A LABORATORY MANUAL IN GENERAL PHYSIOLOGY

A THESIS

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THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

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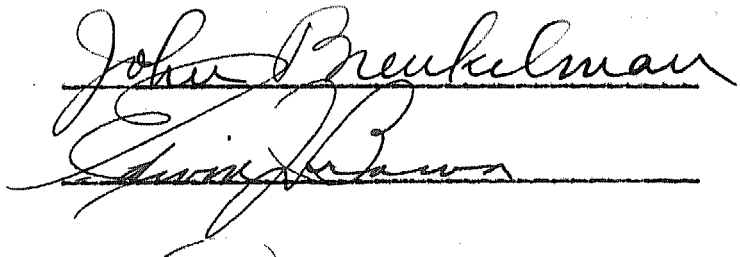

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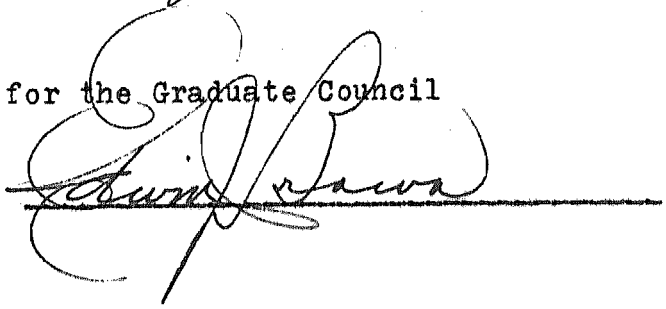

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PREFACE

Laboratory work has come to have a very important place in the teaching of any branch of science. The value of laboratory work in physics and chemistry has long been recognized and no one would attempt the teaching of either of these subjects without ample provision having first been made to secure materials and apparatus necessary for laboratory experiments to illustrate and put into practice the fundamental facts and principles emphasized in class-room procedure. "We learn by doing." Demonstrations by the instructor are not ample. The student must conduct an experiment for himself to learn its importance and see its implications.

It is very important that practical work on a given subject be going on at the same time it is being presented in the lectures. It is the cross-reference from lecture-room to laboratory and from laboratory to lecture-room that makes learning interesting and subject matter vital. It is the multiple sensory avenues of approach utilized by first studying about a phenomenon, then having its intricacies explained, and last of all working with that same object or principle and discovering new facts about it for oneself that makes the educative process natural, fruitful and easy.

H. M. R

INTRODUCTION

The Purpose of the Study

A modern laboratory manual in elementary general physiology is at present not to be found, so this book was planned to meet the need for a new presentation. It is not based on a single book, but may be used with any standard text. It was written as an attempt to meet the rapidly growing need for a group of simple, comprehensive laboratory exercises illustrating the basic physiological characteristics common to all life.

The Problem

The problem was a two-fold one, namely: one of what to include, and one of how to include it. Since physiologists are remarkably well agreed as to what constitutes the field of General Physiology, the task resolved itself into one of selecting a group of exercises from hundreds suggested in various textbooks, properly organizing them, and then presenting them in simple terms. All experiments were rejected which required elaborate equipment or expensive chemicals.

Method of Procedure

A survey was made of the available textbooks on General Physiology to ascertain the scope of material included, in order that the objectives of the course be clearly and adequately formulated. Agreement of authority with authority plus agreement with the prevailing philosophy of education was the primary criterion employed in selecting the major divisions (or sections as

they are herein called)[1]. The next logical step was to obtain experiments, under each section heading, the solving of which would enable the student to vividly comprehend the principles involved in the specific objectives of the course. Some of the experiments have been presented again and again by numerous authors. Others are presented here for the first time.

The Contribution

The writer's chief contribution is one of improved technique in the laboratory presentation of known physiological principles, plus originality in the organization and sequence of the work.

Each experiment has been carefully worked out under actual laboratory conditions. Only those have been retained that will consistently and accurately illustrate what they purport to illustrate.

Previous Works

The writer has been greatly aided in this study by the suggestions and experiments of the following authors: W.M. Bayliss (1914) in his Principles of General Physiology has organized a veritable compendium of physiological experiments. His influence is clearly shown in the work of all modern physiologists and to him the writer gives due credit. Henry R. Barrows (1931) in his laboratory manual entitled, Biological Types and Principles has

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[1]The section headings represent the intermediate objectives of the course, but as this thesis is not written as is typical of the form and terminology of the usual curriculum study, they are not worded as such.

presented an excellent group of experiments on "The Physical and Chemical Nature of Protoplasm". Cleveland Pendleton Hickman (1930) in his Laboratory Manual in College Physiology has included some very good experiments which are general in nature. G. W. Scarth and F. E. Lloyd (1930) in An Elementary Course in General Physiology, Section II, have prepared a group of experiments to supplement their text. W. D. Zoethout (1928) in his Laboratory Experiments in Physiology has a group of experiments quite valuable for the medical student or special student of physiology.

Problems for Further Study

The writer makes no claim for finality in this study. Any curriculum must needs be tentative, flexible, and provide for assimilation and growth. With the exception of the fact that certain portions of electrical experimentation have been necessarily omitted[2], the field of general physiology has been rather thoroughly covered. Additional experiments may be added under each section heading as further experience deems so doing advisable.

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[2]Lack of time and equipment has made it impossible to include experiments on H-ion concentration, bioelectric currents, use of potentiometer, etc.

TO THE INSTRUCTOR

This manual includes sufficient material for one semester (18 weeks) with laboratory classes meeting double periods twice a week. Elementary courses of physics, chemistry and biology are highly desirable, as prerequisites, but not absolutely indispensable.

A laboratory period on the use and care of the microscope[1] is desirable and necessary with most classes.

The instructor must keep at least a week ahead of the class schedule in order that certain supplies be available when needed. Lists of references are found at the close of each group of experiments, with at least one reference to the exact page and book in which information on a given experiment may be obtained. General references in which information on the entire section may be found, are also given. A blank sheet entitled "Additional References" is also found at the close of each group. On this the instructor may note any additional references he may find, or pertinent information in new books which become available from time to time.

Section VII is highly valuable in its content and application, but may be omitted if laboratory facilities do not permit.

Complete lists of the equipment, material, and chemicals required will be found in the appendix.

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[1]The Bausch and Lomb Optical Company, Rochester, New York, publish a little booklet Use and Care of the Microscope which presents in a comprehensive way the necessary information.

TO THE STUDENT

The experiments which follow may seem quite simple in some instances, "but like the traditional falling apple, they have provided thought for great minds". In most cases results are so evident, when instructions are carefully followed, that no aid from the instructor will be required.

Sketch or draw what you actually see. Report what you find out to be the case; not what your neighbor finds out. You may be as near right as he. The concentrations have been carefully worked out. A drop more than the amount stipulated may ruin the result in delicate tests. Use the smallest amount possible at all times. When neutralizing strong acids add the base gradually with a dropper. Test often with litmus paper and thus avoid unnecessary dilutions. Careful handling of materials will prevent ruined clothes and burned fingers. Some solutions that "bump" badly cannot be boiled in test tubes. Most sugar solutions must be boiled in beakers if continued boiling is necessary. Keep the mouth of test tubes, in which liquids are being heated, directed away from your face, and your neighbor's face. Read the entire experiment before starting on any part of it. Reread if it is long or complicated. This is your creed. Read it and then review it occasionally.

Dry substances in powdered form will not pour successfully. The usual result when attempting to pour one of these materials on a filter paper, into a test tube or elsewhere is to get a pound when a gram is called for. Use a small spatula when endeavoring to place small amounts of chemicals in powder form into test tubes, or on the balances for weighing.

Use distilled water, whenever possible, unless otherwise specified. This will prevent evaporation rings in test tubes, beakers, etc., when continued boiling is done, and greatly facilitate washing. Keep your test tubes and all equipment clean. Care and orderliness in procedure and with materials, pays huge dividends in immediate results and in an establishment of correct habits of laboratory technique which if in no other way will find its reward in the "satisfaction of serious effort".

SECTION I

CELLS AND PROTOPLASM

Protoplasm, "the physical basis of life" as Huxley termed it, is a stuff which everywhere has similar properties. It is more than a stuff, however; it is also an organized mechanism. What the least organization compatible with vitality is we do not know. It at least can exist on a very small scale[1]....

Although the cell is not an invariable unit in the differentiation of protoplasm, it is a very important one in that it is the smallest subdivision of a larger living body which is capable of independent existence. Even in the most individualistic organisms, that is, the higher animals, where the parts are so interdependent that a tap on the head, for example, may result in the disintegration of the whole protoplasmic system, the cells are capable of independent existence apart from the body if a suitable environment is provided, as shown by their indefinite growth in tissue cultures. The distinct parts of a cell, on the contrary, cannot continue to live except in combination with the rest. The irreducible organization of protoplasm as we generally know it is therefore the minimum organization of a nucleated cell.[2]

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[1]G. W. Scarth and F. E. Lloyd. An Elementary Course in General Physiology, p. 7.

[2]Ibid., p. 8.

CELLS AND PROTOPLASM

1. Epithelial cells. Gently scrape the inside of the cheek with a sterilized instrument, as directed by the instructor. Transfer the material obtained to a clean slide, and mount in weak methylene blue solution. Prepare another slide and mount in eosin or some other cytoplasm stain. The cells obtained are flat epithelial cells; each includes an outer cell membrane, a cytoplasm, and a nucleus. Sketch a few isolated cells, labelling cell membrane, cytoplasm, and nucleus.

Materials needed:

1. methylene blue
2. eosin

2. Blood cells. Examine a prepared slide of the blood of a frog. The most numerous cells are the red corpuscles. The cytoplasm of these cells contains a protein called hemoglobin, which enables the blood to transport a large amount of oxygen. Sketch a few cells and label the parts as in Exp. 1.

