and water *ad lib.* for 8 days, a fixed daily quantity of the substance to be tested. But during the succeeding 2 days they receive the depletion diet only. In brief, the assay period is 10 days in duration: 8 days on basal diet plus supplement, 2 days on basal only. The last 2 days afford time for the vitamin to complete its effect.

The number of rats used in each group (negative controls, reference controls and experimental assay group) will vary in different circumstances. If the rats of the entire colony show no tendency to rickets resistance, a small number (two–four) may be sufficient for negative controls. The reference control group is made up of 10 rats in some laboratories, and this is a satisfactory number if the rats respond to cod-liver oil with fair uniformity. The numbers required for the experimental assay group may hardly be less than 10, and, if the material to be assayed is to be fed at several different levels, a corresponding number of groups of rats are required. Because of the variability of the rat in succumbing to rickets and in healing, the larger the number employed for any test, the more reliable is the assay. The inaccuracy due to variability may be reduced by placing in the reference control group one or two rats from each litter used. This procedure is regarded in some laboratories as indispensable to a satisfactory assay.

The Line Test.—Proof of healing rickets is established by two criteria. The rats during the 10-day assay period should not decline in weight. The bones, treated as follows, should show a deposit of calcium.

The proximal end of a tibia, or the distal end of a radius or ulna, is used. The end is first dissected out and cleaned of adhering tissue. The bone is then split with a sharp knife to expose a plane surface through the junction of epiphysis and diaphysis. Both sections are rinsed in distilled water and immersed for 1 min. in a 2 per cent solution of silver nitrate. The bones are then rinsed again in distilled water and exposed to bright light until the calcified regions have darkened. Photomicrographs may be used to provide records of the degree of healing (see page 762 of text for diagram showing degrees of healing).

Calculation of Results.—During the period of test, the negative controls must show failure to heal on the depletion diet and water. An assay group must be found whose line test matches that of a fixed dosage of U. S. P. Reference Oil. If this oil contains 95 U. S. P. or International units per gram, calculation may be made as follows: Let R in milligrams = amount of reference oil producing a definite degree of healing. Let A in milligrams = amount of assay substance producing same degree of healing as R. Then $R/A \times 95$ = U. S. P. or International units vitamin D per gram.

Bills gives the following approximate equivalence table:

D CONTENT OF AVERAGE COD-LIVER OIL IN TERMS OF VARIOUS SYSTEMS OF UNITS

International, or League of Nations 1931 system	100 units per gram
U. S. Pharmacopeia 1934 system	100 units per gram
British, or Medical Research Council, 1930 system	100 units per cc.
Steenbock system, 1930	37 units per gram
American Drug Manufacturers' Assn. or Holmes' system, 1931	350 units per gram
Oslo (old) Poulsson and Lovenskiold 1928 system	110 units per gram
Oslo (new) Poulsson and Ender 1933 system	160 units per gram
German system of rat units 1929, 1930, 1931	15 units per cc.
Clinical units (1 clinical unit = 100 rat units)	0.15 unit per cc.
American Medical Association 1931 system	2.8 D potency

APPENDIX

BUFFER SOLUTIONS

Phosphate Buffers, Sorensen, pH 4.49 to 9.18.—Mix 0.066 *M* $Na_2HPO_4 \cdot 2H_2O$ (11.876 grams per liter) solution with 0.066 *M* KH_2PO_4 (9.078 grams per liter) solution as indicated below. The $Na_2HPO_4 \cdot 2H_2O$ is prepared by exposing $Na_2HPO_4 \cdot 12H_2O$ on a porcelain plate to the air for 2 weeks. Use water free from CO_2 in making the solutions.

pH	$Na_2HPO_4 \cdot 2H_2O$ solution, cc.	KH_2PO_4 solution, cc.
4.49	0	10.0
4.94	0.1	9.9
5.29	0.25	9.75
5.59	0.5	9.5
5.91	1.0	9.0
6.24	2.0	8.0
6.47	3.0	7.0
6.64	4.0	6.0
6.81	5.0	5.0
6.98	6.0	4.0
7.17	7.0	3.0
7.38	8.0	2.0
7.73	9.0	1.0
8.04	9.5	0.5
8.34	9.75	0.25
8.68	9.9	0.1
9.18	10.0	0

Phthalate Buffers, pH 2.2 to 3.9.—Mix 0.2 *M* potassium acid phthalate (40.828 grams per liter) solution with 0.2 *N* HCl solution as indicated below and then make up to exactly 200 cc. with distilled water.

