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LABORATORY DIRECTIONS  
IN  
GENERAL PHYSIOLOGY

BY

E. N. HARVEY

PRINCETON UNIVERSITY





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1913



## PART I.

BIOLOGICAL CHEMISTRY :	PAGE.
CARBOHYDRATES .....	9
Polysaccharides (Starch, Dextrine, Glycogen, Cellulose) ..	9
Monosaccharides (Dextrose, Laevulose, Galactose) .....	10
Disaccharides (Saccharose, Lactose, Maltose) .....	11
LIPOIDS .....	12
Fats and Oils .....	12
Lipins (Lecithin and Cholesterin) .....	13
PROTEINS .....	14
Typical protein (egg albumin) .....	14
Derived albumins or albuminates or metaproteins .....	17
Albumoses or proteoses and peptones .....	17
Plant proteins .....	18
EXTRACTIVES .....	18
SALTS .....	19
ENZYMES .....	19
Hydrolysing enzymes (Inverting, Diastatic, Lipolytic and Proteolytic Enzymes) .....	23
Coagulating enzymes .....	23
Oxidizing and reducing enzymes .....	23
Special characteristics of enzyme action .....	25
Enzymes and metabolism in cells .....	27

## PART II.

PHYSICAL CHEMISTRY OF CELLS .....	29
A. SOLUTION AND DIFFUSION .....	29
a. General Phenomena .....	29
b. Semipermeable Membranes and Osmosis .....	30
c. Force of Diffusion—Osmotic Pressure .....	31
B. CELL PERMEABILITY .....	33
C. SURFACE TENSION AND RELATED PHENOMENA .....	35
a. The surface film .....	35
b. Forms of fluids produced by surface tension .....	36
c. Principle of least or minimal surfaces .....	36
d. Internal pressure due to curved films .....	37
e. Changes in surface tension in fluids .....	37
f. Surface tension between several substances .....	38
g. Formation of films under the influence of surface tension .....	39
h. Force of evaporation .....	40
i. Amoeboid movement .....	40

D. COLLOIDAL SOLUTIONS .....	41
a. Suspension colloids .....	42
b. Emulsion colloids or Hydrophilous colloids.....	42
c. Swelling processes .....	43
d. Osmotic pressure of colloids .....	43
e. Effect of salts on colloids in living tissues.....	44

PART III.

PHYSIOLOGY OF MOVEMENT .....	46
I. MUSCLE PHYSIOLOGY .....	46
STRIATED MUSCLE .....	46
a. Methods of Stimulation.....	46
b. Phenomena of Contractility and Irritability.....	48
c. Graphic record of Contractions.....	51
d. Effect of various factors in Muscle Contraction.....	53
SMOOTH MUSCLE .....	58
HEART MUSCLE .....	58
II. NERVE PHYSIOLOGY .....	58
a. Nerve Fibers .....	58
b. Nerve Cells. ( Physiology of the Central Nervous System) .....	63
a. Reflexes .....	63
b. The brain .....	66
III. BIOELECTRIC CURRENTS.....	68
IV. CILIARY MOVEMENT .....	70
V. PROTOPLASMIC ROTATION .....	71

PART IV.

PHYSIOLOGY OF NUTRITION (Including Circulation and Respiration) .....	72
A. METABOLISM .....	72
I. Holophytic Metabolism .....	72
II. Holozoic Metabolism .....	73
B. CIRCULATORY SYSTEM .....	80
I. Physiology of Heart Beat .....	80
II. Physiology of Heart Muscle .....	82
III. Inhibition of Heart.....	83
IV. Effect of various factors on character of rhythm....	84
V. Pressure and Velocity conditions in the circulation..	86
C. RESPIRATION .....	88
I. Respiration by lungs .....	88
II. Oxidation in the tissues.....	88

## INTRODUCTION

The Laboratory Directions in General Physiology contained herein have been used in the course at Princeton in a somewhat modified form for the past two years. They have been printed as an easy solution of the problem how best to prepare directions for the student in laboratory courses. While not attempting completeness in the field of General Physiology they will be found to cover subjects of wider biological interest than those considered in the Physiology Course of the Medical School. Many of the experiments, particularly in nerve-muscle physiology, have been taken from Cannon's "Laboratory Course in Physiology", Porter's "Introduction to Physiology", and Stewart's "Exercises in General Physiology" and I acknowledge my indebtedness to these sources. I express also my deepest thanks to Dr. R. S. Lillie for many suggestions and for correcting the proof. A number of the experiments performed in the General Physiology Course at the University of Pennsylvania under his direction are embodied in this book.

E. N. H.

June 1, 1913



## GENERAL DIRECTIONS

Each student will need a notebook *kept for this purpose alone*. The notebooks will be taken up and examined at intervals.

Students are to work in pairs but each student is to observe the results of every experiment himself. In some cases it will be necessary for the students to observe experiments performed by the instructor. In this case each student is to keep notes exactly as when he performs the experiments himself.

The notes should include an account of the apparatus, preparations made, etc., a statement of what is done in carrying out the experiments or observations, a statement of the results, and finally of what the experiments show. While the notes should not be too voluminous, there is much greater likelihood of erring on the side of making them too brief than too long. *Complete sentences* should always be used and a connected account given, that will be perfectly intelligible to a reader. Sketches, or better, diagrams, of apparatus, figures of structures, etc., should be given wherever possible; they are more important and take the place of long descriptions.

Any pertinent matter in the way of explanations, etc., which the student desires to have on record may be written in the book, but should be distinguished from the results of his own observations, experiments, and reasoning by being enclosed in parentheses. The instructor will then understand that such matter is not put forward as original results.

Enter into each experiment in the spirit of research. Always read the directions carefully and obtain an idea of the general mode of procedure and purpose of the experiment. Do not call upon the instructor at every hitch but endeavor to overcome the difficulty yourself. If you suspect the result of your experiment incorrect consult the instructor before repeating it. Remember that the value obtained from a laboratory course will depend on your own zeal and ingenuity.

In Physiology, as in any experimental science, the best results are only obtained if the apparatus is in good working condition and *clean*. Students should never allow liquids, spilled on the table, or apparatus to remain there, and before leaving the laboratory,

the glassware used must be cleaned and instruments and reagents returned to their proper places. Be careful not to mix the stoppers of reagent bottles. Report any breakage to the instructor at once.

For a description of the apparatus used in this course the student is referred to the catalogue of the Harvard Apparatus Co., makers of physiological instruments. Howell's or Starling's Physiology is recommended for outside reading in connection with both lectures and laboratory work.

## PART I

### BIOLOGICAL CHEMISTRY

*Some characteristics of the substances which make up Organisms*

#### CARBOHYDRATES

Chief of physiological importance are: polysaccharides ( $C_6H_{10}O_5$ )<sub>n</sub>, monosaccharides ( $C_6H_{12}O_6$ ), and disaccharides ( $C_{12}H_{22}O_{11}$ ).

#### I. POLYSACCHARIDES.

##### A. STARCH.

1. *Native Starch.* Mount a scraping from a slice of potato in water and examine under the microscope. Study the structure of the starch grains. Draw. Run a drop of dilute iodine solution under the cover-glass. What is the reaction? Perform the same experiment with corn-starch, and arrow-root or some other type of starch, noting carefully any differences.

2. *Polarization phenomena.* Demonstration—Starch grains exhibit characteristic light and dark bands when viewed by polarized light. Examine the starch under the micropolariscope. Notice the change in position of the bands as the analyzer is rotated. Draw carefully, making the drawing large enough to show details clearly. The principle and descriptions of a polariscope will be found in Carhart's Physics (pp. 323-4). The Nicol prism in the stage of the microscope is the polarizer; that on the tube is the analyzer. Inserting a starch grain between these two prisms is comparable to inserting a selenite plate between two Nicol prisms, except that the starch grain is doubly refractive in crossed bands. Read carefully the account given in the Physics.

3. *Solubility.* Grind a little commercial starch in a mortar and shake with *cold water*. Filter and test the filtrate with iodine. Test solubility in boiling water. Note character of the resulting solution. Cool a portion of a strong solution in a test-tube and note result. Dilute and filter. To dilute starch paste add a drop

or two of iodine solution. Note the result. Heat and cool again, noting results. Try action of (1) alkali (10% KOH) and (2) acid (10% HCl) on the starch iodide.

#### B. DEXTRINE.

A product of the boiling of starch in weak acids (hydrolysis).

4. Note the physical properties, taste, and solubility in water, both hot and cold.

5. Test with iodine, as under starch.

#### C. GLYCOGEN.

"Animal starch." Found in animal cells, particularly liver and muscle.

6. Examine as under dextrine.

#### D. CELLULOSE.

Forms the cell-walls of plant cells.

7. Cotton fibre is almost pure cellulose. Note its insolubility in water and alcohol. Solubility in strong HCl,  $H_2SO_4$ , or  $HNO_3$ ? Strong alkali (10% KOH)? Does it react with iodine? Treat with 50%  $H_2SO_4$  and then add iodine. Result?

8. *Molisch test.* A test for carbohydrates in general, even those combined in the protein molecule. To a few starch grains in 2cc. water add a drop of  $\alpha$ -naphthol solution (20%  $\alpha$ -naphthol in 95% alcohol) and an equal volume of concentrated  $H_2SO_4$ , allowing the acid to run down the side of the test-tube and collect in the bottom without mixing. At the contact of sulphuric acid and starch suspension, a violet ring (furfural) will form indicating carbohydrates.

9. Apply the above test to egg albumin. Any result? What do you conclude?

## II. MONOSACCHARIDES. ( $C_6H_{12}O_6$ ) SUGARS.

A. DEXTROSE (GLUCOSE OR GRAPE SUGAR). B. GALACTOSE.

#### C. LEVULOSE.

Examine each as follows:

10. Note the physical properties, solubility and taste. Make about a 1% solution in hot water. Test solution as follows:

11. Add iodine. Any color reaction?

12. *Moore's test.* Add one-fourth its volume 10% KOH and heat gradually to boiling point. Note color change and odor.

13. Power of reducing metallic oxides is characteristic of many sugars. Some have aldehyde (aldoses), others ketone (ketoses)

structure. Test the reducing power of a simple aldehyde, formaldehyde ( $\text{CH}_2\text{O}$ ): place a few drops 3%  $\text{CuSO}_4$  sol. in a test-tube, add a little formalin, then an equal volume 10%  $\text{KOH}$ . Note each stage carefully. Now heat slowly to the boiling point. Result? Explain.

14. Repeat the last test with dextrose. *Trommer's test*: add a few drops 3%  $\text{CuSO}_4$  to the dextrose solution, then half its volume 10%  $\text{KOH}$  and boil. Note the result carefully. Trommer's test is in principle like Fehling's test but less delicate.

15. Boil a little Fehling's solution in a test-tube. Result? Then add some sugar solution and boil again. Result? Fehling's solution is kept in two bottles. Mix equal parts before using. One contains  $\text{CuSO}_4$ ; the other sodium potassium tartrate (Rochelle salt) and  $\text{NaOH}$ . The sodium potassium tartrate is added to hold the  $\text{Cu}(\text{OH})_2$  precipitated by the alkali in solution. Fehling's test is very important; it is used *e.g.* to detect the presence of sugar in the urine in cases of diabetes. Be sure you understand the reactions underlying the test.

16. *Phenyl-hydrazine test*. An important means of distinguishing different sugars. In a test-tube place a little solid phenyl-hydrazine hydrochlorate with an equal bulk dry sodium acetate; then add 10 cc. dextrose solution and heat in a water-bath at  $100^\circ$  for one hour. Filter hot. Cool the solution and examine carefully under the microscope. Crystallized compounds, *Osazones*, are formed, which are characteristic for each sugar.

### III. DISACCHARIDES ( $\text{C}_{12}\text{H}_{21}\text{O}_{11}$ ).

SUCROSE, LACTOSE, MALTOSE.

17. *Cane-sugar*. (*Saccharose or sucrose*.) Note crystals, taste and solubility as above. Make a 1% solution. Apply Moore's and Fehling's tests. Is it a *reducing sugar*? Why not?

18. Examine *lactose* (*milk-sugar*), and *maltose* (*malt sugar*), a product of starch hydrolysis, in the same manner as sucrose. Note carefully any differences between these disaccharides. Record the results of your tests with the six sugars in the form of a table.

19. *Inversion of cane-sugar*. Boil the solution with a few drops of conc.  $\text{HCl}$  for a few minutes. Cool, neutralize, and apply Fehling's test. Result? Explain. What sugars are formed?

20. *Formation of sugar from starch*. Boil dilute starch paste with a little 20%  $\text{H}_2\text{SO}_4$  till the fluid is clear. Neutralize and test for glucose. Result? Also test with iodine. Result?

21. Has starch reducing properties? Has dextrine? Has cellulose? Test all three with Fehling's solution.

## LIPOIDS (*Fats and Lipins*)

### I. FATS AND OILS.

22. Note the *physical properties*, differences in melting points, etc., of fats, butter, beef-tallow or lard, and olive oil.

23. Test *solubilities* of these fats in (a) *water*, hot and cold; (b) alcohol, hot and cold; (c) chloroform; (d) ether. *Never bring ether near a flame*. Allow the chloroform to evaporate overnight, in a covered glass vessel, and note the fat crystals formed.

24. *Emulsification*. Shake a few drops of olive oil with water in a test-tube. What happens? Set the tube aside for a few minutes. What happens? Now repeat, using 1%  $\text{Na}_2\text{CO}_3$  instead of water and note the difference in result. Explain. Shake *neutralized* olive oil in a test tube with 1%  $\text{Na}_2\text{CO}_3$ . Note and explain difference from ordinary olive oil.

25. *Saponification*. To some emulsified oil in a test-tube add *strong KOH* and boil; formation of soap or saponification results. Is the product soluble in water? Draw off some of the clear solution and to it add  $\text{CaCl}_2$  solution. Result? Explain. Write the equations for these reactions.

26. To a portion of clear soap solution add some 20%  $\text{H}_2\text{SO}_4$  and heat. Note the separation of droplets at the surface. What is the substance separated? Explain.

27. *Salting out of soaps*. To clear soap solution add  $\text{NaCl}$  crystals to saturation. Note the flocculent precipitate. Remove some of this, dissolve in water and test for soap. Result?

28. *Sudan III test for fat*. Place a drop of olive oil on filter paper and immerse in an alcoholic solution of Sudan III. Then wash quickly in alcohol. Does the oil take up the dye?

29. Test the solubility of fatty acid (oleic acid) in pure water, alcohol and chloroform. Add some 1%  $\text{Na}_2\text{CO}_3$  solution to oleic acid and shake. Result? Explain.

30. *Pressure of glycerine in fats*. Place a little glycerine in a test-tube and heat with some powdered acid potassium sulphate ( $\text{KHSO}_4$ ). Note the characteristic odor (acrolein test for glycerine). Repeat with olive oil or other fat in a clean test-tube. Result? What is the chemical composition of a fat?

## II. LIPINS.

31. *Cholesterin*. Demonstration of the iodine-sulphuric acid test. Cholesterin crystals are treated with fairly concentrated  $\text{H}_2\text{SO}_4$  and one drop of a very dilute iodine solution. Note the crystalline form and play of colors. Draw.

32. *Lecithin*. Preparation from hen's egg yolk. To the alcoholic mixture of egg yolk given out add one-half its volume of ether, shake and set aside in a tightly stoppered bottle till the next laboratory period. Then filter into an evaporating dish and evaporate over a *water bath* to dryness, taking care the ether does not catch fire. Dissolve the residue in as small an amount of ether as possible and add four or five volumes of acetone, which precipitates the lecithin. Collect on a glass rod, allow the acetone to evaporate and use in the following tests.

Note the physical properties, consistency, color, etc., of lecithin. Is it soluble in water? in alcohol? in chloroform?

33. *Production of "myelin forms"*. Place a small piece of lecithin in water under a cover-glass. Examine from time to time. Note especially the surface. Draw. Remove the cover-glass and try drawing out the surface with a glass rod or needle. Note the very fine filaments formed.

34. *Presence of glycerine in lecithin*. Try the acrolein test. See under Exp. 30.

35. *Presence of nitrogen*. Heat strongly in a dry test-tube a small piece of lecithin. Suspend in the mouth of the tube a strip of moistened neutral litmus paper and a strip of lead acetate paper. Is the paper changed in color? Due to the formation of what substance? What would the lead acetate paper detect if it blackened in color?

36. *Presence of phosphorus*. Heat a small piece of lecithin with a fusion mixture consisting of 5 parts KOH to 1 part  $\text{KNO}_3$  in a crucible, until colorless. Cool, dissolve in a little warm water, acidify with a few drops of conc.  $\text{HNO}_3$  and add molybdic solution. A yellow precipitate indicates the presence of P.

37. *Presence of fatty acid*. Add a small piece of lecithin to a few cc. of sodium alcoholate in an evaporating dish and evaporate to dryness. Take up with water and add  $\text{CaCl}_2$  solution. A white curdy precipitate indicates fatty acid. What is this precipitate?

Note that lecithin is like a fat in many ways, yet differs in important respects.

## PROTEINS

### I. TYPICAL PROTEIN (EGG ALBUMIN).

38. Carefully pour the white of an egg into an evaporating dish. Save the yolks; they will be collected by the instructor. This is an approximately 12% solution of a protein (albumin). Notice its viscous consistency. Observe that it can be drawn out into rather long cords or cylinders; compare with water in this respect. Test the reaction with litmus paper; is it acid, alkaline, or neutral? Of one-half of the egg-white make a 10% solution. To do this, place in an evaporating dish and cut the egg-white freely with the scissors; this liberates it from the membranes. Then mix with nine times its volume of water, stirring thoroughly. A precipitate of globulin, another protein, forms. Filter through several folds of wet linen. Keep the undiluted half for further use.

#### A. COAGULATION.

39. *Coagulation by heat.* Have a water-bath with water at boiling temperature. Put some of the undiluted egg-white and the 10% egg-white in test-tubes and place in the water-bath. Which coagulates first? What conclusion can be drawn as to the effect of dilution on coagulation by heat?

40. *Coagulation by chemicals.* To 5 cc. of the 10% solution add a few drops of 3% copper sulphate. Note the coagulation. Using a new specimen of solution (10%) at each trial, try in the same way mercuric chloride and lead acetate, recording results. Try also strong nitric, hydrochloric, and sulphuric acids, in the following way: allow a drop or two of the acid to run down the side of the tube till it reaches the solution. Try also 95% alcohol, picric acid, tannic acid, phosphotungstic acid, and  $K_4Fe(CN)_6$ , adding first a little dilute acetic acid in the case of the last four reagents.

41. *Manner in which coagulation takes place.* Dip a thin thread of silk in a 3% solution of copper sulphate and lay the thread on a glass slide, beneath a cover glass. Allow some of the 10% solution of white of egg to run under the cover glass while observing the operation with the microscope (high power). The albumin about the thread will be seen to form small granules, appearing like a fine cloud, and these later run together to form an open network. Draw one or two stages in this process.

#### B. CHEMICAL CHARACTERISTICS.

42. *Presence of C, N, and S.* Place a little powdered dry albumen in the bottom of a dry test-tube, and insert at the mouth of

the tube a small piece of moist red litmus paper and a piece of moist lead acetate paper (strip of filter paper soaked in 2% lead acetate and dried). Heat the lower end of the tube, and note the characteristic odor of burned feathers. If ammonia is evolved (showing the presence of N and H), the litmus paper will turn blue; if sulphur, the lead acetate will turn black, through formation of lead sulphide. If the albumen chars black, the presence of C may be inferred. State the results of your conclusions as to the presence of C, N, H, and S in protein.

#### C. TESTS FOR PROTEIN.

43. *Xanthoproteic reaction.* Dilute some of the 10% egg-white till it is about 2%, and place in a test-tube. Add a few drops of conc. nitric acid. What occurs? Boil. What occurs, as to color and other changes? Cool the solution and add ammonia. Be careful that the contents of the tube do not shoot out. Note the color produced (this is the essential feature in xanthoproteic reaction). Describe and fix in mind this reaction.

44. Does gelatin give the xanthoproteic reaction? Try in the same way a weak solution of gelatin. Does fibrin (obtained from the blood)? Try this reaction also with a small piece of meat, and a small piece of bone or cartilage.

45. *Millon's reaction.* To a little of the dilute solution of albumin add a few drops of Millon's reagent. (This has been made as follows: dissolve one part of mercury in an equal weight of [cold] nitric acid. Then add the solution to twice its volume of water, allow to stand some hours, then decant off the liquor from the sediment.) Note the white precipitate formed when Millon's reagent is added to the albumin. Boil two or three minutes, and observe the result?

46. Does gelatin give Millon's reaction? Does meat? First boil a small piece of meat to destroy the red color. Does bone?

47. *The biuret test.* To a little of the dilute albumin solution add an equal volume of 10% potassium hydrate and add one or two drops (or more is necessary) of ½% copper sulphate. Notice the violet color. Try the biuret reaction with gelatin, caseinogen and peptone solutions.

48. *Adamkiewicz's reaction.* Place in a dry test-tube ten drops of strong sulphuric acid and 20 drops of glacial acetic acid containing glyoxylic acid. To this mixture add a little dried albumin and warm slightly. Notice the reddish violet color, due to tryptophane. Try this with some dry gelatin.

40. *Heller's ring test.* Place a half inch of strong nitric acid in a test-tube. Pour upon this, allowing it to run gently down the side of the tube, a little of the weak albumin solution. Note the white cloud at the junction of the two substances; this is Heller's test for albumin. It is used to test the presence of albumin in the urine.