Materials needed:

1. prepared slides (blood of frog)

3. Liver cells. Pinch off a bit of fresh liver and mount in a drop of water. How do these cells compare with those observed in Exps. 1 and 2? Sketch several cells. Label the parts.

Materials needed:

1. fresh liver

4. Plant epidermis. Carefully peel off a portion of the delicate transparent outer covering (epidermis) from the upper surface of a leaf, and mount in water. Examine under high power. Compare these cells with those studied in the previous exercises. How do they differ in shape? Note the vacuoles in some of the cells. Sketch several adjacent cells.

Materials needed:

1. green leaves (cottonwood or some other leaf with a hard surface)

5. Chromatin. Examine slides of Allium furnished by the instructor. These cells have been stained with special dyes to show the chromatin. Sketch several cells, label cell wall, cytoplasm, nucleus, and chromatin.

Materials needed:

1. slides of Allium

6. Protoplasm movement. Examine under low power a leaf of the water plant Elodea. The cells are alive. Besides the structures already noted in other cells, observe the chloroplasts (green bodies whose color is due to chlorophyll). Observe for a few minutes. Can you detect any movement of the protoplasm in these cells? Is the movement in the same direction in all cells? Is it constant and regular, or not? Do any of the chloroplasts pass through the walls between adjacent cells? Draw several adjacent cells and indicate by arrows the protoplasm movement.

Materials needed:

1. leaves of Elodea

7. Ameba. Prepare a slide and examine for ameba as directed. An active ameba is a naked mass of semi-transparent, granular protoplasm. How does the animal move? (The projections formed on the cell are called pseudopodia--false feet.) Are pseudopodia produced in different directions at the same time? What are the functions of these structures?

Ameba is a one-celled animal. Distinguish the transparent outer layer of protoplasm (ectoplasm) from the inner granular part (endoplasm). Is this a permanent differentiation? Does the ameba have a nucleus? Examine a stained demonstration slide if necessary.

Try to locate the transparent spherical contractile vacuole and note that it seems to close at intervals. What do you think is its function? Food vacuoles are spherical masses containing food particles and a liquid. What is the function of the liquid?

Can you detect any movement of protoplasm in the cell? Can you find any evidence of reactions to stimuli? Do changes in the intensity of light have any effect? Make two sketches: (1) series of outlines showing changes of shape, (2) detail drawing showing all significant parts.

Materials needed:

1. culture of ameba
2. stained demonstration slide of ameba

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Experiment 1. Zoethout, William D. A Textbook of Physiology, pp. 50-55.

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Experiment 4. Maximov, N. A. A Textbook of Plant Physiology, pp. 34-35.

Experiment 5. Zoethout, William D. A Textbook of Physiology, p. 34.

Experiment 6. Bayliss, W. M. Principles of General Physiology, pp. 20-21, 563.

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SECTION II

SURFACE ACTION

Organisms are made up of microscopic cells, cells of various lesser parts, and these again of ultramicroscopic or invisible colloidal particles. Each subdivision involves greater surface. If we estimate that the particles have a mean diameter of 5 uu and that altogether they total one-fifth of the volume of our bodies, each of us contains about 30,000 square meters of internal surface. This fact is of importance because free energy resides at interface, energy which displays itself among other ways in those active movements and in that catalysis of chemical reactions which are among the most characteristic features of life. The operation of surface forces appears as two main types of physical phenomena--Surface Tension and Adsorption--which are now to be studied in turn.[1]

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[1] G. W. Scarth and F. E. Lloyd. An Elementary Course in General Physiology, p. 18.

SURFACE ACTION

1. Surface tension. Fill a shallow dish with water. With clean fingers lower a clean dry needle flat upon the surface of the water. How do you account for the "floating" of the needle? (Examine the surface of the water where the needle is supported.) Touch the surface of the water about half an inch from the needle with a toothpick wet with alcohol. Can you account for the results?

Materials needed:

1. needles (common sewing needles)
2. toothpicks
3. alcohol

2. Surface tension at interfaces. Fill a shallow dish with water and place a drop of oil on the water. Can you account for the spreading of the oil? Now place a thin layer of oil in the dish and put a drop of water on it. How does the spreading of the water compare with that of the oil? Is there any difference between the surface tension of water and that of oil?

Materials needed:

1. lubricating oil

3. Drop method of measuring surface tension. Under proper conditions the weight of a drop which will just remain suspended from the end of a glass tip can be used to determine the surface tension of a liquid. The drop weight can be determined by the number of drops that fall from a known amount of liquid.

Fill the pipette furnished you with tap water up to a certain marked level and allow the liquid to run out so that drops fall slowly and regularly from the tip. Count the drops and repeat several times until a consistent average is obtained. Repeat with olive oil and 50% alcohol. How does the surface tension of these compare with that of water?

Repeat with strong solutions of different salts and of sugar. What is the effect of dissolved inorganic substances on surface tension? Of sugar?

Materials needed:

1. olive oil
2. 50% alcohol
3. calcium chloride
4. sugar (sucrose)
5. salt

Note: Do not use distilled water for surface tension experiments.

If it be wished to obtain a measurement of the absolute surface tension at a water-air interface it is best to use tap water, since this is less likely than distilled water is to contain greasy matter, which has a powerful effect in lowering surface tension, as we shall see later.[2]

4. Capillary tube method of measuring surface tension.

Another method is founded on the rise or fall of the level of a liquid in a capillary tube, according to whether it wets the glass or not. This change of level is due to the curved shape of the meniscus or surface separating the liquid from air.[3]

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[2]W. M. Bayliss. Principles of General Physiology, p. 49.

[3]Ibid., p. 50.

Measure the height to which water rises in a capillary tube (use vernier calipers or centimeter rule). Then as a check fill the tube by sucking on the end of it and measure the height of the water in the tube after it has reached its level. Repeat using strong sodium chloride solution, calcium chloride, sugar, and alcohol. How do these results compare with those found by using the drop method? Where is capillary action found in living protoplasm? What is the effect of lowering surface tension on living protoplasm?

Materials needed:

1. strong sodium chloride solution
2. calcium chloride
3. sugar
4. alcohol

5. Local surface tension changes. Place a large drop of mercury in a clean watch glass. Note form and size. Cover with 2% nitric acid. What change occurs? Touch the edge of the mercury with a small crystal of potassium dichromate. Result? Can you explain the changes that occur in the shape of the mercury drop? What kind of protoplasmic movement does this resemble?

Materials needed:

1. mercury
2. 2% nitric acid
3. potassium dichromate (crystals)

6. Cleavage of a drop by surface tension. Mix a few drops of olive oil, stained with Sudan III, with chloroform until the

mixture is heavier than water. (This can be determined by forcing a drop of the mixture from a dropper, with the mouth of the dropper beneath the surface of water in a clean beaker.) Place a drop of the mixture under water (about 1/4 inch does nicely) in a clean watch glass. Apply a small crystal of sodium carbonate adhering to points of a tweezers about 1/8 inch from each end of the drop. What happens? What causes the constriction?

Note: Droppers, watch glasses and all equipment used in this experiment must be perfectly free from grease or oil. If the drop comes to the surface and spreads, the mixture does not contain enough chloroform (chloroform evaporates very rapidly) or there is surplus oil on the dropper, in the watch glass, or in the water itself.

Materials needed:

1. olive oil
2. Sudan III
3. chloroform
4. sodium carbonate (crystals)

7. Adsorption. Dilute methylene blue with water until it is a pale blue color. Add powdered charcoal until the blue color is removed. Filter off the charcoal. Note color of filtrate. Now pour acetone (made acid with a few drops of hydrochloric acid) over the charcoal in the filter paper. Can you explain what occurs here? Result? What is the importance of adsorption in the human body? In soils?

Materials needed:

1. methylene blue (very dilute)

2. powdered charcoal

3. acetone

4. hydrochloric acid

8. Surface concentration. Make a weak solution of methylene blue. Set aside a sample for comparison later. Place the rest in a test tube and add a little soap solution. Shake and separate the underlying liquid from the foam by repeated decantations. Is there any change in the concentration of dye in the liquid? Add one drop of alcohol to dispel the foam and compare the color of the liquid that settles out. Repeat the shaking of the decanted liquid and again compare. Why does the amount of foam decrease with repeated shakings? Why does soap solution promote foaming and alcohol dispel it? What makes a film of material form on the surface of a cup of chocolate?

Materials needed:

1. methylene blue

2. soap solution

3. alcohol

9. Adsorption and staining.

a. Pour some congo red (prepared with distilled water) into a beaker. Prepare four pieces of old but well laundered cotton cloth. (Cut one pair into squares and the other into rectangles so the pairs can be identified later.) Soak two of the samples in cold distilled water and the other pair in nearly saturated sodium chloride solution. Place the samples soaked in water in the dye for 20 seconds. (Time carefully.) Remove and wash thor-

oughly in distilled water. Rinse well and place aside to dry. Repeat the process using the samples that were soaked in salt solution. (Use the same dye solution as was used for the first samples.)

Some of the students may vary this experiment by placing 1 g. of salt in the dye before the second samples are dyed, instead of soaking them in salt solution. Do not dry the material before placing in the dye for any part of the experiment.

Examine the samples. Is there any difference in color? Does the presence of the salt increase or decrease the strength of adsorption of the dye?

b. Prepare four more samples as in part a. Soak all four samples in distilled water. Proceed exactly as before, but instead of using salt to set the dye in the second pair of samples, heat the dye solution and have it boiling when the second set is placed in it. Examine the samples. Is there any difference in color? Does heat increase or decrease the strength of adsorption of the dye?

Materials needed:

1. well laundered cotton cloth
2. congo red solution (prepared with distilled water)
3. sodium chloride solution (nearly saturated)

10. Adsorption at liquid-liquid interface. Shake vigorously equal quantities of water and xylol or benzol stained with Sudan III and a little powdered charcoal. Examine a drop of this mixture under the microscope without a coverglass. Is it an emulsion? Which is the dispersed phase? Where are the

particles of carbon located?