pH	Potassium acid phthalate solution, cc.	0.2 *N* HCl, cc.
2.2	50	46.7
2.3	50	42.5
2.4	50	39.6
2.5	50	37.0
2.6	50	32.9
2.7	50	29.6
2.8	50	26.4
2.9	50	22.8
3.0	50	20.3
3.1	50	17.7
3.2	50	14.7
3.3	50	11.8
3.4	50	9.9
3.5	50	7.5
3.6	50	6.0
3.7	50	4.3
3.8	50	2.6
3.9	50	1.0

Phthalate Buffers, pH 4.0 to 6.3.—Mix 0.2 *M* potassium acid phthalate (40.828 grams per liter) solution with 0.2 *N* NaOH solution as indicated below and then make up to exactly 200 cc. with distilled water.

pH	Potassium acid phthalate solution, cc.	0.2 *N* NaOH solution, cc.
4.0	50	0.4
4.1	50	2.2
4.2	50	3.7
4.3	50	5.2
4.4	50	7.5
4.5	50	9.6
4.6	50	12.1
4.7	50	14.6
4.8	50	17.7
4.9	50	20.9
5.0	50	23.8
5.1	50	27.2
5.2	50	29.9
5.3	50	32.5
5.4	50	35.4
5.5	50	37.7
5.6	50	39.8
5.7	50	41.9
5.8	50	43.0
5.9	50	44.5
6.0	50	45.4
6.1	50	46.2
6.2	50	47.0
6.3	50	48.1

DOSES, SOLUTIONS, ETC.

Cocaine Hydrochloride.—Frog, local anesthesia of skin: apply a 1.0 per cent solution.

Curare.—Frog: inject 1.0 cc. of 0.5 per cent solution into the lymph sac.

Dilution of Aqueous Solutions.—Measure out the same number of cubic centimeters of stock solution that corresponds to the concentration in percentage that is desired. Add water to bring the volume of the mixture in cubic centimeters up to the same number as the percentage concentration of the stock solution, *e.g.*, to dilute 95 per cent alcohol to 70 per cent, take 70 cc. of 95 per cent alcohol and add 25 cc. of distilled water. This will give 95 cc. of 70 per cent alcohol.

Epinephrine.—Rabbit, to inhibit intestinal peristalsis: inject into vein 0.01 mg. per kilogram weight of rabbit.

Fixative for Kymograph Records.—Dissolve 100 grams of resin in 95 per cent alcohol, bringing the volume up to 1000 cc. finally. White shellac, diluted to five times its volume with wood or ethyl alcohol, is also satisfactory.

Length Units.—1.0 in. equals 2.540 cm.
1.0 ft. equals 30.48 cm.
1.0 μ equals 0.001 mm.
1 millionth micron equals 10^{-10} cm.
1 Ångström unit equals 10^{-8} cm.

Molar Solutions.—A molar solution contains 1.0 gram molecular weight of dissolved substance per liter of solution. To make a 0.1 *M* solution from a 1.0 *M* solution, take 1.0 cc. of the 1.0 *M* solution and add to it 9.0 cc. of distilled water.

Normal Solutions.—A normal solution contains 1.0 gram molecular weight of the dissolved substance divided by the hydrogen equivalent of the substance per liter of solution. To make a 0.01 *N* solution from a 1.0 *N* solution, take 1.0 cc. of the 1.0 *N* solution and add to it 99.0 cc. of distilled water.

Oxalate, Potassium.—Anticoagulant: add about 1.5 mg. of the oxalate per 1.0 cc. of shed blood.

Physiological Saline.—Dissolve 9.0 grams of NaCl in distilled water, bringing the volume up to 1000 cc.

Ringer's Solution.—Frog or turtle: keep on hand 0.7 per cent NaCl, 1.0 per cent $CaCl_2$ and 1.0 per cent KCl solutions. As the Ringer's solution is needed mix these solutions as indicated below:

100 cc. of 0.7 per cent NaCl
1.0 cc. of 1.0 per cent $CaCl_2$
0.75 cc. of 1.0 per cent KCl

Ringer-Locke's Solution.—Mammals: dissolve the following constituents in distilled water as indicated below:

NaCl	9.5	grams
$CaCl_2$	0.1	gram
KCl	0.075	gram
$NaHCO_3$	0.15	gram
Glucose	1.0	gram
Water	1000.0	cc.