#### D. DIALYSIS OF PROTEINS.

50. Add an equal volume of 10% NaCl solution to some undiluted white of egg and place in a collodion or parchment paper bag, or animal membrane. Place the bag in a jar of water in such a way that only the lower part (covered by the membrane) is in contact with the distilled water. After two hours, observe whether any considerable amount of the albumin has passed through the membrane. Determine whether any has passed through by testing the water in the jar by one or two of these tests given above. Has NaCl passed through? Test with 2%  $\text{AgNO}_3$  solution.

#### E. ACTION OF NEUTRAL SALTS. "SALTING OUT."

51. Add NaCl crystals to a solution of egg white to saturation. Any precipitation? Filter and test the filtrate for protein. Is precipitation complete? Test the residue on the filter for protein. Note the similarity to the salting out of soap by NaCl.

52. Repeat Exp. 51, using powdered  $(\text{NH}_4)_2\text{SO}_4$  crystals instead of NaCl. Which salt is more effective as a precipitant? Egg albumen is purified by repeated salting out with  $(\text{NH}_4)_2\text{SO}_4$ , which precipitates completely the protein.

#### F. ACTION OF ACIDS AND ALKALIES.

53. Protein is *amphoteric*, i.e., it will combine with both acid and alkali. Use a solution of Merck's powdered egg albumin in distilled water, filtered. Pour a few cc. into a test-tube and a similar amount of distilled water into another test-tube. Add a drop of neutral red solution to both tubes. Then add very carefully drop by drop from a pipette  $n/50$  NaOH until the neutral red indicator is changed yellow in each tube. Compare the amounts required in the albumin tube with the amounts required for distilled water. What do you conclude from this experiment?

54. Repeat the above but use 1% alizarin as an indicator instead of neutral red and  $n/10$  HCl instead of  $n/50$  NaOH. What does this experiment show?

## II. DERIVED ALBUMINS OR ALBUMINATES OR METAPROTEINS.

55. *Acid albumin.* To a 10% solution of egg-white add an equal volume of 0.2% HCl and heat in the water-bath to about 40° C. for a few minutes. Acid albumin is formed. Heat part of this solution. Any coagulation? Color with litmus and add 0.2% KOH from a pipette to neutralization. Result? Add more KOH. Result?

56. *Alkali albumin.* Add to 10% egg-white solution an equal volume of 0.2% KOH and warm at 40° as before. Heat a part of solution. Any coagulation? Color with litmus and neutralize carefully with 0.2% HCl. Result? Add more HCl. Result?

57. Add 10% KOH to *undiluted* egg-white in a test-tube. Result? "Lieberkühn's jelly" is formed which is solid alkali albumen and will dissolve on warming with water at 40° C. Try similarly glacial acetic acid. Result? (Acid and alkali albumin are the first products formed in the digestion of protein in the stomach and in the intestine, respectively.)

## III. SIMPLER PROTEINS. ALBUMOSES OR PROTEOSES AND PEPTONES.

Use a solution of *Witte's "peptone"*, prepared by dissolving the products of a gastric digest (consisting chiefly of albumoses and peptones) in distilled water. Note carefully the character of the reaction in each case for comparison with the reactions of pure peptones, to be prepared by salting out the proteose. Test as follows:

58. Heat a portion to boiling. Any coagulation?

59. Acidulate with acetic acid and add a little  $K_4Fe(CN)_6$ . Result? Warm. Result? Cool as before. Result?

60. Try precipitation with 2% tannic acid, 95% alcohol, saturated  $HgCl_2$  solution, 3%  $CuSO_4$ , 2% lead acetate, and picric acid. Note in each case the effect of warming and cooling on the precipitate.

61. Try the Millon reaction and Adamkiewicz reaction on a little dry peptone powder.

62. *Salting out of proteoses and peptones.* Saturate a portion of the "Witte's Peptone" solution with solid  $(NH_4)_2SO_4$  and filter. True peptones will be separated from proteoses and be found in the filtrate. Use the filtrate for the following tests.

63. Try the biuret test. Note carefully the color, which is distinctive for peptones. Try the biuret test on the same volume of a dilute albumin solution, in order to compare the color with that given by peptones. Fix carefully in your mind the difference.

64. Repeat experiment 59. Result?

65. Try also precipitation by picric acid, tannic acid, 95% alcohol, sat.  $\text{HgCl}_2$ , 3%  $\text{CuSO}_4$  and 2% lead acetate as before.

66. *Diffusibility*. Determine whether "Witte's Peptone" solution will diffuse through parchment or collodion within a period of two hours.

Compare in a table the reactions of peptone and egg albumin.

#### IV. PLANT PROTEINS.

67. Preparation of an *alcohol-soluble protein (gliadin)* from wheat flour. Flour contains chiefly starch, gliadin, glutenin, albumin, globulin, and a protease. Examine some wheat flour under the microscope and apply the iodine test. Result?

68. Apply Millon's test to flour in a test-tube. Result?

69. Gliadin may be extracted by alcohol. To a small amount of flour in a bottle add some (about 30 cc.) 70% alcohol, and shake at intervals during one hour. Then filter and use in the following tests.

70. Evaporate a small portion in a dish over the water bath. Note the scales of gliadin. Are they soluble in water? Determine this by shaking with water in a test tube, removing the scales and testing the water by the biuret test.

71. Add 100% alcohol in excess to a small amount of the 70% alcohol solution. Result?

72. Pour some of the alcohol solution of gliadin into water. Result:

73. Heat to boiling. Any coagulation?

74. Note that gliadin differs from egg albumin in many important respects. Yet it is a protein. Try the xanthoproteic and Millon's tests with some dry gliadin scales.

#### EXTRACTIVES

Under this head are included a large number of substances such as the alkaloids, glucosides, pigments, purin bases, urea, etc., too numerous to be considered in detail in the laboratory.

## SALTS

All living matter contains salts, chiefly the chlorides, sulphates and phosphates of Na, K, Ca, Mg and Fe. The rôle of the salts in the organism will be considered under the physiology of the various tissues.

### FERMENT ACTION—ENZYMES

In the preceding experiments the breaking down of biological compounds has been studied from the point of view of pure chemistry. The compounds were split by boiling acids or alkalies. That similar decompositions may be effected at low temperatures by substances present normally in living tissues is shown by the following experiments.

It is very essential in these experiments that the temperature be as near that indicated as possible.

#### I. HYDROLYZING ENZYMES.

##### A. INVERTING FERMENTS. INVERTASES.

80. *Invertase*. Thoroughly grind up one-half of a cake of yeast with sand and water in a mortar. Filter. Test the filtrate for sugar with Fehling's sol. Any sugar in yeast? Then mix equal volumes of the yeast extract and cane sugar solution and keep at 40° for 10 or 15 minutes. Then test with Fehling's solution. Result? Conclusion? Invertase may similarly be demonstrated in the intestinal mucosa.

##### B. AMYLOLYTIC OR DIASTATIC FERMENTS. AMYLASES.

81 A. *Plant Diastase*. Crush thoroughly about 5 gms. of germinating barley with sand in a mortar with a little water. Filter. Then mix the filtrate with 10 cc. of starch paste, warm to 40°, and keep in water bath at 40° for an hour. Note any change in the character of the liquid. Test with iodine and Fehling's solution. Note the taste of the resulting liquid. Explain these results. Compare with a control in which the extract was *boiled* before incubating with starch.

81 B. *Salivary diastase*. *Ptyalin*. Collect a few cc. of saliva into a beaker (flow of the secretion may be accelerated by chewing paraffin); dilute the saliva with about 5 volumes of water; filter. Make the following mixtures: A, 5 cc. of equal volumes dilute starch paste and saliva; B, the same, using saliva that has been previously *boiled*; C, the same mixture as A + 5 drops 10% HCl; D,

mixture A + 5 drops 10% KOH. Warm all four tubes to 40° and keep in a water bath at 40° for 10 minutes. Test each mixture for starch (by iodine) and for sugar (by Fehling's solution). Results? Draw conclusions as to the influence of boiling and of free acid and alkali on the activity of ptyalin. Also try the action of ptyalin in the cold. Immerse a test-tube with a mixture of starch and saliva (cooled previously to mixing) in ice water and test for sugar later. What is the influence of *temperature* on enzyme action?

82. *Precipitation.* Collect 1 or 2 cc. of filtered saliva in a test tube and add 5 times its volume of 95% alcohol. When the white precipitate (of ptyalin and mucin) has settled, pour off the alcohol and dissolve the precipitate in the same amount of water as the volume of alcohol previously added. Take about 3 cc. of this, add an equal amount of starch paste and place in the water bath at 40° C. Examine at intervals. Does the starch paste become clear? Test with iodine solution and Fehling's solution. Compare with the preceding experiment.

83. *Stages of Starch Hydrolysis with Ptyalin.* Mix starch paste and dilute (1:10) filtered saliva as above, warm to 40°, and keep warm by holding the tube in the hand. At half-minute intervals transfer with a glass rod a drop of the mixture to a drop of iodine solution previously placed on a white plate. Note the progressive change in color reaction. Explain. Give the reaction which occurs.

#### C. FAT-SPLITTING OF LIPOLYTIC FERMENTS.

84. *Lipase.* Use artificial pancreatic juice made by dissolving an extract of pancreas (commercial pancreatin) in 1%  $\text{Na}_2\text{CO}_3$  solution.

a. Place in each of two test-tubes 2 drops of *neutral* olive oil + 5 cc. of 1%  $\text{Na}_2\text{CO}_3$ ; warm and shake. Result? To one test tube add 5 cc. artificial pancreatic juice; to the other, the same, boiled. Warm and place both in the water bath at 40°. Examine at intervals of a few minutes. Does emulsification occur in either tube? Explain.

b. Place in each of two test-tubes 2 drops of olive oil, and shake. Add to one tube 5 cc. pancreatic extract; to the other 5 cc. pancreatic extract *previously* boiled. Place at 40° C. After 1 hour test each for soap as follows: The emulsified oil will separate to a large extent at the top of the tube. Remove some of the relatively clean liquid from the bottom of the tube with a long pipette. To

it add a drop of *neutral* olive oil and shake. Does emulsification occur?  $\text{CaCl}_2$  cannot be used as a soap test here because pancreatic juice gives a precipitate with  $\text{CaCl}_2$ .

c. Milk test. Into each of two test-tubes, a and b, place 5 cc. *neutral* milk and a drop of litmus solution. To A add 5 cc. neutral pancreatic juice; to B, the same *previously* boiled. Set at  $40^\circ$  for 40 min. Does the litmus become red in one tube? Why? Write the reaction.

85. Does pancreatic juice hydrolyse starch? Perform an experiment to test this. Test also hydrolysis of cane sugar.

86. *Progress of fat-splitting (or lipolysis)*. *Rachford's experiment*. Arrange a series of several watch-glasses each containing 1%  $\text{Na}_2\text{CO}_3$ . Mix in a test-tube 2 cc. neutral olive oil and 1 cc. pancreatic extract. Warm to  $40^\circ$ ; shake the tube, then allow the oil to separate, and transfer a drop by a pipette to the soda-solution in watch-glass 1. Again shake and after an interval allow the oil to separate as before and place a drop in watch-glass 2. Repeat this procedure several times. The changes in the oil produced by allowing the lipase to act for progressively longer and longer periods are seen. Note carefully changes in behavior of the oil on passing along the series of watch-glasses.

#### D. PROTEIN-SPLITTING OR PROTEOLYTIC FERMENTS. PROTEASES.

87. *Pepsin*. Enzyme active in acid solution. Use an artificial gastric juice made by extracting commercial scale pepsin with warm 0.2% HCl.

Take six clean test-tubes labeled A to F, and place in each a few shreds of fibrin, or strips of hard boiled egg-white. To the tubes add the following:

- a. 0.2% HCl.
- b. Artificial gastric juice.
- c. The same, but previously boiled.
- d. Gastric juice carefully neutralized (using as indicator very weak neutral red solution) with 0.2% KOH added from a pipette drop by drop.
- e. Gastric juice neutralized as in d and then rendered alkaline with an equal volume of 1%  $\text{Na}_2\text{CO}_3$ .
- f. Distilled water.

Warm and place in a water bath at  $40^\circ$ . At intervals note any changes occurring in the digests, especially in b. After forty minutes test each solution for protein by the biuret test, after removing

the undigested fibrin. Compare the color of the biuret test given by tubes b to e. Result? Conclusions as to conditions of activity of pepsin? Does 0.2% HCl alone effect hydrolysis of proteid? Note especially difference in appearance of a and f.

88. *Examination of pepsin-HCl digest.* Allow a solution (5 cc.) of egg-white to digest overnight with pepsin-HCl. Then examine the digest as follows: A. Neutralize; is there any precipitate? What is this precipitate? B. Filter; to a part of filtrate apply ferrocyanide or picric acid test; result? Conclusion? C. Saturate the remainder of the filtrate from A with  $(\text{NH}_4)_2\text{SO}_4$  and filter; apply the biuret test to a portion of the filtrate; result? Conclusion? Add bromine water to the remainder of the filtrate. Any coloration?

2. Does *acid alone* digest proteid? Boil a few thin strips of egg-white for some time (at least 20 minutes) in 10% HCl. Cool and apply the biuret test; result? Does pepsin digest proteins in the absence of free acid?

89. *Trypsin*—an enzyme occurring in pancreatic juice and active in alkaline solution. Use a solution of trypsin in 1%  $\text{Na}_2\text{CO}_3$ . Take 5 test-tubes and to each add a strip of fibrin. Then add:

- 5 or 10 cc. 1%  $\text{Na}_2\text{CO}_3$ .
- 5 or 10 cc. trypsin solution.
- The same, exactly neutralized with 1% HCl, using 1 drop of neutral red as an indicator.
- The same as c + equal volume of 0.2% HCl.
- 5 or 10 cc. trypsin solution previously boiled.

Place in the water bath at 40° C., and observe at intervals to see if digestion occurs. After 40 min. remove the undigested fibrin and test for peptone with biuret reagent. Note especially the color of the biuret reaction in each case. In which is peptone formed?

90. *Examination of tryptic digest.* Let 5 cc. egg-white digest overnight with pancreas extract. Examine as follows:

- Filter the digest. Saturate a portion of the filtrate with ammonium sulphate; filter and test the filtrate for peptone.
- To another portion of the filtered digest add gradually *bromine water*. Note the ensuing coloration. Compare with gastric digest. (*Tryptophane* test.)

c. Separation of amino acids (leucin and tyrosin). Evaporate the remainder of the original filtrate to the consistency of a syrup. Then add alcohol gradually to this syrupy solution until no further precipitate forms, stirring continually with a glass rod. What

substances are precipitated? Gather the precipitate together with a glass rod so far as possible and filter the remainder of the mixture through a dry filter. This filtrate contains the amino acids. Concentrate to a syrupy consistency, transfer to a flask and allow to stand until next day for crystallization. Crystals of leucin and tyrosin separate out. Examine under microscope, identify and draw.

91. Examine the preparations of amino-acids; glycocoll, tyrosin, leucin. Test water-solubility, coagulation by heat and response to protein color reactions (xanthoproteic, Millon's, biuret). Note carefully differences from and resemblances with protein.

92. *Vegetable protease. Bromelin.* Cut a pineapple into small portions and express the juice into a mortar. Filter. Test the reaction of filtered juice. Place strips of fibrin in four test-tubes and add as follows: to a: filtered juice (unaltered); to b: the same, exactly neutralized with 0.2% KOH; to c: the same made alkaline with an equal volume of 1%  $\text{Na}_2\text{CO}_3$ ; to d: pineapple juice previously boiled. Place at  $40^\circ$  and examine at intervals as with other ferments. Note carefully any differences from pepsin or trypsin. In which tube is digestion most rapid? After 40 minutes remove the undigested fibrin from each tube and test for peptone by the biuret reaction.

## II. COAGULATING ENZYMES.

93. *Rennin.* Place 10 cc. of milk in each three test-tubes. To a add 3 cc. rennin solution; to b the same, previously boiled; to c add a few cc. dilute  $\text{NH}_4$  oxalate solution and then 3 cc. rennin solution. Place at  $40^\circ$  for 15 minutes, examining at intervals. Result? After 20 minutes add a few drops  $\text{CaCl}_2$  solution to c. Result? Explain.

## III. OXIDIZING AND REDUCING ENZYMES.

94. *Catalase.* To 5 cc. neutralized *hydrogen peroxide* ( $\text{H}_2\text{O}_2$ ) in a test-tube add about 3 cc. crushed liver suspension. Note result. What is the reaction? Repeat using liver which has previously been *boiled*. Result? Also (a) using a mixture of 5 cc. of liver suspension and 5 drops of 10% HCl, (b) 5 cc. of liver suspension and 1 cc. of 1%  $\text{Na}_2\text{CO}_3$ , (c) liver suspension plus equal vol. n/10 KCN solution, (d) liver suspension plus equal vol. saturated  $\text{HgCl}_2$  solution. In which of the above is an evolution of gas

most active? What do you conclude from results of experiments a, b, c, and d?

95. *Oxidases.* Oxidizing enzymes existing in the cells of organisms are readily soluble in water. Vegetable cells yield extracts of special oxidative activity. The initially colorless surface of a pared potato is turned brown by the oxidation of paraoxy-phenyl products such as tyrosin in the potato-cells. The brown pigment produced is a melanin and the enzyme is tyrosinase. Tyrosinase has the power of oxidizing oxidizable compounds, like phenol, added to the juice as well as the tyrosin already present in the juice.

Into each of eight test-tubes place 5 cc. of filtered potato juice, made by scraping potatoes on a grater, expressing and filtering the result through cheese cloth; the starch is allowed to settle and the supernatant fluid removed, filtered and used in the tests. (Place the starch in the vessel destined for it, to be later purified.) Treat the portions as follows:

- a. Potato Extract + 5 drops of toluol.
- b. Extract boiled, then cooled and 5 drops toluol added.
- c. Extract + 5 drops 10% HCl.
- d. Extract + 5 drops of 10% KOH.
- e. Extract + 5 drops phenol (1%).
- f. Extract boiled, then cooled, and 5 drops 1% phenol added.
- g. Extract + 5 drops guaiac solution.
- h. Extract + 5 drops  $\alpha$ -naphthol + 5 drops para-phenylene diamine solution.
- i. To another tube add water + 5 drops  $\alpha$ -naphthol + 5 drops para-phenylene diamine solution.

Thoroughly shake each mixture. Notice any changes in 15 or 30 minutes. Where is the color most marked? Why? Set aside until the next laboratory period and record changes in each. The toluol is added as a preservative. It would kill any cells present but does not destroy the oxidase. Is the phenol a preservative? b, f, and i are control experiments for e and h respectively. Do you see why? Note that potato juice accelerates a process which takes place slowly in the absence of potato juice, as in i.

96. *Catalase and oxidase in animal tissues.* a. Tease finely with a pair of needles portions of the *spleen, liver, lung, kidney,* and *muscle* of the frog. Place portions of these tissues in a series of watch-glasses and add to each dilute  $H_2O_2$ . Which tissue gives the most pronounced catalytic action?

- b. To a similar series of watch-glasses containing the same

freshly teased tissues add an alkaline mixture of *α*-naphthol and *p*-diamino benzene solutions in 0.7% NaCl. Note the results. Arrange the tissues in the *order of their activity* in accelerating this oxidation.

97. *Localization of indophenol formation in blood corpuscles of the frog.* Prepare 4 cc. of a mixture of equal parts saturated solution of alpha naphthol in an alkaline NaCl solution ( $m/8$  NaCl +  $m/100$   $\text{Na}_2\text{CO}_3$ ), and 1% di-methyl para-diamino benzene in 0.7% NaCl. Add this mixture to a few drops of a suspension of frog's blood corpuscles in a solution of  $m/8$  NaCl +  $m/100$   $\text{K}_2\text{C}_2\text{O}_4$  in a watch-glass. Mount two or three drops of this mixture on a slide so as to have several air bubbles under the cover. In the course of a few minutes the indophenol will appear in the cells.

In which cells does it appear first? Where is it chiefly localized in the cell? Is there any relation between the position of the air bubbles and the rate of formation of the oxidation product?

98. *Oxidation test for blood with guaiac tincture.* Apply a little guaiac suspension containing hydrogen peroxide to a blood stain. Note the result. Other dried animal tissues give a similar reaction.

#### IV. SPECIAL CHARACTERISTICS OF ENZYME ACTION.

99. *Specificity of enzyme action.* Collect saliva, dilute with four volumes of water and filter. Place about 3 cc. in each of five test-tubes. Then add:

- a. 5 cc. of starch paste.
- b. 5 cc. of 5% cane sugar solution.
- c. One drop of olive oil + 5 cc. 1%  $\text{Na}_2\text{CO}_3$ .
- d. A shred of fibrin + 5 cc. of 0.2% HCl.
- e. 5 cc. milk.

Set tubes a to e (inclusive) in water bath at  $40^\circ$  for 1 hour, and then test the tubes as follows:

Tube a, for starch and glucose.

Tube b, for glucose.

Tube c, for soap by emulsification.

Tube d, for peptone.

Tube e, note if coagulation occurs.

What do you conclude as to the action of ptyalin in the saliva?