Materials needed:

1. xylol or benzol
2. Sudan III
3. powdered charcoal

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Experiment 2. Bayliss, W. M. Principles of General Physiology, p. 52.

Experiment 3. Bayliss, W. M. Principles of General Physiology, p. 49.

Experiment 4. Bayliss, W. M. Principles of General Physiology, p. 50.

Experiment 5. Scarth, G. W., and Lloyd, F. E. An Elementary Course in General Physiology, p. 28.

Experiment 6. Scarth, G. W., and Lloyd, F. E. An Elementary Course in General Physiology, p. 36.

Experiment 7. Bayliss, W. M. Principles of General Physiology, p. 55.

Experiment 8. Ponder, Eric. Essentials of General Physiology, p. 21.

Experiment 9. Bayliss, W. M. Principles of General Physiology, p. 67.

Experiment 10. Bayliss, W. M. Principles of General Physiology, p. 52.

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SECTION III

THE COLLOIDAL STATE

More, perhaps, of the characteristics of the living substance are explicable on the basis of its colloidal nature than on any other ground.... Our interest is not confined to the behavior of colloids in the mass, but to the potentialities of the elemental particle that constitutes the colloidal state; because the first faint signs of life are displayed by bodies of that order of size and complexity.[1]

[1] G. W. Scarth and F. E. Lloyd. An Elementary Course in General Physiology, p. 150.

THE COLLOIDAL STATE

1. Colloid and crystalloid. Place a crystal of potassium permanganate in a test tube and carefully fill full of water. Note gradual diffusion of color through the water. Similarly, place a bit of corn starch in a test tube and fill half full of hot water. Examine the two tubes by transmitted light. In which tube is diffusion more rapid? Are the liquids transparent or translucent, i.e. does light pass through the liquids without interference or is it diffused? Give reasons for your answer. Which solute forms a colloidal solution in water?

Materials needed:

1. potassium permanganate crystals
2. corn starch

2. Dialysis. Prepare four dialysers as instructed.

> In a short length of parchment dialyzing tube place a quantity of water. Hold up both ends and notice that the tube does not leak. If this is satisfactory, replace the water with some glucose solution. Suspend the tube (by bending it into a u-shape and sticking a glass rod through the upper ends) in a beaker of water. Have the level of the water on the outside the same height as the glucose solution.[2]

In one place a 5% sodium chloride solution, in the second a 1% starch paste, in the third a 2% glucose solution, and in the fourth a 5% albumin solution. At the end of one-half hour test the liquid outside the dialyser for the presence of the materials placed inside, as follows:

Silver nitrate test for chlorides (white precipitate).

Iodine test for starch (blue color).

Benedict's solution for glucose (green precipitate).

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[2] W. D. Zoethout. Laboratory Experiments in Physiology, p. 188.

Nitric acid for albumin (white contact).

Which of these substances are colloidal and which crystalloidal?

Materials needed:

1. 5% sodium chloride solution
2. 1% starch paste solution
3. 2% glucose solution
4. 5% albumin solution
5. silver nitrate
6. iodine solution
7. Benedict's solution
8. nitric acid

3. Brownian movement. Grind a few tiny particles of carmine very finely. Place in a shallow dish. Add a dropper full of water. Mix thoroughly. Mount one drop on a slide and examine under the high power microscope. The vibratory motion observed is called Brownian movement. What causes it? Observe the demonstration slide of India ink. Is there any relation between the type of movement and the size of the particles? Mount some carmine in glycerin. Does this have any effect on the motion? Why?

Mount in water some living root hairs. Can you detect any Brownian movement? What does this tell you about protoplasm?

Materials needed:

1. carmine
2. India ink
3. glycerine
4. grass roots (previously sprouted on blotter paper)

4. Emulsions. If the dispersed substances are in the form of minute droplets of a substance that does not readily mix with the continuous phase the dispersion is called an emulsion. Examine a drop of cream on a slide and observe the large globules of butter-fat. These will stand out more prominently if a little of the dye, Sudan III, is added. Stir the Sudan III (dry) into the cream. Would you expect to find Brownian movement in the fat globules? Why or why not?

Materials needed:

1. cream
2. Sudan III

5. Emulsifiers. Add five or six drops of olive oil stained with Sudan III to half a test tube of water and shake vigorously. Prepare a similar tube and add a little soap solution. Compare results. Has an emulsion been formed in either case? Can you explain the difference in behavior of the contents in the two tubes? Examine a drop from the second tube with the high power for Brownian movement. Why is Brownian movement found here and not in fat globules in cream? (See Exp. 4). Do not attempt to dissolve the Sudan III in water when staining olive oil. Merely stir the stain (dry) into the olive oil.

Materials needed:

1. olive oil
2. Sudan III
3. soap solution

6. Effect of ions on phases. Shake up equal quantities of olive oil stained with Sudan III and a M/100 solution of sodium hydroxide. Repeat with oil and a M/100 calcium chloride. Examine each microscopically without a cover glass. Which phase is internal in each case? While observing allow M/10 calcium chloride to come in contact with the first, and M/10 sodium hydroxide with the second mixture. What is the effect of the sodium and of the calcium ion on a water-oil emulsion? Of what significance is this principle in the behavior of the plasma membrane of the cell?

Materials needed:

1. olive oil
2. Sudan III
3. sodium hydroxide M/10 and M/100 solution
4. calcium chloride M/10 and M/100 solution

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Experiment 3. Ponder, Eric. Essentials of General Physiology, pp. 36-38.

Experiment 4. Ponder, Eric. Essentials of General Physiology, pp. 38-40.

Experiment 5. Zoethout, William D. A Textbook of Physiology, p. 304.

Experiment 6. Ponder, Eric. Essentials of General Physiology, p. 86.

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SECTION IV

THE CHEMICAL NATURE OF PROTOPLASM

The materials of which living things are made are complex, ever-changing substances. No definite chemical formula can be assigned to the structure of protoplasm, but the biochemist has been able to divide it into several constituents which may be identified by applying chemical tests indicating properties common to the members of each of these separate constituents.[1]

[1]Henry R. Barrows. Biological Types and Principles, p. 11.

THE CHEMICAL NATURE OF PROTOPLASM

A. CARBOHYDRATES

1. Molisch's reaction. To 5 cc. of glucose solution in a test tube add 2 drops of 5% alcoholic alpha naphthol. Put 5 cc. of concentrated sulphuric acid in a second test tube. Holding the first tube at a considerable angle, pour the sulphuric acid carefully so that it will form a layer under the glucose solution. Note color at the boundary of the two solutions. Very gentle shaking is sometimes necessary to mix the fluids slightly.

Repeat this test with starch, absorbent cotton, wheat flour, gum arabic, and some other carbohydrates. If a substance does not yield this test it is not a carbohydrate. What is the value of such a test?

Materials needed:

1. 5% alcoholic alpha naphthol
2. sulphuric acid (concentrated)
3. glucose
4. starch
5. absorbent cotton
6. wheat flour
7. gum arabic

2. Composition of carbohydrates.

a. In a small dry test tube heat a small quantity of cane sugar (hold tube horizontally) until it glows. Continue the heating for some time. Cool and break the tube. Notice the color and general appearance of the mass left in the tube. Is it soluble in water? What do you suppose this is? What does

it prove as to the composition of sugar? To what larger class of compounds do the carbohydrates belong?

b. In an evaporating dish or crucible gently heat for three or four minutes about 2 grams of copper oxide (CuO). Stir it with a glass rod. When cool add one-tenth its bulk of cane sugar, mix and place in a dry test tube. Fit this tube with a rubber stopper and a delivery tube which dips into some clear calcium hydroxide solution (lime water). Heat the mixture gently with a small flame. The gases given off pass through the lime water and cause a white precipitate. What is the gas and what is the precipitate? Do you see any evidence of water being formed in the test tube? Where? Why in this place? What is the origin of the water? What did the sugar do to the copper oxide? What happened to the sugar?

Materials needed:

1. cane sugar
2. copper oxide
3. calcium hydroxide (lime water)

3. Fehling's test. Pour 5 cc. each of Fehling's solution no. 1 and no. 2 into two test tubes and mix. Divide the mixture into each of three test tubes. To the first add a few grains of cane sugar, to the second a pinch of starch, and to the third a little glucose. Heat each tube and boil gently for about two minutes. What visible changes occur? For what kind of carbohydrate is Fehling's solution a test? (If it is difficult to remove the copper salt from the test tubes, a little dilute nitric acid will dissolve it.)

Materials needed:

1. Fehling's solution
2. cane sugar
3. starch
4. glucose

4. Benedict's test. To about 5 cc. of Benedict's solution in each of two test tubes add a few drops of cane sugar solution and glucose solution respectively. Heat below the boiling point for two minutes. What difference in the action of complex cane sugar and simple glucose do you note? How does this test compare with that used in Exp. 3 in sensitivity?

Materials needed:

1. Benedict's solution
2. cane sugar
3. glucose

5. Action of alkalies. Heat a few cc. of glucose with a little sodium hydroxide. Color? Odor? This is due to the formation of caramel (a mixture of several compounds) by the decomposition of the glucose molecule.

Materials needed:

1. glucose solution
2. sodium hydroxide solution

6. Inversion. Prepare 10 cc. of a 1% cane sugar solution. To 5 cc. of this add 5 cc. of Fehling's solution and bring to a boil. To the other 5 cc. add 2 drops of dilute hydrochloric acid

and boil for one minute. Neutralize with strong sodium hydroxide and add 5 cc. of Fehling's solution and boil. How does this differ from the untreated cane sugar? What change did the cane sugar undergo by being boiled with acid?

Materials needed:

1. cane sugar
2. Fehling's solution
3. hydrochloric acid (dilute)
4. sodium hydroxide

7. Starch. Make 100 cc. of a 2% starch solution (use cold water). Divide equally into two containers and set one sample aside.

a. Appearance. Mount starch in water and examine microscopically. Note shape and markings of grains. Sketch a few grains.

b. Iodine test. Add a drop of dilute iodine solution to a drop of starch solution on a plate (The white background shows the color most plainly.) Color?

c. Solubility. Pour 10 cc. of the starch solution through a filter. Test the filtrate with iodine. Is raw starch soluble in water?

d. Effect of boiling. Boil the other 50 cc. of starch paste (prepared above) for one minute. Make the iodine test. (See b.) Note change in color given by the iodine test from that found when raw starch was used. To 5 cc. of the paste add several drops of strong sodium hydroxide. Test a drop with iodine. Result? This is due to the hydroxide removing the free iodine,

which is necessary for coloration. Test another of these drops with iodine, then add a drop of dilute hydrochloric acid. Result? What does the acid do? Filter 10 cc. of the boiled starch solution made in d. Test one drop of the filtrate with iodine. What has boiling done to the starch grains? Place about 15 cc. of the starch paste in each of two test tubes. With one make the Fehling test for glucose. Result?

e. Action of acids. To the other add three drops of dilute hydrochloric acid and boil for one minute. Neutralize with sodium hydroxide. Add 5 cc. of Fehling's solution and boil. Result? What has the acid done to the raw starch? Where is this process carried on in the body? In what other ways could starch be thus changed?

Materials needed:

1. 2% starch solution
2. iodine solution (dilute)
3. hydrochloric acid (dilute)
4. Fehling's solution
5. sodium hydroxide solution

8. Dextrin. Make the following tests:

a. Repeat 7-b. Dextrins are generally mixtures and often contain some starch, therefore several dilutions may be necessary to bring out the difference between the starch color and the dextrin color.

b. Repeat 7-c.

c. Repeat 7-e.

Materials needed:

1. dextrin
2. iodine solution
3. hydrochloric acid (dilute)

9. Cellulose. Using a bit of lens paper, absorbent cotton or paper towel, make the following tests:

a. Mount a bit of the material selected in water and examine microscopically. Appearance? Sketch a few cells.

b. Soak a strip of filter paper or paper towel in water. Remove and apply the iodine test to the paper. Result? Tear a small strip of filter paper or paper towel into bits, place into a test tube, add 20 cc. of distilled water and boil for one minute. Make the iodine test (using either the liquid or the bits of paper). Result? Does cellulose contain starch?

c. Action of acid. Tear a quantity of paper toweling into bits (one-half of an average paper towel is sufficient). Place in a beaker. Add 70 cc. of dilute sulphuric acid (6N). Fill a considerably larger beaker one-fourth full of distilled water. Place the first beaker in the second (a double boiler effect) and boil slowly for two hours. Filter about 20 cc. of the contents of the small beaker. Neutralize carefully with sodium hydroxide (15N solution avoids undue dilution) and test with Fehling's solution. Result? Of what is cellulose made? Where is it found in nature? What is its function in plants? In what ways does it differ from other polysaccharides? Discuss briefly its digestibility? Of what significance is this last fact to us?

Materials needed:

1. dilute sulphuric acid (6N)
2. sodium hydroxide solution (15N)
3. Fehling's solution
4. iodine solution (dilute)

10. The identification of carbohydrates. Fehling's solution does not give a reaction if the fluid tested is acid. If the solution to be tested is an unknown, first test it with a strip of blue litmus paper for acidity. If it is acid neutralize it or make slightly alkaline with strong sodium hydroxide. Iodine does not react in an alkaline medium but does so in a neutral or slightly acid one. The detection of boiled starch is more readily made than of raw, so it is advisable to boil a substance or solution before making an iodine test for starch.

You will be given certain substances or solutions by the instructor. Review the tests for carbohydrates and then proceed to determine what the unknowns are. If the unknown is a liquid, first boil for one minute. If the substance is a powder or solid, first dissolve it in distilled water and then boil. If testing food, grind the material in a mortar with a little water, and then strain through a cheese cloth. Tabulate results carefully. Result of iodine test? Result of Fehling's test? If these tests give negative results apply the inversion test to a portion of the liquid (see Exp. 6). If this gives a negative result, apply Molisch's reaction (see Exp. 1).

Materials needed:

1. cane sugar solution

2. glucose solution
3. starch solution
4. Fehling's solution
5. 5% alcoholic alpha naphthol
6. concentrated sulphuric acid
7. sodium hydroxide solution
8. any other unknown substances or solution the instructor may provide.

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THE CHEMICAL NATURE OF PROTOPLASM

B. FATS

1. Spot test. Place a drop of oil on a piece of smooth paper; let it spread and dry. Note appearance, especially when the paper is held up to the light. Now moisten a spot with tap water. How is this different from an oil spot? Rub the paper with the exposed surface of a piece of nut meat. Does this contain fatty material? Repeat with sugar solution, starch paste, and albumin solution. Do these substances produce "grease spots"?

Materials needed:

1. olive oil or lard
2. nut meats
3. sugar solution
4. starch paste
5. albumin solution

2. Solubility. In four test tubes place 3 cc. of water, alcohol, ether, and carbon tetrachloride respectively. To each add a few drops of olive oil. Shake well and set aside for observation. What conclusions can you draw as to solubility? Test a small amount of each of the clear liquids as in Exp. 1. Which is the best solvent for removing grease spots?

Materials needed:

1. alcohol
2. ether
3. carbon tetrachloride
4. olive oil

3. Acrolein test. Place a few drops of olive oil in a test tube and add a little dry potassium bisulphate ($KHSO_4$). Heat carefully and strongly. Keep the fumes away from the eyes. Note odor. The odor is that of acrolein, formed by the burning of fats and fatty tissues. Repeat with lard and butter using the necessary precautions.

Materials needed:

1. olive oil
2. potassium bisulphate ($KHSO_4$)
3. butter
4. lard

4. Saponification. To 2 grams of lard or tallow in an evaporating dish add 25 cc. of 10% sodium hydroxide solution. Boil gently for about 20 minutes, or until no oil globules can be seen in the mixture. The mixture consists of glycerol, excess alkali, and sodium soap. Add about 5 cc. of saturated salt solution and boil 5 minutes. This causes the soap to separate largely from the mixture. Save the soap for Exp. 5. Discuss briefly the relation between saponification and the digestion of fats.

Materials needed:

1. tallow or lard
2. 10% sodium hydroxide

5. Decomposition of soap. Dissolve a piece of the soap from Exp. 4 in hot water, add strong hydrochloric acid until it becomes acid and cool the mixture. The material forming on the surface is the free fatty acid originally present in the fat used

in Exp. 4. Apply the spot and acrolein tests to this material.

Materials needed:

1. soap
2. hydrochloric acid (concentrated)

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THE CHEMICAL NATURE OF PROTOPLASM

C. PROTEINS

1. Millon's test. To about 5 cc. of 1% albumin solution add a few drops of Millon's fluid. Note color change. Boil carefully. Any further change? This reaction is due to the amino acid tyrosine, found in nearly all proteins.

Materials needed:

1. 1% albumin solution
2. Millon's fluid

2. The xanthoproteic reaction. To 5 cc. of 1% albumin solution add 10 drops of concentrated nitric acid. Result? Boil carefully. Result? Cool and slowly add sodium hydroxide until distinctly alkaline. Result? Why does nitric turn the skin yellow?

Materials needed:

1. 1% albumin solution
2. concentrated nitric acid
3. sodium hydroxide

3. Biuret test. In two test tubes put 5 cc. of 1% albumin solution and 5 cc. of peptone paste respectively. To each add 5 cc. sodium hydroxide solution and then copper sulphate solution drop by drop. Shake after each drop. Note color changes. What difference in the color of the simple peptone solution and the more complex albumin?

Materials needed:

1. 1% albumin solution

2. 1% peptone solution

3. 5-7% copper sulphate solution

4. Salting out. To 20 cc. of 1% albumin solution in a beaker, add 35 g. of magnesium sulphate. Mix thoroughly by repeated pourings from one beaker to another. A heavy cloudy precipitate will be formed. Allow to settle one minute. Filter off a little of the clear liquid on top and make the xanthroproteic reaction test. (See Exp. 2.) Result?

Materials needed:

1. 1% albumin solution

2. magnesium sulphate

3. nitric acid (concentrated)

4. sodium hydroxide

5. Salts of heavy metals. Prepare a 1% solution of albumin. Carefully filter it. Then using the clear filtrate, determine whether each of the following salts precipitate albumin: copper sulphate, lead acetate, silver nitrate, mercuric chloride, ferric chloride, and zinc sulphate. Add the salt solution drop by drop. If a precipitate forms add more salt to see whether the protein is soluble in excess salt. Why are large doses of egg white given in cases of mercuric chloride poisoning?

Materials needed:

1. 1% albumin solution

2. copper sulphate solution

3. lead acetate solution

4. silver nitrate

5. mercuric chloride
6. ferric chloride
7. zinc sulphate

6. Milk proteins. To 25 cc. of skimmed milk add an equal volume of water and then carefully add dilute hydrochloric acid, a little at a time and stirring after each addition of acid. Filter off the precipitate that forms and test with one of the color tests for proteins. Is there any protein left in the liquid filtered off? Why does sour milk curdle?

Materials needed:

1. skimmed milk
2. dilute hydrochloric acid
3. nitric acid
4. sodium hydroxide

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SECTION V

DIFFUSION, PERMEABILITY, OSMOSIS

DIFFUSION, PERMEABILITY, OSMOSIS

1. Diffusion through liquid. Below 1.5 cm. (about one-half inch) of water in a test tube introduce, with a dropper, a layer of chloroform containing iodine in solution. Do not allow any of the chloroform to touch the upper surface of the water. The following instructions facilitate proper placement of liquids. Fill the dropper with the colored chloroform. Then wash the end of the dropper free of color by dipping it in water and gently rinsing it. Next insert the mouth of the dropper well beneath the surface of the water and slowly expel the chloroform from the dropper.