100. *Reversibility of enzyme action.* Many enzymes are reversible in their action, i. e. they can accelerate either decompositions or syntheses of the substances on which they act, according to the con-

concentrations of these substances in the solution. Lipase is the most favorable enzyme for the demonstration of this property. Take great care to use the exact amounts called for in the directions.

a. *Hydrolytic*, or splitting, action of lipase. Label 2 small flasks or test-tubes a and b. In a place 10 cc. of neutral pancreatic extract and *exactly* 1 cc. of ethyl butyrate ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{COO.C}_2\text{H}_5$ ). Cork tightly and keep at  $40^\circ$  for 40 minutes. Remove to ice water until quite cold. Color with litmus. What is reaction? Now empty the contents into a beaker and titrate *immediately* with  $n/20$  NaOH, i.e. add the alkali carefully from the burette until the color just changes to blue. Note as exactly as possible the amount of alkali required. To flask b add the same quantities of ethyl butyrate and pancreatic juice *previously boiled* and proceed in the same manner. Compare the results of a and b. Write the equation for the splitting of ethyl butyrate; of glycerol butyrate (butter fat).

b. *Synthetic action of Lipase*. Mix 25 cc. of  $n/20$  butyric acid and 10 cc. 20% ethyl alcohol. Place 20 cc. in each of two flasks, a and b; to a add 5 cc. pancreatic extract; to b 5 cc. pancreatic extract previously boiled. Cork both flasks tightly and place in a water bath at  $40^\circ$  for 40 minutes. Then compare the *odor* from the two flasks. Is the ethyl butyrate to be detected? In which flask? How formed? Write the equation. Empty the contents of each flask into a beaker and titrate with  $n/20$  KOH, using litmus as an indicator. How much is required for each flask? Do your results indicate that some acid has disappeared through synthesis to form the ester?

101. *Influence of temperature on enzyme action. (Quantitative determination.)* Collect saliva, dilute with 7 volumes of water and filter. Add 5 cc. dilute starch paste to each of four test-tubes. Place one test tube in the water bath at  $40^\circ$  C., and keep another at room temperature (about  $20^\circ$  C.). Have two series of iodine drops ready on a porcelain plate. When the test tubes have attained the proper temperatures add an equal volume of dilute saliva to each tube, mix thoroughly, and keep at their respective temperatures. At intervals of one-half minute remove a drop of the mixture and test for starch. One student attend to the tests from  $40^\circ$  saliva mixture; the other to the  $20^\circ$  mixture. Determine the time required to convert the starch into products giving no color reaction with iodine at each temperature.

Perform exactly the same experiment using the *same* diluted saliva but keep one test tube at  $0^\circ$  C. and compare it with another

at 20° C. Record your results in the form of a table. How near in agreement are the two determinations at 20° C.? What is the increase in rate of enzyme action for a rise of 20° C.?

102. *Rate of decomposition of H<sub>2</sub>O<sub>2</sub> by catalase at different temperatures.* Place 25 cc. of dilute filtrate from crushed liver suspension in a bottle provided with a perforated cork and bent or flexible tube; place carefully in the bottle, *without spilling*, a small vial with 5 cc. H<sub>2</sub>O<sub>2</sub> solution. Insert the end of the bent tube below the mouth of a eudiometer or inverted graduate filled with water and placed mouth downward in a beaker of water. Then shake the bottle so as to spill the vial and mix its contents with the liver extract. Measure the volume of O<sub>2</sub> evolved from minute to minute and record the results. Try the experiment with (a) cold mixture (liver-extract and H<sub>2</sub>O<sub>2</sub> cooled just before mixing to about 0° C.), (b) mixture at room temperature (20°), and (c) at 40°. Estimate the relative rates of action at the different temperatures.

103. *Effect of concentration of enzyme on enzyme action. (Quantitative determination.)*

Collect saliva and filter. Add 5 cc. dilute starch paste to each of two test tubes.

Have a series of iodine drops ready on a porcelain plate as in exp. 101. Add as follows to the two test-tubes:

- a. 5 cc. of 1 pt. saliva to 3 pts. water.
- b. 5 cc. of 1 pt. saliva to 7 pts. water.

Keep at 20° C and determine by trials at one-minute intervals when the iodine gives no color reaction with the mixture.

After making the first series of experiments perform the same experiments using the same saliva diluted as follows:

- c. 5 cc. starch paste + 5 cc. of 1 pt. saliva to 15 pts. H<sub>2</sub>O.
- d. 5 cc. starch paste + 5 cc. of 1 pt. saliva to 31 pts. H<sub>2</sub>O.

Keep at 20° C. and determine by trials at two-minute intervals the time required to convert the starch to a substance giving no color with iodine.

## E. ENZYMES AND METABOLISM IN CELLS.

In living cells syntheses, hydrolyses, and oxidations, all of which are under the influence of enzymes, interact in the metabolic processes. The interdependence of syntheses and oxidations is well shown in Pasteur's experiment. The metabolism of the cell varies according to the quantity of oxygen available.

104. *Relation of oxidations to synthetic processes in yeast cells. Pasteur's experiment.* a. Take 1/16 of a yeast cake and mix thoroughly in a mortar with 20 cc. water. Add 10 cc. of this mixture to 100 cc. of nutrient fluid. Mix thoroughly, and while the yeast is uniformly suspended divide into two equal parts, a and b. Place a in a bottle provided with a cork and exit-tube to carry off  $\text{CO}_2$ ; this tube should end below the surface of water in a beaker. Portion b should be poured into one or more wide jars or dishes to form a thin layer well exposed to air; cover loosely with glass plates. Place a and b aside for 2 days or more. Then examine both carefully, comparing the appearance, relative turbidity, odor, and degree of effervescence. Transfer b to a bottle. Shake each portion (a and b); take equal quantities of each. Centrifugalize and compare the amounts of the sediment. Results? Conclusions?

b. *Alcohol formation in presence and absence of oxygen.* Arrange a simple still by allowing a bent tube from a flask to run to the bottom of a narrow test-tube immersed in cold water. Distill off from both a and b (using a moderate flame) a few cc. of the distillate. Cool the distillates. Compare their smell and taste. Warm each with  $\text{K}_2\text{Cr}_2\text{O}_7$  and  $\text{H}_2\text{SO}_4$ . Result? Conclusion?

c. Collect 1 cc. of gas from a fermenting yeast culture in an inverted test-tube. Test the gas by introducing 10% KOH solution, by means of a bent pipette, into the tube in contact with the gas. Absorption indicates  $\text{CO}_2$ . Why? Write the equation.

## PART II

### PHYSICAL CHEMISTRY OF CELLS

#### A. SOLUTION AND DIFFUSION

##### A. GENERAL PHENOMENA.

The purpose of the following experiments and simple observations is to enable the student to form a concrete idea of the reality and character of diffusion and solubility, and to work out some of their general laws for application later to organic processes.

105. Fill a test-tube, supported on a clamp, to within an inch from the top with *distilled* water. Place in a position where it can remain entirely undisturbed. Then with a long pipette drawn to a slender point, place in the bottom of the test-tube 2 cc. of potassium permanganate solution, in such a way as not to disturb the water and to leave all the purple solution at the bottom. On the outside of the tube mark the level of the potassium permanganate solution. Observe that it very slowly rises, by *diffusion*. Mark the position reached by the end of the laboratory period, and leave the experiment in progress until the next period. Mark the point to which the fluid has then risen. What is the approximate rate of diffusion?

106. Seal off a glass tube at one end and fill with hot 1.5% agar-agar, colored red with neutral red solution. Set in an upright position to solidify. When solidified, place in a bottle of 0.2% KOH open end down. Measure by the change in color of neutral red, the amount of diffusion during the laboratory period. Set aside for measurement next day. What is the rate of diffusion of KOH as thus measured?

107. Fill a petri dish with warm agar-agar solution (1.5%) and allow to cool. When solidified place one drop of each of the following solutions on the agar-agar: a. Saturated  $\text{CuSO}_4$ ; b.  $\text{K}_2\text{Cr}_2\text{O}_7$ ; c.  $\text{KMnO}_4$ ; d. aqueous neutral red; e. aqueous methylene blue; f. haemoglobin; g. chlorophyll solution. Compare the rates of diffusion. Is there any difference? Is it related to the molecular weights of the substances? Cover and leave until the next day when the diffusion may be again noted.

108. Place some alcoholic solution of methylene blue, a few drops at a time, on the surface of water in a watch-glass. Does mixture take place slowly or rapidly? (In this case we have an additional factor involved,—changes in surface tension.) Try in the same way an aqueous solution of methylene blue. Are the results different? Why?

109. Have a beaker full of water which is kept undisturbed, and observe from the side. Place on the surface some powdered methylene blue; observe the solution and diffusion. Describe accurately what you see.

110. a. Place a filament of *Spirogyra* in a dilute solution (1 drop to a watch-glass of water) of neutral red in pond water. Is the dye taken up? Does it become more concentrated than in the external solution? Is this contrary to physical laws? Examine the cells under the microscope and determine the condition in which the dye is held within the cell. Describe.

b. Place in another dilute solution of neutral red some *Spirogyra* filaments that have been killed: (a) by immersion for one minute in chloroform-saturated water, (b) by immersion for one minute in boiling water. How do the results compare with those on living cells? Explain.

111. Repeat experiment 110 with *Elodea* leaves. How is the dye accumulated in these cells? Is this in opposition to physical laws? More concentrated dye may be necessary here. How does *Paramecium* take up neutral red. Try a very dilute dye solution.

## B. SEMIPERMEABLE MEMBRANES AND OSMOSIS.

112. What is a *semi-permeable membrane*? Such membranes may be made as follows: (a) Introduce by a fine mouthed pipette 5%  $\text{CuSO}_4$  below the surface of 2%  $\text{K}_4\text{Fe}(\text{CN})_6$  in a watch-glass. What is the membrane formed? Give the reaction. Observe the membrane closely. Does it change in thickness? Do the drops of  $\text{CuSO}_4$  thus formed change in size? If so, why?

(b.) Tannate of gelatin membranes; a solution of 2% tannic acid containing sugar to ca.  $m/2$  concentration is allowed to flow as above below the surface of a non-gelatinizing 10% gelatin solution. Study the character of the membrane, form, and changes of size as before. What part does the sugar play in this experiment?

113. *Growth through absorption of water.* Suspend by a thread a crystal of  $\text{K}_4\text{Fe}(\text{CN})_6$  in a 3%  $\text{CuSO}_4$  solution and place where it will be undisturbed. Observe the general characteristics and

growth of the resulting formation. Do you note any movements? What are they due to? Draw.

114. *Sea-weed-like formations through growth of precipitation membranes.* Place a lump of fused  $\text{CaCl}_2$  in the bottom of a tall jar filled with concentrated  $\text{Na}_2\text{CO}_3$  solution. Set aside for several days and watch the development of a plant-like growth.

115. *Membranes formed through surface action.* a. Shake a few drops of olive oil in a test-tube with the following fluids (a) water, and aqueous solutions of (b)  $\text{NaCl}$  (0.65%), (c) peptone, (d) soap, (e) haemoglobin, (f) albumin (g) sugar, (h) starch (i) lecithin, and (j) gelatin (2%). In which does a permanent emulsion occur? Why?

b. Perform the above experiment using chloroform instead of olive oil. Results?

116. *Formation of artificial cells surrounded by a film of modified protein.* Shake chloroform with an albumin solution. Can the film be removed by repeated washing in water? Pour some of the chloroform globules into a watch-glass of water and examine under the microscope. Can you see the film? Does the chloroform slowly evaporate?

If lecithin is dissolved in the chloroform, it will absorb water as the chloroform evaporates, and a watery solution of lecithin can be obtained surrounded by a protein film. Such a cell resembles in many of its properties a sea urchin egg. Prepare some of these cells by shaking a few drops of a chloroform solution of lecithin (m/80) with albumin solution, washing with water and pouring the globules into a watch-glass of water. Note from time to time during the course of the hour their appearance under the microscope and draw the stages observed. When chloroform has been completely replaced by water add a drop of neutral red to the water in the watch-glass. Is it accumulated by the cells? Try pricking the cell with a fine needle to determine the consistency of the contents and also of the membrane.

### C. FORCE OF DIFFUSION—OSMOTIC PRESSURE.

The energy of diffusion, or the tendency which two substances have to mix, may be measured by separating them by a membrane through which one of them can pass while the other can not. The latter then produces pressure on the membrane and this pressure can be measured. This is partly accomplished by the following experiment.

117. Pour out a thin layer of collodion solution on a mercury surface in a petri dish and allow it to harden somewhat. While still flexible remove the film from the mercury surface and tie tightly on the end of a thistle tube. Prepare two of these "osmometers". Fill one (*a*) with  $m/2$  sugar-formol solution to a point on the stem one inch above the bulb. Fill the other (*b*) with water to a point within one inch of the top of the tube. Place *a* in water and *b* in  $m/2$  sugar-formol solution in a beaker. Note any change in the level of the meniscus of *a* and *b*. How high does the level of sugar in *a* become? Does it then remain stationary? Explain all the phenomena noted in connection with the experiment, leaving the osmometers set up for several days.

118. Prepare two test-tube collodion bags as follows: The test-tube must be cleaned with alcohol and dried thoroughly. Pour 2 cc. collodion solution into a dry clean test-tube and then pour it out slowly into the collodion bottle, revolving the test-tube so as to coat the glass evenly with a thin film of collodion. Allow this to harden somewhat, then gently pry off the edge and allow some water to run down between the film and the test-tube. By carefully prying and pulling the film may be entirely separated from the glass. Fill with water to make sure there are no leaks. If the collodion bags are perfect fill one (*a*) with  $m/4$  salt solution, and tie with a piece of string about the middle so as to obtain a firm salt water filled bag. Fill the other (*b*) half full of albumin solution and tie off so as to leave some air enclosed. Note that it is not firm. Then place *a* in  $2m$  salt solution and *b* in water. Note any changes in rigidity of the bags? Explanation?

119. Place a drop of frog's blood corpuscles in water in a watch-glass and examine *quickly* under the microscope.

Place a drop of suspended frog's blood corpuscles in  $m$  NaCl solution and examine under microscope.

What happens in each case? Explain. Note similarity to the preceding experiment.

120. *Osmotic pressure in living cells. Plasmolysis.* Place Spirogyra filaments in cane-sugar solution of concentrations:  $m/2$ ,  $m/3$ ,  $m/4$ ,  $m/5$ ,  $m/6$ ,  $m/7$ ,  $m/8$ , and in  $KNO_3$  solutions of the same concentrations, in watch-glasses. Note carefully under the microscope any changes of volume of the protoplasm within the cell walls. What solution just fails to plasmolyse in the case of both sugar and  $KNO_3$ ? Take the average of the filaments in the dish. What is the osmotic pressure of the sap of Spirogyra cells as deter-

mined by sugar plasmolysis? Calculate the isotonic coefficient ( $i$ ) for  $\text{KNO}_3$  at the isotonic concentration. Then calculate the degree of dissociation ( $a$ ) of the  $\text{KNO}_3$  using the formula:

$i = 1 + (K - 1)a$ , where  $K =$  number of ions from one molecule, and  $a$  the proportion of molecules split into ions.

121. Perform the same experiment as above, and make the same calculations, using instead of *Spirogyra* the leaves of the water plant, *Elodea*, only two cell layers in thickness: a layer of large cells above and of smaller cells below.

122. Place blood corpuscles of the frog in  $m/2$ ,  $m/4$ ,  $m/6$ ,  $m/7$ ,  $m/8$ ,  $m/10$ , and  $m/16$   $\text{NaCl}$  solutions. In which solution do the corpuscles retain their volume unchanged? What is the osmotic pressure of this solution if the isotonic coefficient is 1.83?

123. Try plasmolysing the cells of a marine plant (*Ulva*) using  $2m$ ,  $1.5m$  and  $m$  sugar solutions. In which solution does plasmolysis take place? Why are stronger solutions needed?

## B. CELL PERMEABILITY

Dead membranes such as parchment, or collodion or cellulose are readily permeable to crystalloidal substances in general, but not to many colloids. The membrane surrounding living cells is permeable to some crystalloids but not to others, thus exhibiting a "selective permeability", which is characteristic of both animal and plant cells. Some of the permeability relations of living cells are brought out in the following experiments.

124. *Permeability to non-electrolytes.* Place *Elodea* leaves in the following solutions of both  $m/2$  and  $m/3$  concentrations in watch-glasses: Cane sugar, grape sugar, urea, glycerine, alcohol. Which of these solutions have the same osmotic pressure? Do they all plasmolyse? Note carefully whether plasmolysis is permanent (till the end of the laboratory period) when it occurs, or only temporary. How do you explain any differences noted? Which substances penetrate the cells most rapidly?

125. *Permeability to salts.* Place *Elodea* leaves in  $m/2$  and  $m/3$  solutions of common salt and  $\text{KNO}_3$ . Have these solutions the same osmotic pressures? Have they the same osmotic pressures as the above mentioned solutions? Is plasmolysis permanent in both salt and  $\text{KNO}_3$ ? Leave in the solutions for 1 hour or longer. What does this indicate?

126. *Permeability to alkalis.* *Elodea* leaves stained in neutral red are to be used. Note if they show protoplasmic rotation. If the

alkali enters, the neutral red will be stained yellow. Place in  $n/40$  NaOH,  $\text{Ba}(\text{OH})_2$ , and  $\text{NH}_4\text{OH}$ , and record the time required for the change of color to occur in each case. As soon as the leaf is wholly yellow place in pond water. Does the red color return? Which leaves have been killed? Does recovery of protoplasmic rotation occur?

127. Impermeability to NaOH may be shown in a striking manner by the following experiment: Place an Elodea leaf stained in neutral red in  $n/40$   $\text{NH}_4\text{OH}$  till yellow; then remove it to  $n/40$  NaOH. Does it become red?

128. Stain Paramecia in a watch-glass of *pond water* to which only a *trace* of neutral red has been added. Place a drop densely crowded with red stained Paramecia in  $n/500$  NaOH and  $n/500$   $(\text{NH}_4)\text{OH}$  in pond water. Note the time of color change and length of life in each case. Are the Paramecia killed in NaOH before the alkali enters? How about  $\text{NH}_4\text{OH}$ ? Which region of the cell must the NaOH attack?

129. *Change in permeability on death.* Place an Elodea leaf stained in neutral red in  $n/40$  NaOH and another, previously killed by 2 minutes immersion in saturated chloroform water, in the same solution. Which is more permeable?

130. Place small cubes, cut from a beet and washed in running water, in a test-tube containing water. Place similar cubes washed and then heated to boiling for a moment in another test-tube of water. Note from time to time the amount of diffusion in each case, of the red pigment from the beet cubes. Significance?

131. The rapid penetration of  $\text{NH}_4\text{OH}$  into cells is probably to be correlated with its *solubility in fat solvents or lipoid substances*. Solubility of  $\text{NH}_4\text{OH}$  in fat solvents may be demonstrated as follows: Shake a xylol solution of lecithin with egg albumin solution containing a few drops of neutral red. Note that the globules of benzol take up the neutral red. Now place some of the latter in  $n/100$   $\text{NH}_4\text{OH}$  and  $n/100$  NaOH in watch-glasses. Which alkali penetrates and why?

132.  $\text{NH}_4\text{OH}$  and NaOH diffuse through dead membranes at the same rate. Demonstrate this by covering the ends of two tubes with a collodion film (made on mercury surface and tied tightly over the tubes), then filling with agar-agar colored with neutral red and immersing the covered end of the tubes in  $n/100$   $\text{NH}_4\text{OH}$  and NaOH respectively. The diffusion rates of NaOH and  $\text{NH}_4\text{OH}$  in pure water are practically the same.

133. *Salts change cell permeability.* Often a pure salt affects the permeability of the plasma membrane so that it cannot be used to determine the osmotic pressure of the cell. A mixture of certain salts (especially salts of Na, K, and Ca) maintains the cell surface in a normal condition.

Place Spirogyra cells in the following solutions:

1. Cane sugar: m/2, m/4, m/6, m/8.
2. NaCl: m/2, m/4, m/6, m/8.
3. NaCl (95 vols.) + CaCl<sub>2</sub> (5 vols.): m/2, m/4, m/6, m/8.

Determine the plasmolytic limiting concentration in each case. The cane sugar will give the true osmotic pressure. Note carefully if at first plasmolysis begins, then disappears and then appears again (false plasmolysis) in any of the NaCl solutions. Compare with the mixtures of Na and Ca. Explanation?

134. *Salts affect the penetration of NaOH.* Place Spirogyra or Elodea, stained in neutral red, in the following mixtures.

1. m/40 NaOH.
2. m/40 NaOH + m/8 NaCl.
3. m/40 NaOH + m/8 (95 vols. NaCl + 5 vols CaCl<sub>2</sub>).

Determine the time required for entrance of alkali in each case. As a control kill the cells in chloroform water and place in the above solutions. Conclusion?

## C. SURFACE TENSION AND RELATED PHENOMENA

Note in all the following observations and experiments that the surface behaves in many ways as if it were a stretched elastic membrane.

### A. THE SURFACE FILM.

Experiments to give a concrete realization of the existence of surface tension.

135. Drop a needle carefully on the surface of still water, so that the entire side of the needle strikes the surface at once. Why does it float? Notice the depression on the surface film close to the needle.

136. Drop water slowly in drops from a pipette. Notice the form of the drops, and how they become stretched or elongated just before they drop. Why do they take the form they do?

137. Form a film on a circular wire frame with soap solution. (This is really a double film.) Does the film pull? Test this by forming a film on a circular wire frame, laying a loop of thread on

the film, and then breaking the film within the loop. What happens?

138. Dip a camel's hair brush in water; notice how it spreads out on the water but closes up when taken out. What pulls the bristles together?

139. *Uses made of surface film by organisms.* Observe any of the following for which there is an opportunity: Spiders, "whirligigs", or other animals moving on the upper surface of the water; flatworms or snails creeping on the under surface of the film.