Place a layer of ether above the water, seal the tube tightly with a cork dipped in melted paraffin, and set aside until the next laboratory period. Iodine is slightly soluble in water but very soluble in ether. What happens? How long does this take?

Materials needed:

1. chloroform containing iodine
2. ether
3. paraffin

2. Negative pole of diffusion.

> A point in a solution at which the concentration of dissolved substance is great, and at which there is accordingly a high osmotic pressure may be looked upon as a pole of diffusion.[2]

Flood a piece of glass with a thin layer of sodium chloride solution. Add a drop of India ink. Result? If the first drop spreads rapidly and makes a thin scum over the entire surface of

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[2]Eric Ponder. Essentials of General Physiology, p. 105.

the water, disregard it and proceed by adding another small drop. Add still another close to the second drop. Result? Why do the drops repel each other? Try this experiment until conditions are ideal for a slow diffusion of the India ink particles. Describe the diffusion pattern. From this experiment, what would you say as to orderliness of the diffusion process?

Materials needed:

1. sodium chloride solution
2. India ink

3. Cell permeability.

a. Carefully wash some slices of beet and place in distilled water. Note whether color comes out. Heat and note any difference.

b. Place a few slices of beet that have been carefully washed in running water, in .25% calcium chloride solution. Note whether any color comes out. Place a like number of slices in 2% sodium chloride solution. What difference? (The beet slices should be in the solutions for at least 24 hours for most evident results.) When making comparisons, pour some of each solution off the beets into test tubes, so that the actual color of the solution may be discerned. Of what significance is this difference? Of what importance are inorganic salts in cell metabolism?

c. Stain some Elodea and Spirogyra in neutral red. Place these in M/40 sodium hydroxide solution. Does the sodium hydroxide penetrate the leaves? (Neutral red is colored by alkalies.)

d. Stain some Elodea leaves in neutral red. Wash the surplus color from them by immersing them in water. Kill half of the leaves by placing them in chloroform water for 5 minutes.

Place one of the live stained leaves and a chloroformed one side by side on a microscope slide. Add two drops of M/40 sodium hydroxide solution and compare under the low power. What is the effect of death on the permeability of the cell wall? Remember that neutral red can penetrate living protoplasm, but is merely an indicator. Sodium hydroxide can penetrate only dead protoplasm.

Materials needed:

1. red beets
2. .25% calcium chloride solution
3. 2% sodium chloride solution
4. M/40 sodium hydroxide solution
5. Elodea leaves
6. Spirogyra
7. neutral red

4. Osmosis. The following method for setting up an osmometer is suggested: Close up the small end of a thistle tube with a short piece of rubber tubing and rubber band. Fill the bell of the tube with corn syrup. Tie a piece of parchment dialyzing paper (previously thoroughly soaked in water) tightly over the flanged end of the tube. Invert the tube, remove the obstruction from the small end, and with a ring stand and test tube clamp arrange the osmometer with the lower end immersed in a beaker of water. Carefully note time of starting and measure the rise of the column of liquid at 30-minute intervals. Compare your results with those of other students, and tabulate the data. Discuss fully the differences and the significance of osmosis in living things. Some of the students may vary the

experiment by using different solutions in the osmometer, or by using different kinds of membranes (egg membrane, collodin, or any other prepared membrane).

Materials needed:

1. corn syrup
2. parchment paper

5. Precipitation membranes. Fill a shallow dish with 3% copper sulphate solution and place under the lowest power of the microscope. Drop into the solution a tiny crystal of potassium ferrocyanide and examine immediately. Try to describe accurately what happens. Is there any resemblance between this and organic growth?

Materials needed:

1. 3% copper sulphate
2. potassium ferrocyanide (crystals)

6. Osmosis in living cells. Place a few filaments of Spirogyra on a slide and cover with a cover glass. While holding a piece of blotting paper on one side of the cover glass add a drop of strong salt solution to the other side and observe what happens in the cells as the salt solution flows under the cover glass and comes in contact with the cells. Now remove the salt solution and replace with distilled water. What result? Explain.

Materials needed:

1. Spirogyra
2. strong sodium chloride solution

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SECTION VI

ENZYME ACTION

There are a large number of reactions which proceed by themselves very slowly, or sometimes, apparently not at all, but which can be enormously accelerated by the presence of small amounts of various foreign substances. The characteristic property of such accelerating agents, known as "catalysts", is that they do not form part of the system in its final equilibrium....

In living organisms there are a large number of substances which behave like catalysts, and are known as "enzymes". They are extremely active, and explain the occurence in the organism of reactions which require, in the laboratory, powerful reagents and high temperatures. Lactose is hydrolyzed by both hydrochloric acid and by an enzyme lactase; but weight for weight the latter is, at least, five thousand times as powerful as the acid. These enzymes are all in the colloidal state... Their action is exerted on the surface, and is controlled by the amount of reagents absorbed.[1]

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[1]W. M. Bayliss. Principles of General Physiology, p. 330.

ENZYME ACTION

1. Salivary digestion (raw starch). Make 100 cc. of a 1% solution of starch paste, using cold distilled water. Test 5 cc. with Fehling's solution for glucose. Result? (If there is glucose present the starch is worthless for this experiment.) Collect saliva (chew paraffin to stimulate the flow of saliva). Dilute about 10 cc. of saliva with an equal amount of distilled water. Stir thoroughly. To 10 cc. of the starch solution add 1 cc. of saliva and after 5 minutes make the Fehling test for glucose. Result? Will ptyalin, the enzyme in saliva, digest raw starch?

Materials needed:

1. 1% starch solution (raw)
2. Fehling's solution
3. paraffin

2. Salivary digestion (cooked starch).

a. Boil the remainder of the starch prepared above, for one minute. Put about 15 cc. into each of five test tubes. To A add a few drops of sodium carbonate solution. To B add 3 drops of hydrochloric acid. Place C in a beaker of ice water. Place D in a water bath at 40° C. Heat E to the boiling point. Add 1 cc. of saliva to the contents of each test tube. Boil E for a few moments. After 5 minutes test a few drops from each tube with iodine, for starch. Test the remainder of the contents of each tube, except C, with Fehling's solution. What is the effect of alkali? Of acid? Test a portion of C. Result? Place the remainder of the contents of C in the water bath at 40° C. for

10 minutes. Test with Fehling's solution. Result? Is the enzyme destroyed by low temperature? By boiling?

b. Place 1 cc. of saliva in a small beaker, and stir 1 g. of bone charcoal[2] very carefully into it. Add 15 cc. of 1% boiled starch paste and stir thoroughly. After 10 minutes filter some of the mixture and test the filtrate with Fehling's solution. Result?

Materials needed:

1. 1% starch solution (cooked)
2. sodium carbonate solution
3. dilute hydrochloric acid
4. Fehling's solution
5. bone charcoal

3. Stages in digestion. Make 50 cc. of 1% starch paste, using cold water. Boil for one minute. Cool the paste to 40° C. or lower. Test one drop, on a white plate, with iodine. Keep this spot for comparison. (When making the tests arrange the drops in succession around the edge of the plate to show the different stages.) Collect saliva and dilute with an equal volume of water. Place 1 cc. of the saliva preparation in 20 cc. of starch paste. Mix thoroughly. After 1 minute make the drop test with iodine. Is there any change from the color shown by the first test? Make Fehling's test for glucose. Result? Repeat the drop test with iodine. Result? Continue the tests at frequent intervals until no further change in color is noted. The

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[2]Bone (or animal) charcoal must be used. Wood charcoal does not absorb the enzyme very effectively.

pinkish reaction with iodine before complete hydrolysis indicates erythrodextrin, an intermediate product. Note the gradual increase of transparency as the colloidal solution becomes crystalloidal. How could one prepare a highly concentrated solution of dextrin? How could the reaction be stopped to prevent its all becoming glucose?

Materials needed:

1. 1% starch paste
2. iodine solution (dilute)
3. Fehling's solution

4. Gastric digestion. Clean four test tubes, A to D. In A put 5 cc. of water and a few drops of pepsin. In B put 5 cc. of 0.2% hydrochloric acid. In C put 5 cc. of acid and a few drops of pepsin. In D put 5 cc. of sodium carbonate solution and a few drops of pepsin. To each add a few small cubes of boiled white of egg. Be sure to use small pieces and of the same size. Observe results the next laboratory period. What effect does alkali have on pepsin digestion? Acid? What digestive effect does acid alone have on proteins?

Materials needed:

1. pepsin
2. 0.2% hydrochloric acid
3. sodium carbonate solution
4. boiled white of egg

5. Oxidation.

a. Prepare two thin cross sections of an apple from the carpellary region. Immerse one in bensidine solution at once. Dip

the other in boiling water and then immerse in bensidine solution. Work fast, so as to expose the cut surface of the apple to the air as little as possible. Note where the color appears, which indicates the location of oxidase, the enzyme that causes the oxidation. Air is the source of the oxidation.

b. Repeat with slices of potato. How long does it take for the color to appear? Make sketches to show what regions are affected first. What is the effect of the boiling?

Materials needed:

1. apples (1 apple to 4 to 5 students)
2. potatoes (1 potato to 5 students)
3. benzidine solution (alcoholic solution)

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SECTION VII

VITAL PROCESSES

All living protoplasm has certain physiological properties. These properties may be exhitited by a simple unicellular animal, or they may appear as specialized functions of separate parts, in a highly organized animal. The maintenance of these properties involves the use and transformation of energy. Energy changes call forth still more involved processes and so it is that the continuity of life is dependent on the co-ordinated execution of certain vital processes.

VITAL PROCESSES

1. The simple muscle contraction.[1]

Note: Do not prepare the muscle until all the apparatus is set up. Isolated tissues and organs lose their irritability rapidly.