## B. FORMS OF FLUIDS PRODUCED BY SURFACE TENSION.

140. *Plateau's experiment.* Half fill a glass tumbler with 60% alcohol. With a pipette drop a few drops of olive oil into this; notice that they sink. (If they do not, add a small quantity of 70% alcohol.) Now add a certain amount of 50% alcohol *without stirring*. The drops will be found not to sink to the bottom. If necessary add more 50% alcohol; or, if the drops do not sink at all, add some 70% alcohol. When a mixture has been obtained where the drops sink part way but not to the bottom, it is ready for observation. What form do the drops take? Add a considerable quantity of olive oil with the pipette, until a sphere an inch or more in diameter is produced. Why does it retain the spherical form? Try changing the shape with a glass rod; does it return to the spherical form? Why does such a mass not keep the spherical form when placed on a flat surface? Place a drop of olive oil on water and note its shape.

## C. PRINCIPLE OF LEAST OR MINIMAL SURFACES.

Owing to the pulling of the surface film in all directions *the surface of a fluid tends to become as small as possible under the limiting conditions.*

141. The spherical form of the oil drops or a soap bubble exemplifies this. Why do they take the spherical form?

142. In a disk of paraffin oil floating on 70% alcohol, increase the surface by pulling out projections with a glass rod. What happens to these?

143. Examine a film of soap solution on a circular wire frame. Note that it is flat. Why? Now bend the frame in the form of an elongated loop curved at right angles to the radii of the loop. What is the form of the film on such a loop? Explain in terms of the above principle.

#### D. INTERNAL PRESSURE DUE TO CURVED FILMS.

144. Blow a soap bubble on a glass tube; why is it spherical? note decrease in size when left to itself, due to expression of air (let it blow against a flame). Why must a curved film press inward? Does this internally directed pressure vary with the degree of curvature? How? Determine this by blowing and balancing two unequally sized bubbles on opposite ends of a Y-tube. Which presses air into the other? Explain. Internally directed pressure in inversely proportional to the radius of the spherical drop or bubble.

145. Try deforming with a glass rod olive oil drops of different sizes in the alcohol. (Experiment 140.) Which show most resistance to deformation, the large or the small ones? Which most quickly regain their shapes? Explain.

146. Is the cylinder a stable form when the principle of minimal surface is considered? The following experiment shows how stability is attained when the form of a cylinder is imposed upon a liquid. Make a cylinder of 70% alcohol in paraffin oil, in the following way: Have a layer of alcohol in the bottom of the tumbler under the oil. Take a glass tube about  $\frac{1}{3}$  to  $\frac{1}{2}$  inch in diameter, and holding the finger over one end, put the other end down to the bottom of the vessel, then remove the finger and allow the alcohol to rise in the tube. Now rather gently lift the tube straight out of the oil, when a cylinder of alcohol will be left in the oil. Observe how it instantly breaks up into spheres. (Why does fluid from a spout usually break up into drops?)

#### E. CHANGES OF SURFACE TENSION IN FLUIDS.

147. Different fluids have different surface tensions. Try dropping slowly water and toluol from a pipette. Which has the greater surface tension? How indicated in this experiment?

148. The surface tension of a fluid may be altered by the presence of another substance. Float a *clean* thin rubber band on the surface of a dish of *clean* water. Now touch the end of a glass rod to the surface within the band, the rod having been previously dipped in oil. Result? Explanation? Now touch similarly the water outside the band. Explain the result. Test the influence of alcohol and chloroform on the surface tension of water.

149. Why does a drop of alcohol break a soap bubble? Drop a drop of alcohol on a thin layer of water on a *clean* glass plate. Result? Explain.

150. Place a small piece of camphor on the surface of *clean* water. Result? Explain movements. What determines the direction of the movements? Touch the surface with a rod containing a trace of oil. Explain what happens.

151. *Changes of form due to chemically induced changes of surface tension.* Place a good sized drop of mercury in a watch-glass; note its form and size; now cover with 2%  $\text{HNO}_3$ ; any change? Place near the Hg a crystal of  $\text{K}_2\text{Cr}_2\text{O}_7$ ; result? Describe the phenomena carefully; draw the drops at intervals showing the nature of the form changes. Note the resemblance to amoeboid movement, ingestion of bichromate crystals, movement of drop toward crystal (analogy to chemotaxis).

152. *Movements and formation of projections, due to changes in surface tension in a drop of olive oil.* a. On a slide fasten some glass rods 1 mm. in diameter, a sufficient distance apart so that they will support a rectangular cover glass near its ends. Make a mixture of two parts glycerine and one part 70% alcohol—place on the slide, and cover it. Then with a fine capillary pipette introduce a small drop of clove oil beneath the cover. Observe that the drop changes its shape and moves about. Why? (If the drop does not move or moves too violently, vary the experiment by changing the proportion of glycerine and alcohol in the fluid.)

b. Into such a drop of clove oil prepared as above introduce with a fine capillary a very little 70% alcohol, close to the edge of the drop. Notice the formation of a projection and movement toward this edge. Why?

c. In the same preparation, or a similar one, touch the upper surface of the cover glass near the drop of clove oil with a hot wire. What happens? Why?

153. *Electrically conditioned changes of surface tension.* The phenomena of Exp. 151 are at bottom due to electrical changes. The influence of the current may be shown thus: Dip wires from two or three dry cells on the opposite sides of a watch-glass containing a drop of mercury in dilute acid. Result? Note the direction of movement of the drop relatively to the poles, and also of particles on the surface of the mercury. Make a diagram showing this.

## F. SURFACE TENSION BETWEEN SEVERAL SUBSTANCES.

When a fluid is in contact with another substance (solid, liquid or gas) the degree of the surface tension depends on both substances.

(Perhaps it may be said that it depends on the degree of attraction or repulsion between the particles of the two substances.)

154. In the case of a solid, if there is a strong attraction between the fluid and the solid, the fluid wets the solid. Will water wet clean glass? Will it wet paraffin? Will mercury wet glass? Try this by dipping a piece of the solid into the fluid.

155. Where there is an attraction the fluid may be lifted against gravity. Try this by dipping a plate of glass into the water. Does the water rise at the sides of the plate? Try with a glass tube. Does the water rise in the tube? What is the form of the water surface in the tube? Try water in a paraffined glass tube.

156. This attraction may likewise pull the solid into the fluid. Place a drop of water on the edge of a glass plate. Then take a very small splinter of wood, and with forceps bring one end of it into the drop. Notice how it is pulled in. Observe that the water rises along the splinter so that the spherical surface of the drop is altered. What pulls the splinter into the drop? Show by a diagram.

157. Place a drop of chloroform on the bottom of a watch-glass full of water. Bring against it a piece of hard shellac. What happens? Why?

158. "*Choice*" in a drop of fluid owing to varying surface tension in contact with different substances. With drops of chloroform as in experiment 157, try bringing other substances into contact with it. Are they accepted or rejected? The following should be tried: shellac, glass, paraffin, gum arabic, chlorate of potash, resin, potassium iodide. Is there any relation to the solubility of the substances in chloroform?

159. "*Artificial Diffugia Shells.*" Grind up some glass finely with chloroform in a mortar. Inject drops of this with a fine-pointed pipette into a watch-glass of water. Notice how the glass grains come to the surface and arrange themselves in a layer,—as in a *Diffugia* shell.

The same experiment may be performed with linseed oil in place of chloroform and 70% alcohol in place of water.

## G. FORMATION OF FILMS UNDER THE INFLUENCE OF SURFACE TENSION.

160. *Laws of Gibbs.* Note in a warmed glass of milk the gradual formation of a film at the surface. Prove that film formation is not due to evaporation. Substances that lower the solution

tension of the solvent tend to accumulate at the surface in higher concentration than in the interior and may there form coherent membranes or films. The formation of cell membranes and similar structures in organisms has been thus explained.

#### 11. FORCE OF EVAPORATION.

161. Fill a porous cup with water and place in a beaker of water for 10 or 15 minutes. Then insert a rubber stopper through which passes a glass tube into the porous cup, and fill the whole apparatus with water. Then place the free end of the water-filled tube under mercury in a glass vessel and clamp in an upright position. Has the mercury begun to rise in the tube by the end of the laboratory period? Why? Leave till next day.

#### I. AMOEBOID MOVEMENT.

162. The preceding experiments have brought out various principles bearing on surface tension. The student should now endeavor to apply them to the study of movement in the living organism, amoeba, whose mode of locomotion is commonly attributed to changes in its surface tension. The experiments and observations should be devised by the student and conducted in the spirit of research, in an endeavor to prove or disprove the surface-tension theory of movement. First make sure of the facts by a careful study of the Amoeba from above. Determine which species of Amoeba you are observing from Plate I of Conn's "Protozoa".

The following are suggested as points worthy of notice:

Can a pseudopodium be thrust out freely into the water, or must it be in contact with the substratum?

Observe as exactly as possible the currents of protoplasm in the amoeba and the currents in the water about the amoeba. The latter, if any, may be observed by india ink granules in the water.

Do particles of soot or debris cling to the surface of amoeba move completely around the animal as if it were a bag rolling about on the slide?

Does the amoeba move forward in jerks or gradually?

What is the character of the movement when viewed from the side? Use the specially prepared slide for this purpose. Study carefully; many of the phenomena connected with the movement become clear when examined from the side. Does the amoeba adhere to the substratum? Can it move up a vertical surface?

After a careful study of amoeba, compare with what you find in the two kinds of movement described below.

163. The movements of some species of amoeba may be imitated by causing a drop of water or glycerine to adhere to the substratum more strongly on one side than the other. This can be done as follows: Place a piece of smooth cardboard in the bottom of a flat dish, and on a certain spot on the paper place a drop of water. Then cover the whole with oil, which soaks into the paper everywhere except in the spot covered by the drop of water. After it is well soaked remove the drop of water and oil and proceed with the experiments. Mix some soot with a drop of water, or better, glycerine, and place this on the surface of the cardboard near the spot that was protected. Allow one side of the drop to come against the protected spot. What happens? How does the movement resemble that of amoeba and how does it differ? Study the movement of particles on the surface of such a drop. Lycopodium powder sprinkled on the drop will make clear the surface movements.

Or does amoeba move in the manner of the drop indicated below?

164. Make a mixture of equal parts glycerine and water, add some bone-black, and place two or three drops on a clean mercury surface. Mercury is *not wet* by the mixture. Powder lightly the surface of the drop with lycopodium. Now place a very small drop of 95% alcohol at one side of the glycerine drop. Which way does the drop as a whole move? Make a diagram of the currents within (as shown by the lamp-black) and the currents on the surface (as shown by the lycopodium). Do they agree with those observed in amoeba?

165. Perform exactly the same experiment but use a *clean* glass surface instead of mercury. The glass surface is *wet* by the glycerine water mixture. Do the results obtained here agree with those observed in amoeba?

What are your conclusions in regard to the cause of movement in amoeba?

#### D. COLLOIDAL SOLUTIONS

In colloidal solutions the particles of solute are large and consist of many molecules (distinction from solutions of crystalloids, e.g., sugar). Hence these solutions resemble suspensions in many of their properties. Usually the colloidal particles are electrically charged. The charge keeps the particles permanently suspended by preventing their union to form larger particles; it also determines many of

the properties of the solution. In some colloidal solutions the particles are *positively*, in others *negatively* charged. The chemical behavior of the colloid is largely determined by the sign of the charge on the particles.

#### A. SUSPENSION COLLOIDS (SUSPENSIDS).

166. The colloids to be studied are ferric hydroxide (positive) and arsenious sulphide (negative). Proceed as follows:

a. Note the physical properties (optical properties, viscosity, diffusion in pure solvent, filterability); diffusion through collodion membranes.

B. *Action of non-electrolytes.* To 3 cc. of each solution add its volume of m-sugar solution. Result?

C. *Action of electrolytes. Ion action.* Using 3 cc. of ferric hydroxide hydrosol in each experiment, add six drops of each of the following solutions. Shake gently and note the result carefully in each case.

a.  $n/20$  HCl,  $n/20$   $H_2SO_4$ ,  $n/20$   $H_3PO_4$  or  $n/20$   $H_3C_6H_5O_7$  (citric acid).

b.  $n/20$   $NH_4OH$ ,  $n/20$  KOH or NaOH,  $n/20$   $Ba(OH)_2$ .

c.  $m/20$  NaCl,  $m/20$   $Na_2SO_4$ ,  $m/20$   $Na_3$  citrate.

d.  $m/20$  NaCl,  $m/20$   $CaCl_2$ ,  $m/20$   $AlCl_3$ .

167. Repeat this series with arsenious sulphide hydrosol. What differences do you find in the action of the acids, bases and salts upon the two hydrosols? What relations do you find between *precipitating power* and *valence* of the ions of the electrolyte? Which ions precipitate the *positive* and which the *negative* colloid? Explain.

168. Mix equal volumes of the two hydrosols. Any result? Explain.

#### B. EMULSION COLLOIDS OR HYDROPHILOUS COLLOIDS (EMULSIDS).

In this class of colloidal solutions the union with the solvent (water) is more intimate. These colloids incorporate or combine water spontaneously; the colloidal particles are thus probably water-swollen or hydrated, and hence not so sharply separated from the medium as in the suspensoid hydrosols. Their solutions are more like crystalloid solutions, and the conditions of precipitation from solution are different from those shown by suspensoids.

## C. SWELLING PROCESSES

169. Arrange a series of test-tubes of uniform diameter and place in each tube 1 gram of granulated gelatin. Determine the relative degrees of swelling undergone by the gelatine in the following solutions. The gelatine is mixed with 10 cc. of the solution and allowed to stand until the height of the swollen gelatine in the tube is constant.

- a. distilled water.
- b.  $n/5$ ,  $n/10$ ,  $n/20$ ,  $n/40$  HCl.
- c.  $n/5$ ,  $n/10$ ,  $n/20$ ,  $n/40$  NaOH.
- d.  $n/5$  NaCl,  $n/5$   $\text{Na}_2\text{SO}_4$ .
- e.  $n/20$  HCl +  $n/10$  NaCl;  $n/20$  NaOH +  $n/10$  NaCl.

Note the influence (a) acid, salt, and alkali acting alone; (b) optimum concentration for swelling; (c) action of acid and alkali in presence of neutral salt. Antagonisms of this latter kind are of great physiological importance.

## D. OSMOTIC PRESSURE OF COLLOIDS.

Direct determinations of osmotic pressure are difficult to make with crystalloid substances because of the difficulty of preparing satisfactory semi-permeable membranes. On the other hand, semi-permeable membranes for colloids are easily prepared, so that, although the osmotic pressure is low, there is no difficulty in measuring it directly. The osmotic pressure of colloids varies with their "state of aggregation", and this varies with the concentration of the electrolytes present in solution along with the colloid, and also with several other conditions: as (1) rate of admixture of electrolyte; (2) degree of mechanical agitation to which the solution has previously been exposed; (3) the temperature; and (4) in general, the lapse of time and the nature of the previous history of the colloid (method of preparation, etc.).

170. A simple and efficient osmometer is made as follows: make a collodion membrane of the shape and capacity of a 50 cc. round-bottomed flask; this is done as follows: Pour a moderate quantity of the 10% collodion solution (in equal parts alcohol and ether) into a 50 cc. flask; invert the flask and turn till an even layer of solution is formed on its walls; pour back the surplus solution into the bottle; blow a current of air into the flask through a glass tube; then add some warm water and change this two or three times. The membrane is then ready to remove from the flask; removal is facilitated

by first running a stream of water between the membrane and the glass wall. Prepare three membranes.

Prepare the following solutions:

- a. 50 cc. 2% egg albumin plus 10 cc. distilled water.
- b. 50 cc. 2% egg albumin plus 10 cc.  $m/8$  NaCl.
- c. 50 cc. 2% egg albumin plus 10 cc.  $m/8$   $\text{NaCl}_2$ .

In the outer vessel of each osmometer add respectively:

- a. distilled water.
- b.  $m/48$  NaCl.
- c.  $m/48$   $\text{CaCl}_2$ .

Use the same volume of outer fluid, e.g., 420 cc. in each osmometer. Fill each membrane with its corresponding solution; insert the rubber cork and manometer tube into the neck of the membrane (excluding air-bubbles) and bind in position with a rubber band. Then place the membrane in position in its corresponding outer fluid and clamp the manometer tube in a vertical position.

Note the rise of the fluid in the manometer tubes and the different rates of rise. What is the maximum pressure in each solution? What do you conclude as to the influence of salts on the osmotic pressure of colloids?

171. The presence of acid and alkali *increases* the osmotic pressure of certain proteins, e. g., gelatine. This action is prevented by the presence of neutral salts in appropriate concentrations. Determine the osmotic pressure of the following solutions:

- a. 1% gelatine.
- b. 1% gelatine containing HCl to  $n/300$  concentration.
- c. 1% gelatine containing NaOH or KOH to  $n/300$  concentration.
- d. and e. Same as *b* and *c* but containing also NaCl to  $m/48$  concentration.

Remember that the outer fluid contains the same electrolytes in the same concentration as in the colloidal solution.

Compare the action of electrolytes on *osmotic pressure* with their action on the *swelling* process.

## E. EFFECT OF SALTS ON COLLOIDS IN LIVING TISSUES.

Since the solid portions of living tissues are colloids, it is to be expected that electrolytes will have a marked influence on vital activities. The following experiments show the importance of electrolytes for the activity of ciliated cells.

172. Separate carefully with a pair of needles a number of filaments from the gills of an oyster or clam. The filaments will remain living in sea-water. Prepare the following solutions. In each experiment transfer several of these filaments with forceps to a clean dry watch-glass; then add several cc. of the solution whose action is to be tested. Examine the filaments in the solutions at frequent intervals and determine as accurately as possible the action of each solution, as follows:

(a) The *character* and *duration* of the ciliary movement. If the cilia are still active at the end of the period cover the watch-glass and examine again next day.

(b) Are there any visible structural changes as a result of its action (swelling of cells, breakdown of cilia, etc.)?

a. Pure isotonic solution of the chief chlorides of sea-water:  $m/2$  NaCl,  $m/2$  KCl,  $m/2$   $MgCl_2$ ,  $m/2$   $CaCl_2$ .

b. Combinations of two chlorides (to show antitoxic action of salts).

(a) 25 vols.  $m/2$  NaCl + 1 vol.  $m/2$  KCl.

(b) 25 vols.  $m/2$  NaCl + 1 vol.  $m/2$   $CaCl_2$ .

(c) 25 vols.  $m/2$  NaCl + 1 vol.  $m/2$   $MgCl_2$ .

c. Combinations of three or four chlorides.

(a) 25 vols.  $m/2$  NaCl + 1 vol.  $m/2$  KCl + 1 vol.  $m/2$   $CaCl_2$ .

(b) 25 vols.  $m/2$  NaCl + 1 vol.  $m/2$  KCl + 1 vol.  $m/2$   $MgCl_2$ .

(c) 25 vols.  $m/2$  NaCl + 1 vol.  $m/2$   $CaCl_2$  + 1 vol.  $m/2$   $MgCl_2$ .

(d) 25 vols.  $m/2$  NaCl + 1 vol.  $m/2$   $CaCl_2$  + 1 vol.  $m/2$  KCl + 1 vol.  $m/2$   $MgCl_2$ .

Note especially the difference between the *pure* solution of NaCl and the *mixtures*. Which solutions are the most favorable? Note the differences between KCl and  $CaCl_2$  or  $MgCl_2$  as antitoxic salt (with NaCl as the toxic salt). The valence of the cation is important in antitoxic action.

## PART III

### PHYSIOLOGY OF MOVEMENT

#### I. MUSCLE PHYSIOLOGY

In the following experiments the catalogue of the Harvard Apparatus Company is to be used as an apparatus reference book. More detailed explanations of the apparatus than can be given in the laboratory direction sheets, will be found there. From now on dissecting instruments will *always* be needed.

#### STRIATED MUSCLE

##### A. METHODS OF STIMULATION.

The apparatus used in electrical stimulation should be carefully studied in all details before the preparation of a frog's muscle for experiment.

173. Batteries. Observe the dry cell. Carbon (+) and zinc (—) plates are immersed in a mass of porous clay permeated with strong  $\text{NH}_4\text{Cl}$  solution. Attach wires to the binding posts of the battery and apply the ends of the wires to neutral litmus paper moistened with  $\text{NaCl}$  solution. (Use insulated platinum-tipped wires.) Note (a) reaction at + and — poles; (b) rapidity with which color change appears with poles (1) close together and (2) one cm. apart, (3) several cm. apart. Explain this. What is Ohm's law?

174. Apply the ends of the wires as in the former experiment to filter paper moistened with starch solution containing  $\text{KI}$ . Result? At which pole does a reaction appear? Explain. Repeat, varying the distance between the two electrodes [anode (positive) and cathode (negative)].

175. Dip the ends of the platinum-tipped wires into weak acid solution. What gases are evolved and at which poles? Try  $\text{NaCl}$  solution, distilled water and sugar solutions. Results? From the above experiments formulate a rule for distinguishing anode and cathode in an unknown circuit.

176. Connect the cell in circuit with a simple *key*. Now without closing the circuit place the platinum electrodes on the tip of the

tongue about 1 cm. apart. Then close the circuit. Note the effect. Is there any perceptible difference between anode and cathode? Explain.

177. *The rheocord.* A device for introducing resistance into a circuit or for obtaining fractions of the electromotive force of a cell. Lead wires from the dry cell through a key to posts 0 and 1 and then from post 0 and the slider to platinum electrodes. Place the electrodes in salt or acid solution and determine the relative amount of electrolysis when the slider is moved toward the 0 or 1 post. Draw a diagram showing the course of the current.