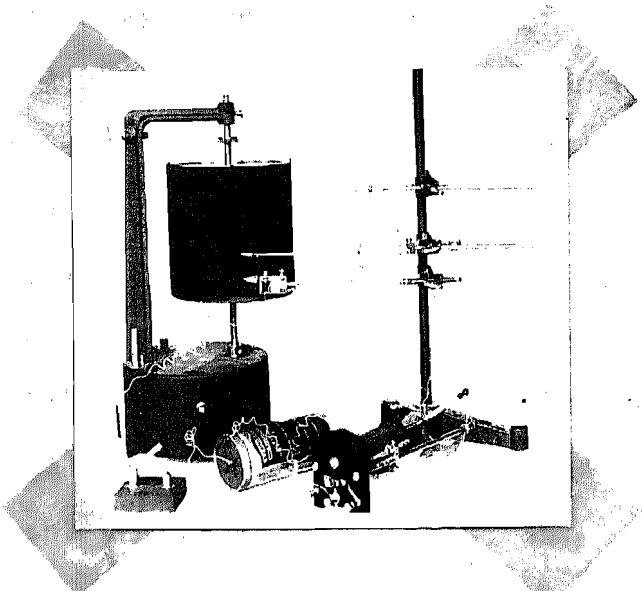

Fig. 1. Set up for Obtaining a Muscle Tracing. From Zoethout--Laboratory Experiments in Physiology.

a. Smoke the kymograph (a smoky kerosene lamp is often used to produce the smoke) and set up the electrical apparatus as shown in Fig. 1.

b. Make a gastrocnemius muscle preparation as follows:

1. Pith brain of a frog in this manner: Hold the frog in the left hand. With the index finger press the nose downward until the head makes a right angle with the trunk. Then stick a dissecting needle forward and down-

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[1]Adapted from W. D. Zoethout. Laboratory Experiments in Physiology, pp. 38-41.

ward through the base of the brain into the skull. Turn and twist the needle from side to side and thus destroy the brain.

2. Make a circular cut through the skin above the knee. Pull the skin downward until the tendon of achilles is exposed. Separate the gastrocnemius from the tibio-fibula bone. Sever the tendon of achilles just above the heel, and make a hole through the tendon.

3. Cut through the tibio-fibula bone just below the knee and cut away all the femur bone but a small portion to which the gastrocnemius is fastened. Lay the preparation on a piece of filter paper and moisten it with 0.7 per cent sodium chloride solution.

c. Place the femur in the jaws of the muscle clamp and through the hole made in the tendon fasten the hook on the muscle lever. Place a 10-30 g. weight on the pan attached to the muscle lever. Be sure the position of the hook upon the muscle lever is constant.

d. Connect one of the wires from the secondary coil with the binding post on the muscle clamp. Run the other wire to the thumb-screw on the muscle clamp and leave four inches of free wire. Wrap the free end tightly around the tendon. The wire wrapped around the tendon should be extra fine wire. Why? Keep the muscle moist with the 0.7 per cent salt solution.

e. Place the ends of the muscle lever and magnet lightly against the drum. Keep the drum high and the recording apparatus low, so if necessary another tracing may be placed above the first one.

Try out the hook-up by closing the key in the primary circuit. With a sufficient strength of induced current, the muscle should contract and cause the writing lever to make a tracing on the drum. The pen must move up and down freely. Why?

f. If all is working properly, start the drum moving at medium speed; close the primary key. Keep it closed for three seconds and open it. What did the muscle do while the primary key was closed? What physiological property is highly developed in muscles? What is a stimulus?

Materials and apparatus needed:

1. kymograph
2. induction coil
3. dry cells
4. simple key
5. muscle clamp and lever
6. stand
7. clamps
8. 0.7 per cent sodium chloride solution
9. frogs (one to each group of students)

2. The relation between the strength of stimulus and height of contraction. What is contractility? Irritability? A stimulus? Do stimuli vary in intensity? Illustrate. From your experience do you surmise that the extent of the response varies with the strength of the stimulus?

a. Set up the apparatus as in Exp. 1. Have the drum stationary during the stimulation so that the tracing produced is merely a vertical line. Between the times of stimulation rotate the drum about one-half inch.

Use the muscle preparation of the previous experiment (three dry cells) in the primary circuit. Place the secondary coil as far from the primary as possible, and stimulate the muscle by closing the key; let the key remain closed for 2 or 3 seconds (stationary drum). If a contraction occurs,

move the drum forward a little, so that no two contractions are superposed. Stop the drum and break the current. After each break move the secondary coil one-half cm. nearer to the primary coil and wait one minute before the next stimulation is made. When the induced current is strong enough, a make or break contraction appears. Continue to increase the strength of the induced current until the secondary and primary coils are flush... What can you say of the strength of the make and break induction shocks?[2]

3. Muscle work; influence of load on height of contraction and on work. What is the function of a muscle? How can mechanical work be measured?

a. Set up the apparatus as in Exp. 1 and provide muscle weights. Use induced current giving a good break but no make contraction (short circuit the make), and keep the current constant throughout the experiment. Why?.. Stationary drum; move it between the periods of stimulation. Make sure that the muscle pen writes without undue friction all the way up as the muscle contracts. Properly "afterload" the muscle by turning the thumbscrew on the muscle lever so that it just touches the short arm of the lever and thus prevents the load from bearing down on the muscle when the muscle is not contracting.

b. With no load (neglecting the weight of the lever) record a contraction. Load the muscle with 20 grams (or with 10 grams, if the muscle is very small) and record the contraction. In the same manner obtain curves for loads successively increased by 20 grams (10, if the muscle is very small) up to 100 grams. Now increase the load by 100 grams up to the point where the load is no longer lifted.... From your experiment discuss the relation between load and work; optimum and maximum loads. Under what two conditions does the active muscle perform no mechanical work? Discuss briefly the energy transformations in these two cases? What factors determine the "strength" of a muscle?[3]

Note: If the students desire, the kymograph tracings may be varnished and kept permanently.--

A good varnish can be made by placing orange or yellow flaked shellac in about four times its volume of wood, or better, denatured alcohol. Let stand for several days, shaking it occasionally. Decant the clear liquid and add alcohol to it, if necessary.[4]

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[2]W. D. Zoethout. Laboratory Experiments in Physiology, pp. 41-42.

[3]W. D. Zoethout. Laboratory Experiments in Physiology, pp. 47-48.

[4]Ibid., p. 244.

4. Graphic record of heartbeat.[5] Set up the apparatus as indicated in Fig. 2. Pith the brain of a frog. Place the frog on a frog board and expose the heart by cutting through the skin over the thorax. Slit open the pericardium. Cut through the

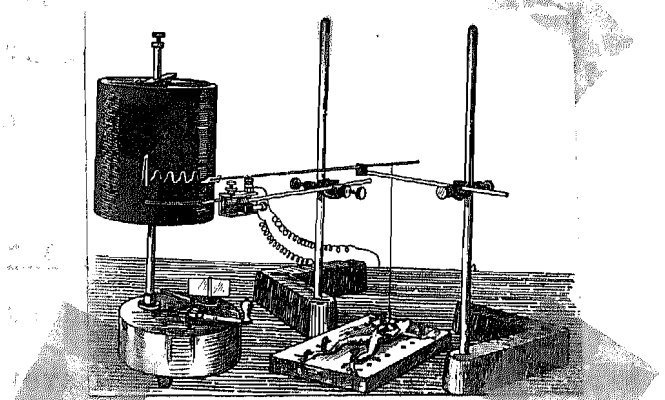

Fig. 2. Set up for Recording the Frog's Heartbeat. From Zoethout--Laboratory Experiments in Physiology.

pectoral girdle and spread the arms out well. Run a small hook through the apex of the ventricle, but do not puncture it. Connect the hook with a fine thread to the heart lever as shown in Fig. 3. Keep the heart moistened with 0.7% sodium chloride solution. Let the point of the heart lever make a light contact with the smoked drum so that a mark will be made when the lever is raised and lowered. Use medium speed drum. Notice that the beat has two parts to it. First the slight contraction of the auricle and then the greater contraction of the ventricle. Is this shown on the tracing? Make a sketch of the kymograph tracing or better still varnish a portion of it for a

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[5]Adapted from W. D. Zoethout. Laboratory Experiments in Physiology, pp. 100-102.

permanent record to be kept in the note-book.

Materials needed:

1. frogs (one to each group of students)
2. frog board
3. kymograph
4. 0.7% sodium chloride solution
5. heart lever

5. Nerve conductivity.

a. Pith brain of a frog. (See Exp. 1.)

b. Reverse the direction of the pin and destroy the spinal cord.

c. Make a muscle-nerve preparation in the following manner: Place the frog back upward in a dissecting tray. Slit open the skin on the side of one thigh.

> Separate the muscles carefully by gentle pressure of the thumbs and observe the sciatic nerve which will look like a glistening thread. Carefully free it as far as possible up into the body, and cut it close to the spinal column. Now proceed to make the usual gastrocnemius muscle preparation, being very careful not to sever the sciatic nerve from the muscle. Call this preparation A. Make a similar preparation of the other muscle and call it B.[6]

Keep these tissues moist with 0.7% sodium chloride solution. Mount the muscle nerve preparation A with apparatus as in Fig. 1, but do not run wires from the secondary coil to the muscle. Lay the sciatic nerve on a glass plate supported by the ring stand. Keep the nerve and muscle moist with 0.7% sodium chloride solution. Stimulate the sciatic nerve with electrodes attached to the secondary coil (break contraction). Result? Now lay muscle-nerve preparation B over the sciatic nerve of A and stim-

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[6]W. D. Zoethout. Laboratory Experiments in Physiology, p. 59.

ulate the nerve on B. Result? What physiological property of protoplasm does this demonstrate?