178. *Induction coil.* Induced currents are usually employed for stimulation. These are momentary currents which appear in any circuit when a current in an adjoining circuit is made or broken, or its intensity altered. In the instrument the wires of the two circuits are arranged in two parallel coils—primary circuit (inducing circuit) and secondary (induced) circuit—to intensify the effects. An automatic interrupter is inserted in the primary circuit. Study the instrument and make a diagram showing its essential construction. Place the primary coil in circuit with a simple key and dry cell.

a. Direction of induced currents. Attach wires to the secondary coil and apply the ends to starch iodide paper. Attach primary circuit wires for single shocks. Close and open the key in primary circuit a number of times. Effect? Note the cross circuiting key at the poles of the secondary coil. Its purpose? Apply the electrodes to test paper as before and close the key in the primary circuit several times in succession but *cross circuiting the secondary coil each time before opening*. Result? Repeat, *cross circuiting before making* so as to allow only the *break* induced current to pass through the electrodes. Result? What conclusions do you draw regarding the *direction* of the induced current on making and on breaking respectively?

b. Separate the primary and secondary coils to some distance. Close the primary circuit and then place the secondary electrodes (platinum-tipped wires attached to secondary coil) on the tip of the tongue. Any result? Now make and break several times. Result? Slide secondary nearer primary, testing as before. Note the effect on the intensity of the shock. Which is stronger, make or break shock? Explain the difference in intensity of shock. Place the coils at right angles to each other. Are shocks perceptible? Change the angle between the coils, and test strength of shocks. Give a generali-

zation as to the relation between the angle of crossing of the coil-axes and the strength of the shocks.

179. *The reversing (or rocking) key or pole changer.* Note the mechanism of the reversing key and draw diagrams showing the connections to be made in order to use it (1) in reversing the direction of the current; (2) as a double key without changing the wires; (3) as a single key.

180. Examine the *non-polarizable electrodes*. They are soaked in physiological salt solution, then filled with  $ZnSO_4$  solution in which is immersed a Zn rod. Take great care not to spill  $ZnSO_4$  on the outside of the boots. Zinc is dissolved at the anode (+ pole), and is deposited on the Zn rod at the cathode (— pole). The Na and Cl ions carry the current through the tissue.

Set up the non-polarizable electrodes, place on litmus paper moistened with physiological salt solution, and determine the effect of passing a current through them. Does electrolysis take place? Do you see why they are used in physiology?

Immediately after using, wash out the  $ZnSO_4$  very carefully and place the electrodes in physiological salt solution to soak. Wipe off the Zn rods so that they will be ready for another experiment.

## B. PHENOMENA OF CONTRACTILITY AND IRRITABILITY.

*Muscular Contractility.* Muscle cells are typically stimulated to contraction by impulses conveyed through tracts of conducting tissue called nerves. A muscle with its attached nerve represents the chief motor organ of higher animals.

181. *Nerve-muscle preparation.* A muscle with nerve attached (gastrocnemius-sciatic) can be isolated as follows: Destroy the brain and spinal cord of a frog by pithing, as demonstrated. All spontaneous movement should cease. Do you know why? Remove the skin from the whole body of the frog except the head, as secretions of the skin injure the muscle. The object now is to remove the gastrocnemius muscle (still attached to the femur), and the whole sciatic nerve (still attached to the gastrocnemius muscle) from its origin in the spinal cord. Note on the dorsal side of the thigh a longitudinal depression between the vastus externus and semimembranosus muscles (see Ecker's Frog, p. 95). The sciatic nerve lies in this groove along with the blood vessels. Lift up the nerve very gently with a glass seeker and carefully isolate it as far as the knee in a downward direction. Then separate well the

thigh muscles with forceps and isolate the nerve upward, taking care not to injure it where it passes over the dorsal side of the pelvic bones, and thence forward ventrally to arise from the cord by several roots, clearly visible when the intestine and kidneys have been removed.

Cut the roots as near the spinal cord as possible. What happens? Cut through the femur in the middle and remove the thigh muscles without injuring the nerve, cut the tendon of Achilles *below* the ankle, separate the gastrocnemius muscle from the other muscles of the calf and cut the calf just below the knee. You now have a gastrocnemius-sciatic preparation. Keep moist with physiological salt solution (why?) and avoid touching muscle or nerve tissue with forceps

Fix the cut end of the femur in a femur clamp and lay the nerve on a glass slide supported by another clamp. Attach with thread a 10-gram lead weight to the tendon of Achilles.

182. *Mechanical stimuli.* Pinch the end of the nerve, or tap with a glass rod. Result? Try tapping a muscle directly, using some other muscle of thigh. Result?

183. *Thermal stimuli.* Touch the nerve with a warm glass rod. Also a muscle as in the preceding experiment.

184. *Chemical stimuli.* Place a few drops  $n/10$  HCl on the extremity of the nerve. Result?

185. *Electrical stimuli.* Galvani's experiment. Prepare a nerve-muscle preparation and lay the nerve across a piece of filter paper, soaked in physiological salt solution, on the china plate.

a. Gently touch the nerve with a copper wire and the muscle with an iron wire. Any result? Now touch the ends of the Cu and Fe wires together. What happens? Can you explain this? If the wires are corroded, file a clean surface at the points of contact. Try the same experiment with two wires of the *same* metal.

b. Touch the muscle alone and the nerve alone with the Cu and Fe wires in contact at the opposite ends. Result?

c. Touch the filter paper near the nerve (but do not touch the nerve itself) with Cu and Fe wires about 1 cm. apart with their line of junction parallel with the nerve. Now bring into contact the opposite ends of the wires.

d. Try the same experiment, but place the wires on each side of the nerve, but not touching it.

e. Try the same experiment with the muscle. Do you obtain the same result? Explain. If the gastrocnemius muscle does not

respond, try the sartorius or some other muscle of the leg. (Ecker, *The Frog*, p. 98.)

f. *Osmotic stimuli.* (a) Place a few salt crystals on the nerve or dip in  $2\frac{1}{2}$  m. NaCl. Result? Wash off the salt with physiological salt solution. Result?

(b) Allow the nerve to dry. What is the effect on the muscle? Does the nerve lose its irritability? Wash with salt solution to see if the power of functioning returns?

(c) Remove a sartorius muscle and suspend it half immersed in distilled water. Note carefully any movements or changes in length or color?

186. *Independent irritability of muscle.* A muscle is stimulated by the electric current, but we cannot be certain that nerves in the muscle are not also stimulated. These nerve endings can be paralyzed by curare. Proceed as follows: Etherize a frog *lightly* with ether soaked in cotton under a glass jar. Expose the sciatic nerve in the thigh by a small slit in the skin over the course of the nerve; be especially careful *not to injure the femoral artery* which runs close to the nerve. Carefully separate the nerve for a length of half an inch; pass a strong thread *under* the nerve, and tightly ligature the whole leg except the nerve. The circulation is thus interrupted below the ligature without injury to the nerve. Now inject into the dorsal lymph sac a few drops of a 1% solution of curare. When paralysis is complete (15-30 min.), expose both sciatic nerves and stimulate with tetanizing currents. Note the difference between the two legs, and explain. Is the nerve trunk affected by the curare? Where is the point of action? Is the muscle itself affected? Stimulate the curarized muscles *directly*. Do they contract? What do you conclude as to the independent irritability of muscle? Place the non-poisoned muscle with its attached nerve, in a watch-glass with curare solution. At intervals test its irritability through the nerve. Is its *direct* irritability affected? Test the direct irritability of a curarized muscle to the make and break of the constant (or galvanic) current, using non-polarizable electrodes applied at opposite ends of the muscle. Try similarly make and break single induction (or faradic) shocks, and tetanizing shocks (with interrupter). Is there any difference from indirect stimulation with regard to the relative readiness or response to the different forms of electrical stimulation?

187. *Polar stimulation of muscle.* a. Stimulation at cathode and anode. Slit a curarized sartorius from its lower end about

two thirds of its length. Then apply to each of the two halves thus separated a non-polarizable electrode. Use a galvanic current. *Make*, and note which limb contracts. After an interval *break*. Where does contraction start at *make* and *break*, respectively?

b. Cool a curarized muscle by placing on ice covered with paraffin paper (to protect the muscle). When thoroughly cool place in a Gaskell clamp and bathe with ice cold salt solution. Bring non-polarizable electrodes against the opposite ends of the clamped muscle and stimulate as before. Results?

c. Remove the rectus abdominis muscle from a frog, lay on a dry glass or porcelain plate and apply non-polarizable electrodes to either end. Stimulate with the galvanic current and note what occurs (close observation is required here) in the region of the tendinous bands which divide the muscle into segments? Is polar stimulation indicated? The effect is most distinct with a *cold* muscle.

188. *Does muscle change volume in contraction?* Remove the skin from the hind limb of a frog and place the limb in the volume tube. Hook electrodes into the muscle at opposite ends of the limb. Fill the tube quite full of isotonic NaCl solution, and replace stopper in such a way that *air is absolutely excluded* and fluid is forced part way up the capillary tube. Adjust the position of the meniscus by the glass rod. Stimulate the muscle by an interrupted induction current. Note movements, if any, of the meniscus and draw conclusions as to the nature and extent of the change of volume during contraction.

### C. GRAPHIC RECORD OF CONTRACTIONS.

189. *The graphic method of recording muscular contractions and other physiological processes.* The muscle is so arranged that its own contraction describes on a uniformly moving surface a curve from which the extent, character and time-relations of the movement can be seen. Usually smoked paper is used wrapped around a drum on a vertical axis moved by clock work. Such an instrument is a kymograph. Examine thoroughly. Learn how to wind it, regulate speed, etc., from the description in the Harvard Apparatus Company catalogue. Learn how to cover the drum with paper and smoke it.

Examine also the following pieces of apparatus and learn their use: Light muscle lever, writing lever, scale pan and signal magnet.

To prevent drying during experimentation, the muscle is often kept

in a *moist chamber*. Examine the moist chamber; note the muscle clamp in which the femur may be placed, and the binding posts.

Make diagrams of all the pieces of apparatus.

Adjust the muscle lever and moist chamber on the support and arrange your whole apparatus in a convenient position for taking a record of contraction. (See demonstration.) Diagram.

190. *Curves of single contraction, summation of twitches, and tetanus.* Each student is to preserve one set of records, so two records should be made by a pair of students working together. When the paper on the drum is covered it should be removed, clearly labelled, shellacked, and hung up to dry; then the records should be cut out and pasted in the laboratory book, with the description of the experiment. Always draw a base line under the muscle curve before shellacking. The student's name and data of the experiment should also be written *on the record*.

a. *Single contraction.* Connect the inductorium with two cells only and push the coils near enough together to give a good single shock. Set the drum on high speed and allow it to revolve after pressing the writing lever and signal magnet lever lightly against the drum. Take a record of a single twitch on the make of the current, and another on the break. Which is greater? Why?

b. *Summation of stimuli.* Send in two shocks in rapid succession by making and quickly breaking, so as to obtain a curve showing what happens when a muscle is stimulated at the height of contraction. If you fail in obtaining the correct time interval between shocks the first time, try again.

c. *Incomplete tetanus.* Repeat, making and breaking by hand in rapid succession. Summation of several shocks should be obtained, giving an incomplete tetanus.

Better records of incomplete tetanus can be obtained by means of the spring interrupter. Study the instrument and make a diagram of it. Connect with an inductorium whose coils are so far separated that only break shocks stimulate, and obtain records showing different degrees of incomplete tetanus.

d. *Complete tetanus.* Describe a curve of contraction with tetanizing current, i.e., with interrupted faradic or induced current. Do not stimulate for longer than 3 seconds.

e. *Fatigue curve.* With very slow speed of drum, fatigue the muscle by prolonged tetanus. Note the gradual relaxation in spite of continued stimulation. When completely fatigued, allow to rest. Wash with salt solution. Then take curves of single twitches with

maximal break shocks. Compare with the curves from the fresh muscle.

191. The preceding experiments will give practice in handling the apparatus. The student should now make a neat record of a single muscle twitch, as before, but introducing also a tuning fork which will make a time curve on the drum so that the duration of the phases of a contraction may be recorded. Turn the drum a *single revolution* fairly rapidly *by hand* instead of clockwork and while revolving stimulate the muscle with a single induced shock or break shock. The writing levers and vibrating tuning fork with writing point attached should be pressed against the drum before turning. The writing points should all be in a vertical line. After the record has been obtained place the lever point of the signal magnet over the point of stimulation as indicated on the record and with the drum stationary, stimulate the muscle to contract. This will give the exact latent period in case the writing points are not exactly over each other. Practice may be necessary to obtain a good record.

#### D. EFFECT OF VARIOUS FACTORS IN MUSCLE CONTRACTION.

192. *Influence of repeated stimuli—Treppe.* Fasten the muscle in a moist chamber and arrange the apparatus for recording contractions on a drum. Connect the muscle through a key with the binding posts on the desk. A current will be made and broken by a revolving key which automatically excludes the break shocks from the inductorium. Thus the muscle will be stimulated at a certain rate by make induced shocks alone. Record the contractions on a slowly moving drum until nearly fatigued. Allow to rest several minutes and again record the contractions till fatigued. Mark the rate of stimulation on the record. One record for a pair of students will be sufficient.

193. *Repeated stimulation at the moment of relaxation.* Records can be obtained by means of a special muscle lever. Consult the instructor for directions.

194. *Influence of strength of stimulus on height of contraction.* Arrange the muscle as in experiment 190 for direct stimulation with single induction shocks. Separate the coils (connected with one dry cell) to a distance at which both make and break shocks are ineffective. Then slowly increase the strength of stimulus by moving the coils nearer until the break shock just begins to be effective. Now

record the height of make and break contraction on a stationary drum with gradually increasing stimuli—moving the coils 1 cm. closer at each trial, until both make and break shocks give maximal stimuli. Rotate the drum by hand about 5 mm. between each contraction record. Take also a tetanic contraction. Write on the drum below each contraction line the distance between the coils in centimeters. Note carefully any relation between height of contraction and intensity of stimulus. What is the general law describing this relation? Compare the height of a single contraction with that of a tetanus. *From the height of the curves and the relative lengths of the two arms of the lever, estimate the actual distance through which the muscle contracts, both in single twitch and tetanus. What proportion of its own length does the muscle contract in both forms of contraction?*

195. *Influence of load on height of contraction.* Same arrangement as in the preceding experiment. Use a fresh unexhausted muscle. Use the same stimulus (maximal break shock) throughout. Attach the large weight pan to the lever and take contractions with the following loads: (1) unloaded, lever alone; (2) lever and scale pan; (3 and following) same + 10, 20, 30, 40, etc., grams up to 100 gms.; then increase by 20 gms. each until the limit of contraction is reached. Describe the relation between load and height of contraction. Estimate the work performed by the muscle in each contraction in gram-centimeters. At which load is the work done maximal? What is the absolute lifting power ("absolute force") of the muscle stimulated by a single twitch? Is the lifting power increased in tetanus?

196. *Influence of temperature on contraction.* The muscle is fastened in the "muscle warmer" by binding the femur to the metal rod with fine wire. The tendon of Achilles is connected by a bent pin and fine wire with the short arm of a special light muscle lever. Place a signal magnet in the primary circuit, and stimulate with single induced make or break shocks. Place a thermometer loosely in the "muscle warmer" so that the fluid may be stirred occasionally and the temperature maintained even. See that the muscle is irritable and then lower the temperature to 0—1° C. but *do not freeze*. Take a record of contraction on a rapidly moving drum. Then raise the temperature slowly and take records at 5°, 10°, 15°, 20°, 25°, 30° and 35°. Note carefully the differences in *height, duration* and *general form* of contraction curve at the different temperatures. Note also the variation in the *latent period* with temperature.

197. *Heat rigor.* Using the muscle of the last experiment, disconnect the inductorium and attach to the signal magnet wires from the desk binding posts which will give time intervals of 15 seconds. Revolve the drum *very slowly* and at the same time raise the temperature of the "muscle warmer" about one degree in 2 minutes. Mark on the rigor curve thus obtained the temperatures. Note especially the temperature at which marked shortening or heat rigor of the muscle begins.

198. *Action of salt-solutions on muscle.* a. Use *curarized muscles*. Remove small muscles (sartorius, biceps, tibialis, etc.) from the leg of the frog, *with as little injury as possible*, and place in the following solutions, which should be *changed two or three times* to remove all foreign substances.

- a. m/4 sugar solution (non-electrolyte).
- b. Mixture of 4 vols. m/4 sugar + 1 vol. m/8 NaCl.
- c. m/8 NaCl pure.
- d. m/8 NaBr pure.
- e. m/8  $\text{Na}_2\text{CO}_4$  (calcium precipitant).
- f. Mixture of 24 vols. m/8 NaCl + 1 vol. m/8  $\text{CaCl}_2$ .
- g. m/8 KCl pure.
- h. m/8  $\text{CaCl}_2$  pure.

Note the following points: (1) Any immediate change on placing the muscle in the solution; (2) behavior of the muscle after it has been in the solution for several minutes; (3) changes in the irritability of the muscle: test with single induction shocks at ten-minute intervals.

(a) Which solutions cause the muscle to contract or shorten permanently?

(b) Which produce rhythmical contractions or twitches? Which do not? Which solutions have the greatest effect of this kind?

(c) Which solutions deprive the muscle of irritability most rapidly? Compare especially solutions a and b.

b. After irritability has disappeared try the effect of transferring the muscle to normal saline solution (NaCl in tap water). Does the irritability return?

c. Compare the behavior of the muscle in m/8 NaCl, m/8 NaBr, m/8  $\text{Na}_2\text{SO}_4$ . Any difference? Then try the effect of adding to each solution a few drops of m/8  $\text{CaCl}_2$ . What is the effect? What do you conclude from this experiment and from the action of solution f above, as to the influence of Ca on the spontaneous activity of skeletal muscle? Try returning the muscle after an interval to the pure m/8 NaCl, etc. Does the former behavior return?

d. Try the effect of adding to the pure  $m/8$  NaCl or  $m/8$  NaBr a little  $m/8$  KCl solution: e. g., 9 vols.  $m/8$  NaCl + 1 vol.  $m/8$  KCl. How does the KCl influence the behavior of the muscle?

199. *Muscular twitching in salt solutions. Sensitizing and desensitizing action of various salt solutions. Graphic record.* The muscle (gastrocnemius) is attached to the extremity of a bent glass rod by a wire encircling bone and rod, and is arranged to pull vertically downward on the short arm of the light muscle lever. Connection is made with the lever by a silk thread attached to an S-shaped hook passing through the tendon. The glass rod with the attached muscle is immersed in the solution contained in a beaker, which stands on a block so that the solution can be readily withdrawn without disturbing the muscle (see demonstration). Test the action of the following solutions. The muscle is arranged for the lever to write on a slowly moving drum. Take a base line with the muscle in Ringer's solution.

a. Transfer the muscle from Ringer's solution to a mixture of 7 vols.  $m/8$  NaCl + 1 vol.  $m/8$  KCl by substituting a beaker with 250 cc. of this solution for that containing the Ringer. Make the change quickly, but be careful to avoid jarring. Note the behavior of the muscle and the characteristics of the contraction-curve in this solution, tone-change, etc. At the end of 2 minutes return to Ringer. Note the effect.

b. After the muscle has been in Ringer 2 or 3 minutes transfer to pure  $m/8$  NaBr; leave in this solution exactly 4 minutes. Then, while the drum is moving transfer to the NaCl-KCl mixture. Note the difference in the resulting activity. After 2 minutes return to Ringer as before. Result?

Repeat this experiment using  $\text{Na}_2\text{SO}_4$  instead of  $m/8$  NaBr. Note the difference in action. Note carefully the behavior of the muscle both *in* the solution and at the moment of exposure to the air when the transfer is made. (*Contact-irritability*, shown especially after treatment with solutions of salts whose anions precipitate calcium; as NaF,  $\text{Na}_2\text{C}_2\text{O}_4$ , etc.) Note also carefully the behavior in the NaCl-KCl solution. Leave here 2 minutes and then transfer to Ringer.

c. *Desensitizing salts.* Repeat using  $m/8$   $\text{CaCl}_2$  instead of  $m/8$  NaBr. Note the difference in effect. Is this action of  $\text{CaCl}_2$  reversible? If there is time try also  $m/8$   $\text{MgCl}_2$ .

200. *Influence of muscle poisons—Veratrin.* Inject one or two drops of a saturated solution of veratrin into the dorsal lymph sac

of a frog. Note from time to time the condition of the frog. What is the effect on reflexes and on general activity?

When the animal is "veratrinized" remove the gastrocnemius muscle *plus sciatic nerve* and mount in a moist chamber. To stimulate the nerve it is laid across "needle electrodes" in the moist chamber; to stimulate the muscle, connections are made as usual through the muscle.

a. Record contractions when the *muscle* is stimulated by the make or break of the induced current and a short tetanizing current.

b. Record contractions when the *nerve* is stimulated by the make or break of the induced current.

Note character of curve. What part of the neuro-muscular mechanism is affected by veratrin?

201. *Unipolar method of stimulation.* To stimulate human nerves a large "indifferent" electrode is placed over the skin where there are no large nerves and a smaller "stimulating electrode" over the nerve to be stimulated. Place the indifferent electrode, covered with cotton soaked in physiological salt solution, over the biceps muscle and connect with the inductorium for single induced shocks. Explore the inner surface of the forearm with the stimulating electrode, also covered with salt-soaked cotton, and note the contractions of the muscles of the fingers when their nerves are stimulated. (See fig. in Howell, p. 93.) Draw an outline of the arm and mark the "motor points" of four or five muscles which you have been able to find on your arm.

202. *Record of human contractions—The ergograph.* Tie all the fingers of the right hand except the index finger in the wooden block of the ergograph and adjust the rod between index finger and ergograph lever so as to record *isotonic* contractions of the abductor indicis muscle. A celluloid writing point should be attached by wax to the lever, or an aluminium point if the movement is very slight. Take the following records:

a. Unipolar single make or break induced shocks. The indifferent electrode should be placed in the palm of the hand and the nerve stimulated at the angle between first and second metacarpals. Try also tetanizing shocks and record the contraction.

b. Voluntary contraction, of a very short duration. Voluntary contraction of several seconds duration.

Then shift the rod toward the cast iron support of the spring and take an *isometric* record of:

c. Voluntary contraction of very short duration.

d. Continued contraction until marked fatigue results.

Note difference between voluntary tetanus and tetanus due to electric stimulation.

203. *Production of acid in muscle.* a. *On contraction.* Pith a frog and remove the skin from the hind legs. Stain these in physiological salt solution containing neutral red. Then stimulate *one* leg with the tetanic current for 2 or 3 minutes. Any change in color? Now place both legs in  $n/200$   $\text{NH}_4\text{OH}$  in physiological salt solution. Any difference between stimulated and unstimulated legs?

b. *On heat rigor.* Heat the unstimulated leg in the  $\text{NH}_4\text{OH}$  salt solution gradually till heat rigor sets in. Note any change in color and also in position of the leg as a whole. What do you conclude as to the relative strength of the flexors and extensors of the leg?

### SMOOTH MUSCLE

As material for experiment, rings about 3 mm. broad cut from the stomach of a frog or strips from a cat's bladder may be used.

204. Attach by fine copper wire one end to an L-shaped glass rod, the other to the short arm of a light muscle lever as in experiment 196. Immerse in Ringer's solution and record any changes in length on a very slow moving drum. Do you obtain rhythmical contractions or tone changes? Try raising the temperature of the Ringer's solution to about  $30^\circ\text{C}$ . Result?

205. Make appropriate electrical connections and study the response of smooth muscle as under striated muscle. (Exps. 190-191.)

### HEART MUSCLE

The properties of heart muscle will be examined in studying the physiology of the heart (p. 81).

## II. NERVE PHYSIOLOGY

### A. NERVE FIBERS.

Stimulation of a muscle, suspended in a moist chamber, through its nerve will give exactly the same type of contraction and muscle curve as direct stimulation of the muscle fibers themselves.

Many of the fundamental phenomena of stimulation, however, can be demonstrated to greater advantage on nerve. Muscle shows irritability, conductivity and contractility. Nerve shows only irritability and conductivity; these two properties are interconnected and

only partly separable. Great care must be taken not to injure the nerve in removing it from the frog. Never pinch a nerve with forceps.

206. *Effect of alcohol and CO<sub>2</sub> on nerve. a. Carbon dioxide.* Arrange the inductorium for single induced currents. Connect the secondary coil with the main posts of the pole changer (used in this experiment as a double key). Connect the two other pairs of posts with the usual stimulating electrodes and the electrodes of the small gas chamber. Join the inflow tube of the gas chamber with the outflow tube of the CO<sub>2</sub> bottle. The gas chamber should be clamped in position on a glass plate. Make a nerve-muscle preparation, *preserving the full length of the sciatic nerve up to the vertebral column.* Pass the nerve through the holes of the gas chamber so that it lies on the electrodes. The nerve should be drawn through until the muscle is close to the gas chamber. Stop the holes through which the nerve passes with normal saline clay. Bring the outer pair of electrodes against the central (i.e., towards spinal cord) end of the nerve near its exit from the gas chamber. Determine which position of the double key corresponds to each pair of electrodes. Stimulate the nerve first within the chamber, and then on the central end of the nerve, using a current just sufficient to cause tetanus. What is the result? Now pour 10% HCl on the marble in the generator and pass the gas through water and then through the chamber. After a few minutes stimulate as before. Result? What is the explanation?

b. *Alcohol.* Disconnect the rubber tube from the gas generator, and blow through the gas chamber until the CO<sub>2</sub> is driven out. Does the nerve recover its irritability? Determine this by stimulating from time to time. When the nerve has recovered, drop a little alcohol through the long glass tube of the gas chamber, *being very careful that only the vapor of the alcohol comes into contact with the nerve.* Stimulate both within and without the chamber. What results do you now obtain? Which property of nerve does the alcohol affect? To obtain good results, the electrodes within the gas chamber should not be too far from the opening through which the nerve passes to the muscle.

207. *Threshold value of Stimulation.* Prepare a gastrocnemius muscle with the sciatic nerve from its point of origin in the spinal cord down, and place in the moist chamber. Hang the nerve over the needle electrodes. Determine the single break induced stimulus which just causes contraction. This is the threshold value. Now

apply the same needle electrodes to the muscle directly. Is the threshold value for muscle (or the nerve fibers in muscle) the same? Determine the threshold value for different points along the nerve. Is there any difference? (Allowance must be made in this experiment for differences in the electrical resistance of the two tissues, or of the different regions of the nerve.)

208. *Summation of subminimal stimuli.* Using the last nerve-muscle preparation, place the coils of the inductorium just far enough apart to prevent contraction on stimulation of the muscle with needle electrodes. Allow the muscle to rest a few minutes. Now stimulate. If no contraction results keep stimulating about twice a second. Does the muscle eventually contract? Does it contract with a tetanizing current? Does your result indicate that the excitations outlast the stimulus and reinforce subsequent stimuli?

209. *The excitation wave remains in the muscle or nerve fiber in which it starts.* In order to limit the stimulus to one or two fibers, the method of unipolar stimulation may be adopted. Fasten in one post of the secondary coil of the inductorium arranged for tetanizing currents a wire soldered to a blunt needle. Expose the sacral plexus in a brainless and spineless frog in which the skin has been removed from the hind limbs. Connect the preparation by means of a copper wire to the earth through the gas or water pipes by connecting with the desk binding pests. Touch the sacral nerves here and there with the needle electrode, watching meanwhile the sartorius muscle. Do all fibers contract? Stimulate the sartorius directly. Do only the fibers touched by the needle contract?

210. *The same nerve fiber may conduct impulses both centripetally and centrifugally.* a. The nerve of the sartorius divides at the muscle, part going to each half of the muscle. Microscopical examination shows that the division is not simply a parting of individual nerve fibers, but that each axis cylinder forks, one limb going upwards, the other downwards. If the muscle is severed between the forks, no impulse started in one half of the muscle could reach the other half, except by going up one branch to the original axis cylinder and down the remaining branch; for it has been shown that the nerve impulse does not escape transversely from one axis cylinder to other neighboring ones.

Remove a sartorius muscle with great care. Split the muscle in the middle line for one-third of its length, beginning at the broad end. Stimulate the right segment by snipping it with a pair of scissors. Note carefully if the fibers of the left segment contract.

b. The gracilis muscle of the frog is divided by a fascia into an upper shorter part and a lower longer part. Remove carefully the muscle *with its attached nerve* and note that the nerve and blood vessels divide so as to go on each side of the tendon. Cut the muscle in half at the tendon *without injuring the nerves*. Then stimulate one half. Does the other half contract?

211. *Electrotonus*. a. *Effect of constant current on irritability*. Two currents are to be sent through the nerves, a *galvanic polarizing current* whose effect on irritability is to be studied and a *stimulating current of single induced shocks* for testing the irritability. Set up the nerve muscle preparation in the moist chamber for recording on a drum, placing the nerve over non-polarizable electrodes about 1-2 cm. apart, connected through the rheocord and a reversing key with the desk binding posts which will give a strong galvanic current. Place the stimulating needle electrodes (see that they are clean) on the muscle side of the boot electrode nearest the muscle and connect with the secondary of an inductorium. Stimulate the nerve with a minimal break induced shock and record the contraction on a stationary drum. Now send a weak constant current through the nerve and, disregarding the contraction on making the galvanic current, stimulate again. Is the height of contraction increased or diminished? Indicating what? Break the galvanic current and in a minute or so stimulate again. Result? The record should show the result of stimulation in all phases of the experiment. At which pole is irritability increased?

Repeat the above but reverse the direction of the constant current through the nerve. Result and conclusions?

The best results are obtained with a certain strength of polarizing current which must be determined by experiment. Do you see now why the make galvanic stimulus is greater than the break?

b. *Effect of constant current on conductivity*. Apparatus the same as in the preceding experiment except that the stimulating electrodes are placed midway between the boot electrodes and a piece of muscle is introduced in the stimulating circuit to increase resistance. Do you see why? No record need be taken. Use minimal stimuli as before. Determine if a weak galvanic current can block the passage of a nerve impulse and the pole at which the block occurs. Increase the strength of the polarizing current and determine its effect on the blocking of the impulse.

212. *Speed of the nerve impulse*. Adjust the drum for turning by hand. Place two pairs of needle electrodes in the moist chamber

connected through a double key with the secondary of an inductorium. Make a nerve muscle preparation, preserving *the full length of the sciatic nerve*, and place in the moist chamber with the electrodes under the nerve and as far apart as possible. With a tuning fork and signal magnet pressed against the drum, stimulate with a maximal make shock and record the latent period and contraction (as in exp. 191), *first*, with the electrode far from the muscle in circuit, and *then* with the near electrode in circuit. The time differences in the latent periods must be the time required for a nerve impulse to pass along a space equal to that between the two cathodes (why?). Measure this distance and calculate the rate of the nerve impulse per second.

213. *The seat of fatigue.* Pith the brain of a frog (but *not* the spinal cord) and plug the cavity with cotton to prevent bleeding. Expose the sciatic nerves of both sides and pass a thread underneath so that they may be lifted readily for stimulation. Stimulate the right sciatic (with a current strong enough to cause contraction of the left leg) and note the time required for the muscles of the left leg to relax, completely fatigued. Have the right leg muscles also relaxed? Call this time A. Then stimulate the left sciatic (do not fatigue it) to see if the muscles still contract. Result? Where has fatigue occurred? Quickly remove the skin from the right leg, tie the thread about the sciatic and cut centrally to the ligature, remove the muscles from the thigh, cut the femur and fasten in a clamp. Stimulate the sciatic with a weak stimulus (tetanizing) until fatigue occurs, recording the time required. Call this time B. Now stimulate the muscle directly until fatigued. Call this time C. What has been fatigued here?

To prove that the sciatic nerve has not been fatigued at the point of stimulation proceed as follows: Remove the left leg, *retaining the whole length of the sciatic nerve*, and place in a femur clamp with the nerve across non-polarizable electrodes near the muscle. A galvanic current (from desk electrodes) is to be passed through the nerve in order to block the nerve impulse, and the far end of the nerve is stimulated with a *weak* tetanizing current for a length of time equal to  $A + B + C$ . If the muscle contracts when the polarizing current is turned off, but the tetanizing stimuli are still given, we can safely assume that no fatigue has occurred at the part of stimulation during the time of the experiment. Which are fatigued in order in this experiment—nerves, nerve endings, nerve cells, muscle fiber?

## B. NERVE CELLS. (PHYSIOLOGY OF CENTRAL NERVOUS SYSTEM.)

### A. REFLEXES.

214. In a normal frog observe the following: Movements of the head when the animal is revolved in (1) a vertical plane parallel to the axis of the body, (2) in a vertical plane perpendicular to axis of body, (3) in a horizontal plane. Make a general statement defining movements in the above cases.

215. Pith a frog (brain only) and stimulate by pinching or touching the following regions: a toe of right foot; a toe of left foot; a finger; an eye; the skin of the abdomen. Record the movements resulting in each case.

216. Record what happens in the following reflexes in yourself or partner: *Pupil reflexes.* (1) Light reflex. Close one eye for several seconds, then open it quickly. Note any change in pupil. (2) Consensual reflex. Close one eye as before, but watch the pupil of the other eye when the first is opened again. (3) Accommodation reflexes. Look, alternately at a near and a far object. Note any change in pupil. This experiment cannot be performed on yourself. (4) If you are not already familiar with the "knee jerk", demonstrate this.

217. *Purposiveness of reflexes.* Suspend from a hook a frog with its brain pithed. Dip in acetic acid a piece of filter paper about a quarter cm. square. Shake off the excess of acid, then apply the paper to the front of the frog's body. What movements result? Remove the paper, dip the frog in water to remove the acid from his skin, and again suspend the animal from the hook. After five minutes repeat the experiment, but apply the acidulated paper to the inside of the thigh. If only one foot is drawn up hold that foot. Does the other foot now move? After washing off the acid and waiting again for five minutes, apply the acidulated paper to the back near the tip of the urostyle. To what region is the response now directed? Are the directions of the reflex movements sufficiently different in these three instances, and pointed toward a definite end with sufficient clearness, to indicate purposive action?

Are the reflexes in sections 214, 215, and 216 obtained when the spinal cord is also destroyed?

218. *Summation.* Suspend by a hook a frog with brain pithed. Tie two fine copper wires 1 cm. apart around the left foot, near the toes, and attach the wires to a secondary coil of the inductorium.

Connect the primary coil through a simple key to a dry cell. Do single make and break shocks evoke a reflex response? Be very careful in this case to distinguish between a direct stimulation of the muscle by the electric current and a reflex stimulation from the central nervous system. Stimulate with regularly repeated *weak* make and break shocks, and test whether under these circumstances reflex action can result from the summation of afferent impulses. If the stimuli are repeated more rapidly, does the reflex occur sooner? What is the effect of increasing the strength of the stimuli and maintaining the same rate of stimulation?

219. *Inhibition.* Use the frog and apparatus as described in the foregoing experiment, but arrange the inductorium to deliver a tetanizing current.

Provide a vessel of water. Immerse the toes of the right leg of the frog in 0.5% sulphuric acid and note the time required before reflex action occurs. Without any delay wash off the acid in water. After an interval of 3 minutes, stimulate the left foot with a weak tetanizing current as the right is again immersed in the acid. If the foot is not withdrawn from the acid after 20 seconds, stop the tetanizing currents. What has been the effect of the afferent impulses from the left foot on the efferent impulses to the right leg? After again washing the leg in water, prove that the sensory endings in the skin are still irritable to the acid.

220. *Irradiation.* Use a tetanizing current and arrange as in experiment 219. Start with a subminimal stimulus and then gradually increase its strength, determining the effect on the reflex movement produced. Does the reflex become "crossed"? Does it extend to anterior regions? Record the order of spread with increasing strengths of stimulus.

221. *Augmentation.* Determine the ease with which the "knee jerk" is given, using your partner as a subject. Let him then pull upon his clasped hands in a maximum muscular effort and again determine the activity of the "knee jerk". Is there any difference?

222. *Modification of reflex response by altering condition of nerve-endings.* Production of hypersensitiveness of cutaneous nerve-endings can be induced by sodium citrate. Suspend the frog so that the feet dip in  $m/8$  Na citrate solution. After one to two minutes withdraw the feet from the citrate solution and dip in ordinary tap water. Note the effect. Replace in citrate solution. Note the effect.

After producing the sensitive condition as before, dip the frog's

feet into m-cane-sugar-solution. Note the effect. What is the general physiological effect of such a solution? Then dip the feet into the water as before. Is there any response?

Can the hypersensitive condition be restored by the citrate solution? i. e., are these changes of sensitivity reversible?

223. *Modification of reflex by altering condition of cord. Effect of strychnine on reflex action.* Inject with a fine-pointed pipette a few drops of 0.5% solution of strychnine sulphate into the dorsal lymph sac of a frog whose brain has been destroyed. After a few minutes test the reflex excitability of the animal by touching the foot with a needle. Note carefully the character of the response and how it differs from that of an unstrychninized frog. Note evidence that as the influence\* of the strychnine becomes more marked the afferent impulses spread more and more readily throughout the entire cord. Then destroy the spinal cord and stimulate the animal as before. What is the essential nature of the change produced in the cord by strychnine?

224. *Production of hyper-irritability of the nerve-trunk.* Under some conditions the nerve-trunks become abnormally sensitive, and a reflex response may be modified by this cause. Hypersensitiveness to contact may be induced as follows: Immerse the nerve of a nerve-muscle preparation in m/8 Na citrate solution for about 5 minutes. (Let the muscle rest on moist filter paper on a glass plate from the edge of which the nerve hangs down into the beaker containing the solution.) Is there any effect on the muscle while the nerve remains in the solution? Then remove the nerve from the solution and let it hang in the air. Any effect? Touch it with a glass rod or the handle of a scalpel. What is the result? Dip the nerve in Ringer's solution for a short time and again test its contact reaction. Can the hypersensitiveness be produced a second time? Muscle may be similarly rendered hypersensitive.

225. *Reaction time.* Place a signal magnet in circuit with two simple keys and the primary of an inductorium arranged for single shocks. The signal magnet is arranged to write on a drum (turned by hand) just above a vibrating tuning fork. One student is to place the stimulating electrodes from the secondary terminals of the inductorium on his tongue and his right hand on one of the keys which must be closed. He should close his eyes and concentrate attention on the stimulation of his tongue. When stimulated he should instantly open the key in his right hand. The other student must start the tuning fork, rotate the drum and close the second key

which gives the stimulus. Take at least two records and determine the average time required to react to a stimulus.

226. *Reaction time with choice.* Apparatus as in the preceding experiment except that three keys are placed in the circuit and the stimulating electrodes are held on the tongue by the lips. Right and left hands are placed on two of the keys (both closed) and if a strong stimulus is received the left hand opens a key; if a weak stimulus the right hand. Strong and weak stimuli must be determined beforehand in terms of coil distances and of course the subject must not know which he is to receive. Take two records and compare with the preceding experiment.

227. *Reflex touc of muscles.* Pith the brain only of a frog and suspend from a hook by the lower jaw. Note especially the position of the legs. Now make a small slit in the abdomen and cut the roots of all the nerves going to the right leg where they leave the spinal cord. Again suspend the frog from the hook and note the position of the legs. What does this experiment indicate?

## B. THE BRAIN.

In the following operations proceed very slowly and cautiously, as the frogs are to be kept alive for as long a time as possible to recover from "cerebral shock", due to the operation. Use well sharpened instruments. At intervals during the operation wash the skin with antiseptic salt solution ( $\text{HgCl}_2$  1:2000).

228. *Removal of hemispheres.* Select a male frog, characterized by a thickened pad on the innermost digit of the front limb. Anaesthetize by placing him under a battery jar with some absorbent cotton wet with ether. If during the following operation the effect of the anaesthetic diminishes, place under the jar again.

The cerebral hemispheres of the frog extend back to a line connecting the front margins of the two tympanic membranes. Cut the skin along this line over the top of the skull. From this cross cut make a median incision forward nearly to the nostrils. Lay back the flaps. With scissor points separated to either side of the top of the skull, immediately in front of the transverse skin incision, cautiously bring the points together, cutting barely through the bone. Insert the sharp blade forward and at one side under the bony covering of the cerebrum, and snip the bone. Repeat the operation on the other side. Raise the bone with small forceps and carefully cut forward, alternately on one side and the other, until the cerebrum is entirely exposed. Sever the connections between the optic

lobes and the cerebrum and remove it. With silk thread sew together the flaps of the skin.

Note the posture of the animal immediately after the operation. To what factors may this be due? While the animal is recovering perform other experiments. During the interval, however, keep the frog's skin moist, for he breathes in large part through his moist skin. In about an hour test the capabilities of the frog as follows: (a) Posture. Record the difference between the decerebrate frog and a spinal frog as to posture. (b) Locomotion. Similarly record the differences in leaping and swimming. (c) Respiration. Is there a difference in respiratory activity? (d) Vision. Compare the eyelids. Place an opaque object between the decerebrate frog and a source of light. With the animal facing the object, which should be only 6 or 7 cm. distant, stimulate him to jump. Does the frog jump against the object, or avoid it? (e) Equilibration. Turn the decerebrate frog on his back. Compare his reaction to that of a spinal frog. Place him on the palm of the hand or on the frog board. Slowly tilt his support. What happens as his equilibrium is disturbed? See if the frog can be made to crawl to the other side of the hand or frog board as the support is further turned. (f) Croak reflex. Hold the decerebrate frog gently between the thumb and first finger, placed immediately behind the front limbs. Apply slight pressure for a moment. The frog should croak in response to each application of stimulus. Stroke with the moistened finger the skin of the back or flanks, and note if this also evokes a reflex.

(g) If the operation is successful the animals live for several days. They will be kept in the laboratory, and if possible the student ought to examine their general reactions on the day after the operation.

229. *Influence of optic lobes on reflexes.* Endeavor to find some marked difference between a decerebrate and a normal frog. a. Expose the brain according to the directions already given. Immediately posterior to the hemispheres lie the optic lobes, two gray spherical bodies. Separate the cerebral hemispheres from the optic lobes by a transverse incision, and carefully remove the hemispheres. Wait until the shock of the operation has passed. Now suspend the frog without injury so that the tips of the toes hang above a shallow dish containing 0.5% sulphuric acid. Determine the reflex time. Wash off the acid and, after a moment's rest, sprinkle a very little finely powdered common salt on the cut surface of the optic lobes. Again determine the reflex time. Is it markedly changed by the stimulation of the optic lobes?

b. Remove carefully the optic lobes, wash off any excess salt with physiological salt solution, and again determine the reaction time. Any change? Now sew carefully together the flaps of skin over the brain cavity. Compare its reactions with those of the decerebrate frog of experiment 228. Place the frog in the box to be kept for observation next day.

### III. BIOELECTRIC CURRENTS

230. *Capillary electrometer.* The inertia of the coil of the ordinary d'Arsonval galvanometer is so great that it is unsuited to record rapid changes in potential such as are produced by actively functioning plant and animal tissues. For this purpose the capillary electrometer is used. The wires are attached to two surfaces of mercury, one large and one small (in a capillary tube), separated from each other by 20% sulphuric acid. When a current passes, the mercury in the capillary moves in the direction of the current. Movement is proportional to the strength of the current and depends on a change in the surface tension of the mercury. Its surface tension is greatest when the potential difference across the surface is least. Draw a diagram showing the construction of the capillary electrometer. A detailed description of the instrument will be found in Howell, p. 98.