Materials needed:

1. frog (one to each group of students)
2. dissecting tray
3. 0.7% sodium chloride
4. induction coil
5. dry cells
6. simple key
7. muscle clamp and lever
8. stand
9. clamps

6. Determining the amount of oxygen consumed by a small animal. Carbon dioxide will dissolve very readily in potassium hydroxide. In fact, several volumes can be taken up by the hydroxide with no appreciable increase in volume. Thus any change in volume in the apparatus in this experiment is due to the oxygen consumed by the animal used.

Set up the apparatus as in indicated in Fig. 3. A two-quart Mason jar with a small stop cock soldered on the lid for a hose connection does nicely for an animal cage. (Make a hole through the lid under the stop cock.) Test the apparatus carefully for leaks. Place three small beakers in the bottom of the cage and fill them nearly full of 50% potassium hydroxide solution. Cover the beakers with a screen so the animal cannot get into the potassium hydroxide. Fill the pipette (100 cc. size) and burette (50 cc. size) until the pipette is about one-fourth full of

water. Place the animal (a small mammal) in the cage, screw the lid on tightly and check all hose connections. What is the reading on the burette? Nearly subjerge the cage in a large container of water so the temperature may be held constant. What would an increase in temperature do to the pressure in the apparatus? Be sure the temperature of the water in the container is the same as the room temperature.

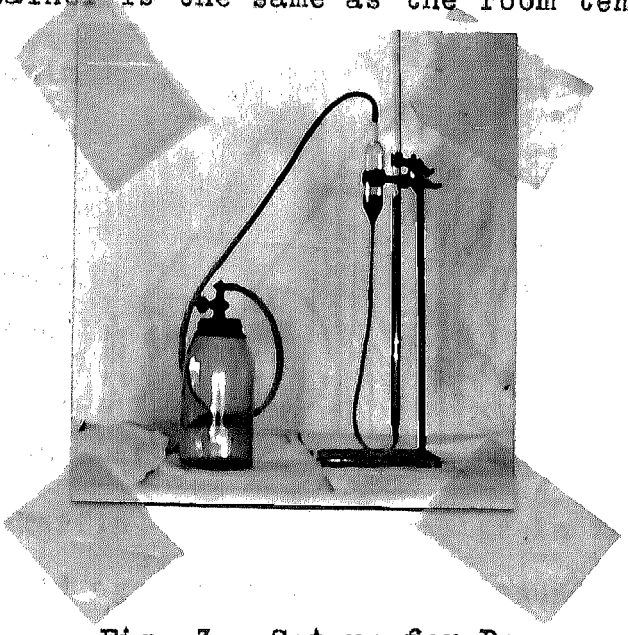

Fig. 3. Set up for Determining Oxygen Consumption of a Small Animal.

Watch the water level in the burette. As the water level lowers in the burette raise the burette so the water level is the same as in the pipette. This keeps the pressure in the system uniform (atmospheric). Read the burette at frequent intervals. Do not keep the animal in the cage for periods longer than twenty minutes. If it is necessary to repeat the experiment, use another animal. How much had the water in the burette lowered in 5 minutes? In 10 minutes? At the end of the experi-

ment? What is the source of the carbon dioxide that unites with the potassium hydroxide? Animals also give off water vapor as another waste product of the respiratory process. Do you see any evidence of this? Where?

Materials and apparatus needed:

1. 50% potassium hydroxide solution
2. rats (or other small mammals)
3. an animal cage for each group of students
4. rubber tubing

7. Methylene blue as an oxygen indicator. Fill two test tubes with pond or aquarium water. Stain the water in each with two or three drops of methylene blue. Place a small tadpole in one of the tubes and seal each tube tightly with a cork dipped in melted paraffin. Compare the color in the tubes at 15-minute intervals (hold against a white background). Methylene blue is an oxygen indicator. What became of the oxygen? How can the color be restored?

Materials needed:

1. tadpoles
2. methylene blue
3. paraffin

8. Effect of changes in temperature on heart action of daphnia. Mount a live daphnia, in a thin film of water, in a clear watch glass. Observe under the low power microscope. Try to ascertain the rate of heart beat. (The heart is the clear disc-shaped organ on the dorsal side of the animal.)

Place a number of daphnia and some watch glasses, in which the daphnia are to be observed, in the refrigerator for ten minutes. Then mount one daphnia as before and observe under the low power. Result? What has the low temperature done to the heart action? Work rapidly. Make observations before the temperature increases. Keep the watch glasses and daphnia ice cold! Watch the animal as the temperature increases. Is the heart action sensitive to slight temperature changes?

Materials needed:

1. culture of daphnia

GENERAL REFERENCES[7]

Muscles (Contractility)

Bayliss, W. M. "Contractile Tissues"; in Principles of General Physiology, Chapter XIV.

Ponder, Eric. "Muscle"; in Essentials of General Physiology, Chapter VII.

Zoethout, William D. "Muscle-Nerve Physiology"; in Textbook of Physiology, Chapter V.

Blood (Circulation)

Bayliss, W. M. "The Circulation of the Blood"; in Principles of General Physiology, Chapter XXIII.

Ponder, Eric. "Circulation"; in Essentials of General Physiology, Chapter XIV.

Zoethout, William D. Textbook of Physiology, Chapters VII and VIII.

Nerves (Conductivity)

Bayliss, W. M. "Excitation and Inhibition"; in Principles of General Physiology, Chapter XIII.

Ponder, Eric. "Nerve"; in Essentials of General Physiology, Chapter IX.

Zoethout, William D. "Muscle-Nerve Physiology"; in Textbook of Physiology, Chapter V.

Respiration

Bayliss, W. M. "Respiration"; in Principles of General Physiology, Chapter XXI.

Ponder, Eric. "Respiration"; in Essentials of General Physiology, Chapter XIII.

Zoethout, William D. "Gas Exchange: Respiration"; in Textbook of Physiology, Chapter X.

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[7]These experiments are so general in nature that reference to a specific experiment is not profitably made.

ADDITIONAL REFERENCES

APPENDIX I

COMPLETE LIST OF SUPPLIES NECESSARY FOR THE COURSE

A. Chemicals

Acetone

Albumin (egg)

Alcohol (95%)

Automobile oil (a few cc.)

Benzidine

Benzol

Calcium chloride

Calcium hydroxide (lime water)

Carbon tetrachloride

Carmine

Charcoal

Chloroform

Congo red

Copper oxide (CuO)

Copper sulphate

Crystal violet

Dextrin

Eosin

Ether

Fehling's solution (1 and 2)

Ferric chloride

Gelatin

Glucose

Glycerin

Gum Arabic

Hydrochloric acid

India ink

Iodine solution

Lead acetate

Magnesium sulphate

Mercuric chloride

Mercury

Methylene blue

Nitric acid

Neutral red

Olive oil

Paraffin

Pepsin

Peptone

Potassium bisulphate

Potassium chloride

Potassium dichromate

Potassium ferrocyanide (crystals)

Potassium hydroxide

Potassium iodide

Potassium permanganate

Silver nitrate

Soap solution

Sodium bicarbonate

Sodium carbonate (crystals)

Sodium chloride

Sodium hydroxide

Sucrose

Sudan III

Sulphuric acid

Xylol

Zinc sulphate

B. General Apparatus

This list includes equipment most of which is commonly found in any physiological laboratory. The articles enumerated in the first column are used only in the last group of experiments--Section VII. Each piece of equipment in this column will suffice for from three to six students, depending on the wishes of the instructor or equipment available. The list follows:

Burette (50 cc.)

Dry cells (3)

Electrodes

Electro magnet

Frog board

Heart lever

Induction coil

Simple key

Kymograph

Kymograph paper

Mason jars (2 qt. size. Hose connection in lid)

Muscle clamp

Pipettes (100 cc.)

Balances and weights

Capillary tubes

Evaporating dishes

Flat pieces of glass (such as old lantern slides)

Mortar

Parchment paper (for dialyzers)

Plates (white)

Rubber tubing

Thermometers

Water bath

C. Miscellaneous Supplies

Many of the articles enumerated in this group are perishable and can be obtained, the day they are to be used, from any grocery store. Cultures of ameba and daphnia may be secured locally, in some pond or pool; or if this is not the case, they may be ordered from a Biological Supply house[1]. The following list contains all articles of such nature as was suggested above or of some irregular classification.

Ameba culture

Apples

Beets

Butter and cream

Cotton (absorbent)

Cotton cloth (well laundered)

Corn starch

Corn syrup

Daphnia

Eggs

Elodea leaves

Grass roots (freshly sprouted on filter paper)

Green leaves (cottonwood or other hard leaves)

Lard or tallow

Liver (fresh)

Milk

Nut meats

Potatoes

Rats

Slides
- Allium
- Ameba (prepared)
- Blood of frog

Spirogyra

Tad poles

Wheat flour

D. Individual Equipment

Some articles will be needed repeatedly and for this reason they are not included with the lists of materials found at the end of each experiment. Other supplies and materials will be

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[1]See page 81 for a list of firms from which biological supplies can be secured.

obtained from the stock room or from shelves provided for them. Students will be charged for excessive use and breakage. The following list of materials should be provided for each student, or group of students:

Beakers (3)
- small
- medium
- large

Blotting paper

Bunsen burner

Celluloid millimeter scale

Corks (for test tubes)

Dissecting instruments
- scalpel
- fine pointed scissors
- small forceps
- pair of dissecting pins

Filter paper

Glass funnel

Graduated cylinder (25-50 cc.)

Lens paper

Litmus paper

Medicine droppers (3)

Microscope
- slides (3)
- cover glasses (12)

Pipette (1 cc.)

Ring stand and clamp

Sewing needles (2)

Stender dishes (3)

Test tubes (12)

Thistle tube

Tooth picks

Watch glasses (3)

Wire gauze

E. Reagents and Solutions

Benedict's Solution

Fehling's Solution

Millon's Fluid

Molisch's Reagent

APPENDIX II

AIDS FOR MAKING SOLUTIONS AND REAGENTS CALLED FOR IN THE COURSE

A. General Instructions for Making Solutions

In making the usual type of solution called for, such as a 3% solution of sodium chloride, place 3 g. of sodium chloride in a beaker, dissolve, and fill up to 100 g. with water. (In all cases distilled water will be the solvent used unless otherwise specified.)

In making solutions of egg albumin, starch, or other substances of colloidal nature, put the desired weight of solute in suspension and fill up to 100 cc. with water.

A molar solution contains 1 gram molecular weight of dissolved substance per liter (1000 cc.) of solution. Thus a molar solution of sodium chloride would contain 58.5 g. of sodium chloride in a liter of solution; a M/10 solution would contain 5.85 g., a M/100 solution .58 g., etc.

A normal solution contains 1 gram molecular weight of dissolved substance divided by the hydrogen equivalent of the substance per liter of solution. Thus in solutions such as NaOH, KOH, and others with just the hydrogen equivalent of the substance, a normal solution is also a molar solution.

The following steps will aid in making a dilute solution (in per cent concentration) from a stronger one of a known concentration:

1. Take as many cc. of the more concentrated solution as will be the desired per cent strength in the distilled solution.

2. Dilute with water until the volume in cc. equals the per cent strength of the original solution.

Thus, if a 30% solution of alcohol is desired, and only a 95% solution is available, take 30 cc. of the 95% alcohol and dilute it to 95 cc. with water.

B. Table of Formulae and Molecular Weights of All Inorganic Compounds Used

Name of Compound	Formula	Molecular Wt.
Calcium chloride.....	$CaCl_2 \cdot 6H_2O$	219.1
Calcium hydroxide.....	$Ca(OH)_2$	74.1
Carbon tetrachloride.....	CCl_4	153.8
Copper oxide.....	CuO	79.6
Copper sulphate.....	$CuSO_4 \cdot 5H_2O$	249.7
Ferric chloride.....	$FeCl_3 \cdot 6H_2O$	270.3
Hydrochloric acid.....	HCl	36.5
Iodine.....	I_2	253.8
Lead acetate.....	$Pb(C_2H_3O_2)_2 \cdot 3H_2O$	379.3
Magnesium sulphate.....	$MgSO_4 \cdot 7H_2O$	246.5
Mercuric chloride.....	$HgCl_2$	271.5
Mercury.....	Hg	200.6
Nitric acid.....	HNO_3	63
Potassium chloride.....	KCl	74.6
Potassium dichromate.....	$K_2Cr_2O_7$	294.2
Potassium ferrocyanide.....	$K_4Fe(CN)_6 \cdot 3H_2O$	422.4
Potassium hydroxide.....	KOH	57.1
Potassium iodide.....	KI	166
Potassium permanganate.....	$KMnO_4$	158

Name of Compound	Formula	Molecular Wt.
Potassium bisulphate.....	$KHSO_4$	136.2
Silver nitrate.....	$AgNO_3$	169.9
Sodium carbonate (crystals).	$Na_2CO_3 \cdot H_2O$	124
Sodium bicarbonate.....	$NaHCO_3$	84
Sodium chloride.....	NaCl	58.5
Sodium hydroxide.....	NaOH	40
Sulphuric acid.....	H_2SO_4	98.1
Zinc sulphate.....	$ZnSO_4 \cdot 7H_2O$	387.6

C. Reagents and Solutions[1]

Benedict's Solution is a modification of the Fehling solution, superior in that it does not deteriorate upon long standing. It reacts in the presence of uric acid and yields a precipitate with surprisingly small quantities of glucose present. It is made up of the following:

Copper sulphate..... 17.3 grams

Sodium citrate.....173.0 grams

Sodium carbonate.....100.0 grams

Fehling's Solution is composed of two very definite solutions--a copper sulphate solution and an alkaline tartrate solution--which may be prepared as follows: Copper sulphate solution 34.65 grams of copper sulphate dissolved in water and made up to 500 cc. Alkaline tartrate solution 125 grams of potassium hydroxide and 173 grams of Rochelle salt (sodium-potassium tartrate) dissolved in water and made up

[1]Note: These solutions may be obtained in the prepared form from chemical supply houses.

to 500 cc. These solutions should be kept in rubber-stoppered bottles and mixed in equal volumes when needed for use. This is done to prevent deterioration.[2]

Iodin Solution for starch tests is prepared by dissolving 4 grams iodin and 6 grams potassium iodide in 100 cc. of water. (This may be diluted still more for delicate starch tests.)

Millon's Fluid is made by dissolving 1 part (by weight) of mercury with 2 parts (by weight) of nitric acid (sp. gr. 1.42) and diluting the resulting solution with 2 volumes of water.

The Molisch Reagent is used as a general test for carbohydrates. It is composed of a 15 per cent alcoholic alpha naphthol solution.

D. Comparison of Metric with English Measures

Length

Metric	English
1 meter	39.37 inches
1 centimeter	.4 inch
1 millimeter	.04 inch
1 micron	.00004 inch
2.5 centimeters	1 inch

Volume

1 liter	1.05 liquid quarts
1.1 liters	1 dry quart
.95 liter	1 liquid quart
28.35 centimeters	1 fluid ounce
16.38 cubic centimeters	1 cubic inch

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[2]Philip B. Hawk. Practical Physiological Chemistry, p. 25.

Weight

1 gram	15.43 grains
1 kilogram	2.2 pounds
453.6 grams	1 pound
28.35 grams	1 ounce

BIOLOGICAL SUPPLY HOUSES

The following list of well-known biological supply houses is suggested, for the convenience of those using this manual:

Central Scientific Co., Chicago, Illinois.

Chicago Apparatus Company, Chicago, Illinois.

General Biological Supply House, Chicago, Illinois.

Marine Biological Laboratory Supply Department, Woods Hole, Massachusetts.

Southern Biological Supply Co., Natural History Building, New Orleans, Louisiana.

Western Biological Laboratories, Lincoln, Nebraska.

ANNOTATED BIBLIOGRAPHY

Barrows, Henry R. Biological Types and Principles. Richard R. Smith, Inc., New York, 1931, 395 pages.

A laboratory manual in college biology. Proceeds by the "type-principle" method.

Bayliss, Sir William Maddock. Principles of General Physiology. Longmans, Green, and Co. Ltd., London, 1927, Fourth Edition, 882 pages.

The general physiologists "Bible". A monumental compilation of material, with a very complete bibliography.

Cohen, Julius B. Theoretical Organic Chemistry. Macmillan and Co., Limited, London, 1925, 604 pages.

A college text of organic chemistry. Useful for information on all organic compounds used in preparation of this thesis.

Haupt, Arthur W. Fundamentals of Biology. McGraw-Hill Book Company, Inc., New York, 1928, 358 pages.

An elementary college textbook in Biology.

Hawk, Philip B. Practical Physiological Chemistry. P. Blakiston's Son and Co., Philadelphia, 1923, Eighth Edition, Revised, 693 pages.

A book designed for use in course in practical physiological chemistry in schools of Medicine and Science.

Hickman, Cleveland Pendleton. Laboratory Manual in College Physiology. The Macmillan Company, New York, 1930, 116 pages.

A manual for elementary physiology in colleges and normal schools.

Loeb, Jacques. Studies in General Physiology. The University of Chicago Press, Chicago, 1905, Part I, 423 pages.

A collection of papers, on diverse subjects, all pertaining to General Physiology. Contains Papers I to XV.

_____ Part 2, 782 pages.

A continuation of Part 1. Contains Papers XVI to XXXVIII inclusive.

Maximov, N. A. A Textbook of Plant Physiology. McGraw-Hill Book Company, Inc., London, 1930, 381 pages.

An English translation of a great Russian text. It is edited by A. E. Murneek and R. B. Harvey, two authoritative American workers in plant physiology.

Ponder, Eric. Essentials of General Physiology. Longmans, Green and Co., New York, 1929, 497 pages.

A very satisfactory textbook for college students of general physiology.

Raber, Oran. Principles of Plant Physiology. The Macmillan Company, New York, 1928, 377 pages.

A teachable text for elementary students in plant physiology.

Rosenthal, I. General Physiology of Muscles and Nerves. D. Appleton and Company, New York, 1881, 325 pages.

A pioneer attempt to write a connected account of the General Physiology of Muscles and Nerves.

Scarth, G. W., and Lloyd, F. E. An Elementary Course in General Physiology. John Wiley and Sons, New York, 1930, 258 pages.

A theoretical textbook to be used by teachers, or as a reference by students. Laboratory course included.

Stewart, G. N. A Manual of Physiology with Practical Exercises. W. B. Saunders and Company, Philadelphia, 1900, Fourth Edition, 894 pages.

The title signifies the nature of the book.

Stirling, William. Outlines of Practical Physiology. P. Blakiston's Son and Co., Philadelphia, 1901, Third Edition, Revised and enlarged, 402 pages.

A chemical and experimental physiology with reference to practical medicine.

Verworn, Max. General Physiology. Macmillan Co., New York, 1899, Second Edition, 695 pages.

Deals with the history and method of physiological research, elementary vital phenomena, and the general conditions of like.

Williams, Jesse Feiring. A Text-Book of Anatomy and Physiology. W. B. Saunders Company, Philadelphia, 1924, 523 pages.

A physiology text for schools of nursing, normal schools and colleges.

Zoethout, William D. A Textbook of Physiology. The C. V. Mosby Company, St. Louis, 1928, Third Edition, 664 pages.

A very teachable book admirably suited to more advanced students of physiology.

_____. Laboratory Experiments in Physiology. The C. V. Mosby Company, St. Louis, 1928, 251 pages.

A detailed and well illustrated laboratory guide for physiology laboratory work.

INDEX TO EXPERIMENTS