Note the short circuiting key on the instrument. Non-polarizable electrodes must always be used in leading off the wires from the tissue to the galvanometer.

Fill the tubes of the capillary electrometer with mercury and 20% sulphuric acid and set up on the stage of a microscope as demonstrated. Handle the parts *very carefully*, as the instrument is expensive and easily broken. Wax cement may be used to hold the tubes firmly in the block support. The pressure tube on the right will not be used for these experiments.

231. *The action current of the heart.* Non-polarizable electrodes are applied to ventricle and auricle of the frog's heart while in the body and connected to the capillary electrometer on the microscope. Heart muscle, like every other muscle, becomes during contraction electrically negative relatively to inactive portions of the tissue. A wave of contraction accompanied by a wave of negative potential passes over the heart and is recorded by the electrometer. Do the excursions of the mercury correspond to the heart beats?

232. *The current of injury (current of rest or demarcation current) of muscle.* A sartorius muscle is carefully prepared and one

end cut off. Non-polarizable electrodes are placed one on the *uninjured* surface, near one end, and the other on the *injured* surface, near the other end, and led off to the capillary electrometer. A deflection may be noted on opening the short circuiting key, indicating differences in the electrodes. Note its direction and amount. Now one of the non-polarizable electrodes is placed on the injured end, the other is left near the uninjured end of the muscle. Again note the deflection of the galvanometer. Is it greater than before? In which direction does it indicate that a current is flowing?

233. *Measurement of current of injury.* Connect injured and uninjured surfaces of a cut sartorius, with an electrometer in circuit, to the slider and O-post of a rheocord. Connect a dry cell through a key to the O and 10-meter post of the rheocord. Either both negative or both positive poles of muscle and cell must be connected to the O-post. (See fig. in Harvard Apparatus Company Catalogue, p. 22.) Move the slider to a position where no current flows through the galvanometer when the cross circuiting key is opened. The fractional voltage of the dry cell (1.4 volts) can then be read directly from the rheocord and will just balance the voltage of the muscle. Result?

234. *Current of action.* Stimulate the muscle by pinching while a current of injury is flowing. How is the electrometer affected? Do you see now why the action current was called the "negative variation" of the current of rest?

235. *The bio-electric currents are strong enough to stimulate nerve. The "Rheoscopic Frog Preparation".*

a. Make two nerve-muscle preparations, A and B. Lay the nerve of A lengthwise over the muscle of B. Stimulate B through its nerve. Does the muscle of A contract as well as that of B?

b. Cut the B muscle near its tendon end. Lay nerve of A carefully on muscle of B, touching injured and uninjured surfaces. Is there any contraction? Now stimulate the nerve of B. Does the A muscle also contract? How do you interpret each of the results obtained in the above experiments?

c. Lay the nerve of A lengthwise across the beating heart of the frog. The heart is left in the body of the frog but exposed by cutting away the pectoral girdle and pericardium. What happens? Explain. This experiment succeeds best if the frog furnishing the nerve muscle preparation is previously kept on ice for some time.

236. *Polarization current.* Connect two keys (A and B) in circuit with two dry cells. Connect a frog's muscle, by means of

non-polarizable electrodes about one cm. apart, with the binding posts of one of the keys (A). Close the circuit through the muscle for several minutes by means of key B, leaving key A open. Now open key B and immediately make and break key A several times. Does the muscle contract? Where is the source of the current in this experiment? The muscle acts as a delicate galvanometer.

In what other way may a polarization current be obtained?

## IV. CILIARY MOVEMENT

236. Pith a frog, destroying both brain and spinal cord, and pin it on its back on the frog board. Cut away the ventral body wall and remove all of the viscera except the oesophagus and stomach. With the scissors cut through the lower jaw in the middle line and continue the cut back to the stomach. Draw back the flaps of the lower jaw, and pin out the oesophagus to form a flat surface on a level with the roof of the mouth. Keep the oesophagus and the mucous membrane of the roof of the mouth moist with normal saline.

a. Lay one of the small pieces of cork on the exposed mucous membrane. In which direction does the cork move? Lay a weight on the cork block and repeat the observation. Repeat again after tilting the frog-board so that the weight must be carried up an incline.

b. Remove the weight, and determine the time in seconds in which the cork moves one inch. Make a second determination after warming the preparation with saline solution at 30° C.

c. Saturate a piece of filter paper with ether and blow the fumes down upon the preparation. After a few seconds make a third determination.

d. Similarly test the effect of vapor of ammonia, but in this case it will be sufficient to blow across the open mouth of the bottle.

Record the results of the four determinations.

237. Open a clam, mussel or oyster shell and catch the contained fluid in a beaker. Cut a small piece of tissue from the mantle, tease it well with needles and mount under cover-glass. India ink may be added to ascertain the direction of currents. Study and draw under the high power various phases in the beat as it becomes slowed through lack of oxygen. Do you see any individual cilia beating which are not attached to cells?

238. Mount a piece of mantle on a slide between two non polarizable electrodes in sea water. Place a cover-glass on the preparation and study with the high power. What is the effect of make and break of the galvanic current? Make and break single induced and interrupted shocks?

239. Paramecium is a good form for a study of ciliary action. Study carefully if you have not already done so. Can the effective stroke of the cilium be reversed?

240. *Effect of  $NH_4OH$ . Cytolysis*—Place Paramecia in  $n/1000$   $NH_4OH$ . Note immediately the changes undergone by cilia and vacuoles. Does swelling occur? Note especially if the surface of the animal is lifted off while the cilia still remain beating on the surface. Describe the changes during cytolysis.

## V. PROTOPLASMIC ROTATION

A type of movement closely allied to amoeboid movement (see p. 39). The leaves of Vallisneria, Chara, Nitella, Elodea, and the stamen hairs of Tradescantia are well adapted for study of protoplasmic rotation. Use Elodea leaves in the following experiments.

241. Study under the high power. Note time for a complete revolution and the direction of rotation in adjacent cells. Draw a diagram indicating the direction by arrows. Try warming slightly the slide. Effect on rate?

242. Place a leaf in isotonic sugar solution. Does the movement cease? Cut leaf in half with sharp scissors and note if the cells near the cut edge are affected in any way. Can you observe any protoplasmic fragments moving in the fluid?

243. Place a leaf in isotonic sugar solution and add a few crystals of sugar. What is the effect of the resulting plasmolysis on rotation? Does it finally cease?

244. a. Place leaves in water one sixth saturated with chloroform. Effect on rotation? Remove to pure water again. Result?

b. Try also ether water. Note that practically all vital processes are slowed or abolished by these anaesthetics and that the effect is reversible.

245. Mount a leaf on a slide in physiological salt solution. Effect? Place non-polarizable electrodes at the ends of the leaf and determine any effects of stimulating by galvanic and faradic shocks. Result?

## PART IV

### PHYSIOLOGY OF NUTRITION (INCLUDING CIRCULATION AND RESPIRATION)

#### A. METABOLISM

A very important division of metabolism (the action of enzymes) has already been studied. Certain general features remain to be considered.

#### I. HOLOPHYTIC METABOLISM.

246. *Oxygen formation.* Place in a test-tube with clean water a healthy branch of Elodea (or some other water plant that has not finely divided leaves). Do not use water from the hydrant, for this contains too much air; use if possible water in which the plants are found, or other water that has stood for a time in tanks, and be careful not to get any bubbles of air in the tube. Invert the test-tube in a vessel of the same water. Place in a bright light,—where the direct rays of the sun reach it.

At the same time prepare another experiment, in exactly the same way, but place this in complete darkness.

Try the same experiment, but using water that has been *boiled* thoroughly and cooled quickly without disturbance. Wash the plant in this water before placing in the tube. Place in sunlight as before. Any difference from above result? Explain.

Allow the three experiments to stand for some hours or for a day, if necessary. In which one are bubbles of gas produced? If in all three, which shows the larger quantity? Test for oxygen by the spark test.

247. *Photosynthesis in plants.* Examine Spirogyra filaments that have been well exposed to light. Study the chlorophyll bands. Sketch. Then run under the cover-glass of a second preparation a little iodine solution and examine. Compare carefully with the untreated preparation. Note the distribution of starch. Examine similarly filaments that have been kept in dark some days. Note any difference? Explain.

248. *Photosynthesis in leaves.* a. Pin two flat pieces of cork together over a portion of a suitable green leaf (to exclude light). Place the plant in a bright place and leave two days. b. Then pick leaf; dip in boiling water for a minute or so; extract chlorophyll with 95% alcohol (some time will be necessary), and treat with weak iodine solution. Note distribution of starch. Explanation? c. Perform the same experiment with a leaf enclosed in a bottle containing strong KOH to absorb  $\text{CO}_2$ . The petiole is passed through a slit in the cork and the whole closed airtight with vaseline. Note difference from b? Explanation? d. Note distribution of starch in a variegated leaf. Place the leaf (after momentary boiling) in 80% alcohol to extract the chlorophyll and treat as before. Result? Conclusion?

## II. HOLOZOIC METABOLISM.

The following study of certain of the processes of metabolism in a number of organisms will be carried on partly as laboratory work, partly as seminary work. The processes are to be observed by the student as far as possible. Where this is not possible, descriptions of them are to be read, in the references that are given. The essential point is to have after study a clear idea how the process in question takes place; be ready to describe and explain to the instructor. The books and papers referred to will be placed on the desk in the laboratory, and are to be referred to as a part of the regular work.

In organisms in general we can distinguish a number of factors and processes concerned in metabolism. These are listed in the following, together with suggestions for their study in the organisms examined.

a. The taking of food. Organs or processes involved. Make sketches and descriptions if possible.

Most organisms have either some process of bringing food to the mouth, or of going to the food. Determine which is true in the given case (or whether both or neither are true), and describe.

b. The digestion of food. This usually takes place in an enclosed region, the alimentary canal. Make a diagram of this when possible. The processes involved are usually the subjection of the food to certain chemicals. This is usually not directly observable, but has been imitated experimentally. Such experiments we have already carried on in our study of enzyme action. Sometimes the changes in the food can be traced; this should be done where possible.

In certain cases references will be given to descriptions of these processes.

c. The discharge of the unused parts of the food (defaecation). Observe and describe if possible. In some cases there is a definite opening for this purpose, in other cases not.

d. Absorption, assimilation and dissimilation. These are usually not observable.

e. Distribution of food within the body. There is usually some method of carrying the food about within the body; sometimes a definite set of organs for this purpose (circulatory system of higher animals).

f. Respiration, the taking of oxygen and giving off of  $\text{CO}_2$ . The processes involved are usually movements produced in the surrounding atmosphere or water, to bring  $\text{O}_2$  and carry away  $\text{CO}_2$ , and internal currents or movements (same as mentioned in last paragraph). Study both carefully. Often special organs are present,—respiratory organs (gills, lungs, tracheae, etc.). Draw and describe.

g. Excretion,—the discharge of the waste products of dissimilation (distinguish clearly from defaecation). Study the organs involved and how they act; make drawings. Study also the processes involved. Notice that often currents are produced, for carrying off the waste materials (as in f).

It will not be possible to study all these processes in each organism we take up. Some are lacking in various organisms, and others are unfavorable for study. In each case suggestions will be made, or questions asked, indicating the points to be studied.

#### A. PARAMECIUM.

Study Paramecium first, as a type showing how most of these processes are carried on in a simple way.

In many cases in the study of Paramecium, the animals may be mounted to advantage in gum tragacanth, which makes their movements slower.

247. a. *The taking of food.* By the use of india ink observe how Paramecium brings food to its mouth. Sketch a quiet individual, showing the currents, in relation to the mouth and oral groove. Observe the passage of the particles into the mouth, and the formation of the food vacuoles.

b. *Distribution of food.* Observe the circulation of the food vacuoles. Do the vacuoles alone move, or does the internal protoplasm move with them? Follow the circulation of the vacuoles.

Make a drawing of Paramecium, showing mouth, oral groove and food vacuoles and indicate the path of circulation of the food vacuoles by means of arrows. (Watch carefully for defaecation; if observed, indicate in your figure where it occurs.)

c. *Digestion.* Feed the animals on green alga cells. Observe the changes in color in the food vacuoles after they have been some time in the body. They become yellowish in place of bright green. The difference in color among the different food vacuoles is commonly easily seen.

Stain some of the living Paramecia with neutral red, in the following way. Make a 1/1000% solution of the neutral red. To a small quantity of this add twice this quantity of culture water containing many Paramecia, and allow to stand for ten minutes or more. The animals will now be found to be partly stained red. Since this substance stains only structures having an acid reaction, the staining gives an opportunity to determine the nature of some of the chemical processes in development. It will be found that some of the food vacuoles are strongly stained, indicating the presence in them of an acid. Others will be found colorless, while others are of a pale yellowish tinge. The latter are those in which digestion is about finished, and the acid, together with the nutritious parts of the food, have been absorbed, and the remaining material has taken an alkaline reaction (to which the yellow color is due). Such a mass is usually found in the posterior part of the body, about half way between the mouth and the posterior end. It consists of waste matter ready for discharge, the discharge taking place at about this point.

Note the red stained zymogen granules about the vacuoles. These are thought to be the substances giving rise to the ferments of digestion.

The reaction of the vacuoles can also be followed by feeding on finely divided litmus particles. Try this experiment as a check on the above.

d. *Defaecation.* Observe this in Paramecia that have been fed with India ink. With a little patience in watching it is usually easily seen in such cases. Represent the place where it occurs on your figure.

e. *Excretion* takes place by means of the two contractile vacuoles. Observe the method by which these are filled; draw two or three stages in the process, showing the canals, etc. Observe the discharge of the vacuoles in the following way. Place the Paramecia in a thick solution of India ink, so that they appear white against

a dark background. Withdraw enough of the water with filter paper so that the cover shall rest upon them and hold them still. Find a specimen with contractile vacuoles near one edge (not above or below). Under these circumstances the discharge of the vacuole contents into the surrounding black fluid is easily seen. Sketch. Is the discharged fluid carried away in any way, or does it remain against the surface of the animal?

f. *Respiration*. This is difficult to observe or demonstrate. It has been done in the following way. Make a weak, slightly reddish solution of rosolic acid (rosol). Place a dense mass of *Paramecia* in this, on a slide, with a supported cover-glass. Often the animals gather in dense groups. When this occurs, enough carbon dioxide may be given off in the respiration of the large number to decolorize the rosolic acid. When this occurs, if the preparation is placed on a white background the region where the *Paramecia* are gathered appears like a white spot in the red preparation.

Is there any method in *Paramecium* of continually renewing the water that is in contact with the body, so as to have at all times a fresh supply of oxygen?

250. Types of substances digested. *Paramoecia* which feed on algae or bacteria must digest proteins. Determine if starch is digested, as follows:

Add a little dilute corn starch paste to some of the organisms in a covered watch-glass. At intervals remove a few *Paramecia* to a slide and add iodine solution. Does the reaction indicate digestion of starch? Keep for a day or two and test with iodine again.

The digestion of fat is difficult to demonstrate but it is probable that *Paramecium* contains lipolytic enzymes.

### B. AMOEBÆ.

If material and time permit, make a study of the same processes in *Amoeba*. If you are unable to see the facts yourself, read the references given below. Form an idea of the way each of the processes mentioned on pp. 72 and 73 occur.

251. a. *The taking of food*. This can usually be observed, with some patience, in a culture where *Amoebæ* are numerous. If observed, describe and sketch. If you do not see it yourself, read the accounts given in the following:

Carnegie Institution, Publication 16, Jennings: Contributions to the Study of the Behavior of the Lower Organisms, pp. 193-202.

Leidy, Fresh water Rhizopods of North America, pp. 85-89 (in *Dinamoeba*, which is practically an *Amoeba*). See also Plate VI.

b. *Digestion*. If you have many favorable specimens, stain with neutral red, in the same way as with *Paramecia*; determine whether an acid is present, and if it disappears before the waste matter is discharged.

c. *Defecation*. This is not easily observed. Do you know how it occurs?

d. *Excretion*. Contractile vacuole, as in *Paramecium*. With many favorable examples the external discharge may be observed by the use of India ink, as in *Paramecium*.

e. The following questions you may not be able to answer by direct observation, but you should be able to answer them from what you have seen or read: Is there any special method of distributing the food within the body? How does it probably occur? How is oxygen probably taken? Carbon dioxide given off? Is there any way of producing a current of water, to bring food and oxygen, and carry away waste matter?

#### C. HYDRA OR A SEA ANEMONE.

252. a. Study and describe the taking of food, by feeding Hydra a small animal or a bit of meat. Keep the specimen in a vessel with a bit of plant, to furnish oxygen, and determine if you can about the length of time taken for digestion.

b. Does Hydra exercise any choice of food? Try feeding pieces of plant, or bits of paper. Are they taken?

c. After a Hydra has been well fed, will it take more food, or do hungry Hydras act differently from well fed ones in this respect?

d. If you have opportunity, observe the egestion of the waste matter. How does it take place? Make a diagram of the alimentary canal in Hydra. Is there any way of distributing the food? Examine the cavity within the tentacles with high power; are there any internal currents?

e. Are there any external currents, for bringing food and oxygen, or carrying away waste matter?

f. How do respiration and excretion probably occur? Are there any special respiratory or excretory organs? Determine whether oxygen is required, by leaving Hydra in a vessel of boiled water and keeping it tightly closed. Does it live as long as in other water?

#### D. ANNELIDS.

Study the processes and organs of metabolism, so far as it is possible, in two of the lower annelids, as follows:

253. *Aelosoma*. a. Observe the straight alimentary canal, with an opening at each end. Distinguish mouth, short muscular pharynx, narrow oesophagus, wide stomach, and narrow intestine, and the anus. If necessary stupefy the animals partly by placing them for a time in a dish containing a weak solution (about  $\frac{1}{4}$  saturated) of chloretone (use a weaker solution if this injures them.) Notice the ciliary movement within the alimentary canal; in what parts is it found? Are there any peristaltic movements? Notice especially the large glandular cells which make up the walls of the stomach. Are there any separate glands connected with the alimentary canal?

b. Is there any method of causing an external current in the water for bringing oxygen and carrying away carbon dioxide? Test with India ink. How are the currents produced, and where are they found? Make a figure of the animal, showing the alimentary canal, and indicating by arrows the external currents.

Are there any indications of internal currents, i.e., of a circulatory system?

c. In *Aelosoma* we find definite excretory organs, of a very simple character. They consist of small coiled tubes, which are found in pairs at intervals attached to the body wall below the alimentary canal. Study these carefully. They can best be seen when the animal is placed with ventral side up; and in a region where the alimentary canal is not too wide (oesophagus or intestine). Notice that one end of the tube is open and bears cilia, which can be seen in lively movement; this end is called the funnel. Are there cilia elsewhere in the tube? The opposite end of the tube is attached to the body wall, and opens to the outside. The whole organ is a nephridium. Make as complete a drawing of the nephridium, on a large scale, as you can and indicate the ciliary movement. The nephridium takes up waste substances from the fluid of the body cavity, through its walls. These are washed out by the ciliary action, a certain amount of fluid which aids the washing out being introduced into the tube through the opening of the funnel.

254. *Dero*. a. This is studied particularly for its very peculiar respiratory organs. Notice however the simple alimentary canal, like that of *Aelosoma*, but perhaps still simpler. Observe also that there is a method of producing internal currents, i.e., a circulatory system, with reddish blood. How is this blood caused to move?

b. Are there any external currents? (Determine with India ink.) Observe that these are at the *posterior* end in this case (compare with *Aelosoma*). Study carefully the large posterior

extension. Notice the finger-like extensible and retractile points, covered with a sort of hood. How many of the points are there? Are they ciliated? Observe if possible that the whole structure is supplied with much blood, which circulates through it. In some species the points are much longer, extending out like fingers. What is the function of this structure? Why is it ciliated? Why is it so well supplied with blood? Draw the structure carefully.

c. Nephridia are present in *Dero*, but are not favorable for study.

255. Study, if opportunity is given, the living nephridium of the earthworm. Observe especially the ciliary movements in the funnel. Distinguish funnel, secretory portion, and reservoir. Observe the ciliary movement within the secretory portion. Observe also, if it is possible, the blood vessels covering the nephridium. Examine the figures in Beddard's *Monograph of the Oligochaeta* (or copies of these); note in these the blood vessels.

#### E. CRUSTACEA.

256. Study the respiratory and circulatory processes in *Daphnia*. Where does respiration occur? How is the respiratory current produced? (Use India ink if necessary.) Observe the beating of the heart, and the circulation of the blood. See the movements of the blood corpuscles in the head, appendages, etc. By figure, diagram, or description, indicate the external (respiratory), and internal (circulatory) currents. What uses do these currents serve?

Can you see the alimentary canal in *Daphnia*? If so, sketch or describe.

257. Study, if opportunity is given, the respiratory current, by means of India ink, in a crayfish or crab. Where does it occur, and how is it produced? Examine in a preparation the structures producing it. Examine also the gills. Observe that they are simply expansions of the body wall, that have taken a complicated form. Compare them with a figure on the chart, showing their relation to the blood vessels. Draw one of the gills.

258. Examine, and describe, if there is opportunity, the respiratory organs and respiratory movements in *Limulus*.

#### F. INSECT.

260. Examine and draw the respiratory organs of an insect larva. In what fundamental way do they differ from the other respiratory organs we have studied?

#### G. FROG.

261. Examine the alimentary canal of the frog, and identify the

different parts. Measure its total length, and compare with the length of the body. Examine the alimentary canal of a tadpole. What is its length, measured in units of length of the body (without the tail)? How does it compare in relative length with that of the frog? Why the difference?

Examine also the lungs of the frog. Inflate them. Note their sac-like character, and the internal ridges, which contain blood vessels.

## B. CIRCULATORY SYSTEM

### 1. PHYSIOLOGY OF THE HEART BEAT.

262. *The frog's heart.* Pith a frog, being careful to avoid loss of blood. Plug the brain with cotton if any occurs. Now expose the heart by cutting through the body wall to one side of the middle line (to avoid abdominal vein), turn the pectoral girdle to one side and remove if necessary. Note the pericardium surrounding the heart. Carefully cut through the pericardium exposing the heart. Note a slender band of connective tissue running between the dorsal surface of the heart and the pericardium—the fraenum. Tie a thread tightly around this; then divide the fraenum dorsal to the ligature. Use the thread for turning the heart over or otherwise handling it. Study carefully the anatomy of the organ; draw from dorsal and ventral aspects. Note right and left auricle, junction of three venae cavae to form the sinus venosus, single truncus arteriosus, bifurcation of the latter and subdivision of each branch into three arterial trunks—carotid, aortic and pulmonary. Be sure you understand the anatomy of the organ. Observe the whitish crescent at the junction of sinus and right auricle.

a. Study carefully the sequence of the beats of the different chambers. Close observation is needed here. Note any change in color, size and form of the ventricle during systole and diastole respectively.

b. Count the number of beats per minute. Now excise the heart, cutting wide of the sinus after lifting by the ligature. Place the organ in a small glass vessel with Ringer's solution. Has excision altered the rate of beat? Keep the heart for use in experiment 265 b.

263. *The turtle's heart.* Expose the heart of a turtle without loss of blood and familiarize yourself with its structure and sequence of beat as in the frog. That may be done as follows: Pith, by

making a transverse slit through the heavy muscles on the back of the neck. Continue the slit through a joint between the vertebrae and destroy the brain with a wire. Plug the cavity with cotton. Sever the union between plastron and carapace at the sides with bone shears and cut the skin and muscles as near the plastron as possible so as to remove the plastron from the body. On pulling the forelimbs straight the heart will be seen beating and by a little careful dissection can be freed from the pericardium. The latter is attached to the tip of the ventricle, and this strand should be used to take hold of in handling the organ.

264. *Conduction of impulse in the heart.* In the frog and turtle the impulse originates in the sinus and spreads to the auricle and ventricle; in the mammal the impulse starts in the right auricle near the venae cavae and spreads to auricles and ventricles and also to a certain distance over the veins opening into the auricle. On reaching the auricle-ventricular junction there is a distinct pause termed the auriculo-ventricular interval; finally, the excitation reaches the ventricle, and the contraction wave is seen to traverse the ventricular muscle. The auriculo-ventricular interval may be lengthened by any natural or artificial hindrance to the excitation wave.

a. Place the Gaskell clamp about the auriculo-ventricular junction. Very carefully turn the screw until the rubber edge makes a gentle pressure on the cardiac tissues at that point. With careful work a degree of pressure will be reached that diminishes the conductivity of the muscle fibres joining the auricle and ventricle so far as to allow only every second or third excitation to pass. The auricle will beat without change of frequency, but the ventricle will beat only when the excitation succeeds in passing the block.

b. Repeat experiment a, but place the screw clamp across the middle of the ventricle. The passage of the excitation from one part of the ventricle to another will be delayed or interrupted by the lowering of the conductivity in the compressed portion. Many irregularities in the frequency and force of the heart can be explained by variation in conductivity of its several parts.

265. *Automaticity of different chambers of the heart.* a. Careful observation is required to detect contractions in small pieces of the heart. Determine the rate of the whole heart. Cut off the sinus venosus. Does it beat? Rate? Does the remainder of the heart beat? Rate? Cut the sinus into small pieces. Rate of each piece? It is best to tie a ligature (of Stannius) between

sinus and auricle while the heart is in the body and *full of blood*, so that sinus can be distinguished from auricles when excised.

Separate (1) the two auricles from the ventricle, (2) the auricles from each other, (3) the tip of the ventricles from the base, and determine in each case if the piece of heart isolated is automatically rhythmic and its rate. In which region is the beat fastest? Are the now automatically beating regions independent of each other in rhythm.

b. Repeat the above experiment with the frog's heart used in experiment 262. Does the conus arteriosus of a frog's heart beat when isolated?

## II. PHYSIOLOGY OF HEART MUSCLE.

266. *Graphic record.* Use the frog's heart still beating in the body. Pass a bent pin, to which has been fastened a fine wire, through the tip of the ventricle. Fasten the wire to the heart lever by wax, and adjust the lever on the support against a slow moving drum. Record the contractions.

267. *Refractory period and compensatory pause.* Place the signal magnet in the primary circuit of the inductorium and arrange the latter for single induced shocks. Attach one wire from the secondary posts of the inductorium to the heart lever and place another about the auricles. Record the normal beat of the heart on a slow moving drum, and stimulate at various phases of the beat with make or break shocks. From your record determine at what period the heart is non-irritable (i.e., refractory toward stimuli). Note the compensatory pause. At what phase is the maximum extra-contraction obtained? Is there any difference in the latent periods of the extra contractions? Try tetanizing. Result?

268. *All contractions are maximal.* Inhibit the heart by a ligature or a Gaskell clamp placed at the auriculo-ventricular junction. Find the least strength of stimulus that will cause the ventricle to contract. Increase the strength of the stimulus, but do not stimulate oftener than once in ten seconds (to avoid the stair-case contractions described below). Record the contractions. Does the force of ventricular contraction remain the same, notwithstanding the increased stimulus? How is this expressed in words?

269. *Stair-case effect or treppe.* Find the least stimulus that will cause the ventricle to contract. Repeat this minimal stimulus after every relaxation, recording the contractions on a slow moving drum. How does this result agree with the above experiment?

270. *Inhibition of ventricle by constant current.* No record need be taken. Place an indifferent electrode in contact with the muscles of the frog's throat or other indifferent region; the other electrode is placed in contact with the tip of the ventricle by means of a thin strip of cotton soaked in 7% NaCl. The two electrodes are connected through a key and pole-changer with the battery. With the *anode* in contact with the ventricle, *make* the current. Note the change in appearance of the ventricle; explain. Then reverse the current and break just before the beginning of systole. The cathode is now in contact with the ventricle. Any result? At what poles does the inhibition appear (a) at make and (b) at break of constant current? Compare with the conditions for stimulation.

271. *Stimulation by constant current.* Bring the ventricle to rest by a ligature at the auriculo-ventricular junction. Using the same arrangement as before, determine at which pole stimulation appears (a) at make, and (b) at break of constant current.

### III. INHIBITION OF HEART.

272. *Reflex inhibition.* Etherize a frog *lightly* by placing under a glass jar with a piece of ether soaked cotton. Fasten the animal in the holder ventral surface upward. Apply ether at intervals if needed. Expose the heart, preventing loss of blood. Now tap the abdomen rapidly with the handle of a scalpel, noting any change in the number of beats per minute. The normal rate of the heart should be determined before tapping begins. Now expose the intestine and try the effect of direct stimulation of the intestine, both mechanical and electrical. What is the effect on the rate of heart beat? If the above method of reflex inhibition fails, expose the sciatic nerve, ligature, and stimulate the central end. Effect on the heart?

273. *Situation of cardio-inhibitory mechanism in central nervous system. (Cardio-inhibitory center.)* Remove carefully the cerebrum and optic lobes. The medulla is left intact. Now stimulate the intestine as before, after allowing time for shock to pass off. Result? Stimulate the medulla directly by platinum electrodes. Effect on the heart beat? Now destroy the medulla and repeat reflex stimulation. Result?

274. *Intra-cardiac inhibitory mechanism.* Stimulate the heart directly at the "white crescent" marking sinu-auricular junction. Result? After inhibition note carefully the manner in which the beat is resumed.

275. *Inhibition by direct stimulation of vagus.* Expose the vagus nerve in frog as follows: Pass a rather wide glass tube down the oesophagus (to put the tissues on the stretch). This will expose three large nerves at the side of the neck; these are, in order from above down, *glosso-pharyngeal, vagus, hypoglossus*. Stimulate the vagus with a weak tetanizing current, noting the effect on heart. If no effect is shown try a stronger current, or try the vagus on the other side of the body, since the two are often unequal in their action. Note (1) latent period of inhibition, (2) duration of inhibition, (3) manner in which the beats are resumed.

Take a record of vagus inhibition, placing a signal magnet in the circuit to record the moment of stimulation. Then connect the signal magnet with the desk binding post and revolving the drum at the same speed take a second's time curve.

If the frog's heart is weak use a turtle. The vagus lies in the side of the neck and may be exposed by putting the tissues on the stretch, and recognized by stimulation.

To make sure that the effect is not reflex, ligature the vagus as near the central end as possible, cut centrally to ligature, lift by ligature and stimulate. Test the direct irritability of the heart while in a state of inhibition. Does it respond readily?

#### IV. EFFECT OF VARIOUS FACTORS ON THE CHARACTER OF THE RHYTHM.

276. *Graphic record of the influence of temperature on the rate of heart beat.* Expose the heart of a frog. Pass a small hook attached to a thread through the tip of the ventricle. Then excise the whole heart, cutting widely around it, and pin the tissues surrounding its base to a small cork plate. Fasten the plate to a glass rod by inserting the latter into a hole cut with a cork borer. The heart, thus attached to the rod, may then be immersed in any desired solution, and its action recorded by the thread, which is tied to the end of the short arm of the heart lever.

The heart is immersed in Ringer's solution contained in a glass or beaker supported by a block, as in the experiments with voluntary muscle. Take tracings of the beat on a slow drum with the heart surrounded by Ringer's solution at the temperatures  $5^{\circ}$ ,  $15^{\circ}$ ,  $25^{\circ}$ . Proceed thus: Have ready a glass filled with the cold Ringer, e.g.,  $5^{\circ}$ ; bring the heart into the solution in the usual manner (by removing the block, bringing the solution up from below, and then replacing block). Let the heart make a tracing at this tempera-

ture for two or three minutes; mark the minute intervals on the drum immediately below the writing point. Then replace the solution by Ringer at a temperature of  $10^{\circ}$  higher (i.e.,  $15^{\circ}$ ), and let the heart record the beats at this temperature, marking the minutes as before. After two or three minutes replace this second solution by a third  $10^{\circ}$  warmer than the second (i.e.,  $25^{\circ}$ ), and take a similar record at this temperature. Count the number of beats per minute at each temperature. What is the relative increase of rate between  $5^{\circ}$  and  $15^{\circ}$ ? Between  $15^{\circ}$  and  $25^{\circ}$ ? What is the average "temperature coefficient of acceleration" for a rise of  $10^{\circ}$ ? How does this compare with the temperature coefficient of chemical reactions?

277. *Actions of salt-solutions on the heart-beat.* Take records of the following: Bring the heart, arranged as before, from Ringer's solution (at room temperature) into pure  $m/8$  NaCl; after a minute change this solution for fresh to remove all traces of K and Ca. Note any changes in the rate and character of the beat in this solution. After some minutes change the  $m/8$  NaCl for a mixture of 100 vols.  $m/8$  NaCl + 2 vols.  $m/8$   $CaCl_2$ . Is there any change in the beat? After a few minutes return to pure  $m/8$  NaCl and note the effect. Then transfer to a mixture of 100 vols.  $m/8$  NaCl + 2 vols.  $m/8$  KCl. Leave in this solution for some five minutes; note any differences from pure  $m/8$  NaCl. Finally return the heart to Ringer's solution. What is the importance of Ca and K for the heart?

278. *Action of  $CO_2$ , acid, alkali, alcohol, and KCN on heart.* Using the same arrangement as before, test the action of the following solutions on the frog's heart. Take records.

a. Ringer's solution saturated with carbon-dioxide. The solution should be drawn from the Sparklet siphon bottle shortly before using.

Try also half-saturated and third-saturated solutions. Return the heart to Ringer's solution soon after definite effects have appeared.

b. Ringer's solution plus  $n/400$  HCl.

c. Ringer's solution plus  $n/400$  NaOH.

d. Ringer's solution plus  $n/400$  KCN.

e. Ringer's solution plus 4 vol. % ethyl alcohol.

In all cases determine if the effects produced are reversed by return to normal Ringer's solution.

*Effects of some alkaloids on the heart.* Pith a frog or turtle

without loss of blood and expose the heart. Determine which vagus contains the inhibitory fibers.

279. *Action of nicotine.* Apply nicotine solution (0.2%) to the ventricle. After a few minutes, stimulate the trunk of the vagus nerve. No curve need be written. Is the heart inhibited? Now lift the heart with a glass rod, and stimulate the intra-cardiac inhibitory nerves, i.e., at sinu-auricular junction or white crescent. Result? Nicotine paralyzes some inhibitory mechanism between the vagus and the intra-cardiac inhibitory nerves. But it is known that nicotine does not paralyze nerve trunks. Hence it is probable that the cardiac inhibitory fibers in the vagus do not pass to the cardiac muscle directly but end in contact with nerve cells which take up the impulses, and transmit them through their processes to the muscular fibers of the heart.

280. *Atropine.* With a clean pipette apply a few drops of a solution of atropine (0.5%) to the heart. After a few moments lift the ventricle and stimulate the crescent. Is the heart inhibited? Atropine paralyzes the intra-cardiac inhibitory nerves.

281. *Muscarine.* With a fine pipette put upon the ventricle a few drops of salt solution containing a trace of muscarine. Effect?

282. *Antagonistic action of muscarine and atropine.* With a fresh pipette apply a little salt solution containing atropine (0.5%). Result?

## V. PRESSURE AND VELOCITY CONDITIONS IN THE CIRCULATION.

283. *Circulation in the web of the frog's foot.* Etherize lightly a frog and adjust on the frog board with the web between the toes stretched over the hole in the board. Study the circulation under the microscope and draw. Observe the following points:

a. Veins, arteries, capillaries. How can you distinguish them?  
b. Which pulsate and in which is the velocity fastest and in which slowest? Why?

c. Observe under the high power movements of individual red and white corpuscles. Are these found in particular regions of the vessel? Why?

d. Watch for a white corpuscle passing through the wall of the capillary.

284. Place a tiny drop of glacial acetic acid (from the point of a pin) on the web. Note the effect of the irritant, changes in size of vessels, collection of leucocytes, etc. *Do not get acid on the lens of the microscope.*

285. *Artificial circulation scheme.* Examine the scheme, following the description given in the Harvard Apparatus Company Catalogue, and make a diagram labelling the features presented. Fill with *distilled* water, tipping the tube so as to allow escape of air through the arterial path, and attach the manometers filled with Hg, connected with the tubes of the scheme by water. Note especially the following: Effect of (1) increasing the rate of heart beat on (a) arterial pressure, (b) venous pressure, (c) character of flow. (2) Pulse in the aorta. (3) Action of the mitral and aortic valves.

286. *Graphic record of blood pressures.* Blood pressures of living animals may be estimated and recorded by various methods. The principles involved can be determined from records given by the artificial circulation scheme. Place a writing lever in the arterial manometer from which the thistle tube has been removed, and adjust against a drum. (See demonstration.) Take a record showing the effect of (1) a rapidly beating heart on the blood pressure, (a) with high and (b) with low capillary resistance; (2) -a slow beating heart with (a) high and (b) low capillary resistance. A base line should be drawn showing the point of zero blood pressure, and a time curve (in seconds) taken from the desk binding posts.

287. *Pulse record. Sphygmograph tambour.* Examine the instrument, and set up as in the demonstration, cementing the aluminum angle to the rubber membrane and using a straw for the writing lever. Draw.

Cover a small thistle tube with rubber membrane on which has been cemented a bone button. Connect the tambour by rubber tubing with a side branch and clamp. Place the thistle tube over the "aorta" of the circulation scheme and take a record of its pulse with (1) slow heart with (a) large and (b) small capillary resistance. (2) Rapid heart with (a) small and (b) large capillary resistance. A time curve (seconds) should be recorded at the same time.

288. *Human pulse.* Place the thistle tube without the button over the carotid artery just below the angle of the jaw, having the side branch of the connecting tube open. Adjust against a slow moving drum. Now close the side and record. If no pulse shows, adjust the thistle tube until the correct spot is obtained. Compare the curve with that obtained from the artificial circulation scheme.

## C. RESPIRATION

### I. RESPIRATION BY LUNGS.

289. *Respiration scheme.* Study the mechanics of mammalian respiration in the artificial respiration scheme, following the description given in the Harvard Apparatus Company Catalogue, p. 74. The manometers should be filled with distilled water and the pressure condition should be such that the lung is always slightly stretched even in expiration. This is done by closing the cavity in the pleural tube with the water level near the lung.

Note especially the following:

a. Pressure relations in thorax and lung cavities during inspiration (lowering water level) with tracheal tube open. Same during expiration.

b. Same as above but with tracheal tube partially closed. Raise and lower the water level rapidly and note the effect on intrapulmonic pressure. Close the tracheal tube more and more and note the effects on intrapulmonic pressure.

c. During inspiration open the pleural tube. Note the effect on intrathoracic pressure. This is what happens when a bullet enters the chest.

d. Coughing or sneezing and hiccough can be imitated in the artificial scheme. Do you see how?

### II. OXIDATION IN THE TISSUES.

291. *Gases given off in respiration.* Expire through a glass tube and  $\text{Ba}(\text{OH})_2$  solution in a bottle. What does the precipitate indicate? To make sure that the  $\text{CO}_2$  is actually increased in expired air arrange another bottle with the same amount of  $\text{Ba}(\text{OH})_2$  solution and draw air through it by inspiration the same number of times as in the previous experiment on expired air. Which bottle contains the most precipitate?

Breathe against a cold plane pane of glass. What gas is indicated? Is this an oxidation product?

291. *Independence of  $\text{CO}_2$  production and  $\text{O}_2$  consumption.* "Intramolecular respiration." Carefully remove the seed-coats from 6 peas that have been soaked in water over night. Fill a small vial with mercury, and invert in a small vessel containing mercury, taking care to admit no air. Now place the peeled peas one by one under the rim of the vial so that they float to the top. Let stand one or two days. Observe the production of gas. Test by introducing

a little strong KOH with a bent pipette. Since no free  $O_2$  was present in the vial what must we conclude the origin of the  $CO_2$  to have been? This phenomenon is a *general* characteristic of metabolism.

292. Review Pasteur's Yeast Experiment (p. 27) which shows very clearly the difference between aerobic and anaerobic respiration.

293. Organisms can oxidize substances through oxidizing enzymes. Review the experiments on oxidation under the head of enzyme action (p. 23). Organisms have also a strong reducing power as indicated in the following experiment:

Cut thin slices of the tissues (muscle, kidney, sex organs, brain or spinal cord, etc.) of a frog and stain in salt solution plus methylene blue. Kill a thin piece of muscle tissue in hot water to serve as a control and stain in the same way. Place all on slides with salt solution under cover-glasses. Does the blue color disappear after a time, thus indicating reduction? Note if the color is localized in any region. Lift the cover-glass and determine the effect enzyme action (p. 23). Organisms have also a strong reducing power?

294. *Simultaneous reducing and oxidizing actions.* Inoculate the beef-broth-dextrose culture-medium with a collection of bacteria from sewage and place in a fermentation tube. Allow to stand a day or two and note the collection of gas. Gas formation is due to *Bacillus coli*.

Fill the tube with 10% KOH solution, excluding air, place your finger over the end and invert. Any absorption? Allow the gas to collect in the upper part of the tube and estimate the per cent absorbed. Again fill the tube with water to exclude air. Allow the gas to collect at the open end of the tube, covered by your finger. Remove your finger and quickly apply a match. What gases are indicated by the tests?

295. *Effects of lack of oxygen on Paramecium.* The Engelmann gas chamber is used in this experiment. The *Paramecia* are examined in a hanging drop of culture fluid on the under surface of a cover-glass placed over the aperture of the gas chamber. Oxygen is removed by passing a current of hydrogen through the chamber. The gas is generated from zinc and dilute  $H_2SO_4$ , and is passed through two wash bottles, one containing  $KMnO_4$  solution, the other 20% NaOH, and then through the chamber; from the exit tube of the chamber a rubber tube opens under the surface

of water to serve as indicator of the rate of gas flow. All junctions must be rendered gas-tight, using paraffin where necessary.

Note the effects of lack of oxygen (1) on the activity of the cilia and contractile vacuoles (compare at intervals with the "control", i.e., *Paramecia* in normal culture fluid; (2) on the consistency of the protoplasm; (3) on the absorption of water by the protoplasm.

Determine the degree of reversibility of the effects. After the movements have almost ceased, expose the drop to air by removing the sliding top from the gas chamber. Are the movements renewed? Try the same experiment with *Paramecia* that have entirely ceased movement.

296. *Effects of carbon dioxide on Paramecium.* Generate the gas with marble and dilute HCl. Pass through two wash bottles with water, and through the chamber as before.

Study the effects of  $\text{CO}_2$  as above. Note carefully any differences from the  $\text{H}_2$  experiment. Determine the reversibility of the effects as before.

297. *Effects of lack of oxygen and carbon dioxide on the ciliated epithelium of the oyster gill.* Mount portions of gill filaments in sea-water in the Engelmann's gas chamber as above, and study the effects produced by a stream of hydrogen and of  $\text{CO}_2$  respectively. Note carefully all differences between the effects of these two gases. Compare with the conditions in *Paramecium*.











