COMPARATIVE PHYSIOLOGY

AND

URINE ANALYSIS.

FISH.

QP
44
F53p

~~Fdg~~
~~F52~~
~~1898~~

QP
44
F53P

THE
Roswell P. Flower Library

THIS BOOK IS THE GIFT OF

Pierre A. Fish

3 1924 001 042 252

yes

DATE DUE

FEB 18 1969

GAYLORD

PRINTED IN U S A.

Cornell University Library

The original of this book is in
the Cornell University Library.

There are no known copyright restrictions in
the United States on the use of the text.

PRACTICAL EXERCISES

IN

COMPARATIVE PHYSIOLOGY

AND

URINE ANALYSIS

BY

PIERRE A. FISH, D.Sc.,

Assistant Professor of Comparative Physiology and Pharmacology,
New York State Veterinary College,
Cornell University.

PUBLISHED BY THE AUTHOR.

PRESS OF ANDRUS & CHURCH
ITHACA, NEW YORK
1898

QP
44
F53p

~~Ida J52p~~
Copyright 1898 by
PIERRE A. FISH.

no. 965

PREFATORY NOTE.

This little manual has been designed, especially, to meet the needs of those students who desire to become physicians or teachers of science. While not intended as an exhaustive treatise, it has been the endeavor to concentrate a number of useful experiments into a small compass. For a few of these experiments a somewhat special equipment may be needed ; but the majority of them may be as easily performed in a preparatory school as in a college, with a little experience and ingenuity on the part of the instructor.

It has been the aim to explain clearly the essential steps of the experiments and the reasons for them ; but, at the same time, to leave opportunities for observation on the part of the students themselves, and to have them record their own inferences of the phenomena observed.

In the preparation of this laboratory guide, the standard and recent books and papers bearing on practical physiology have been largely drawn upon, among which may be mentioned the works of Stirling, Halliburton, Waller, Brunton, Foster and Langley, Gamgee, Stewart, Long, Hall, Chase, and others.

In a sense, all physiology is comparative since we are dependent upon the lower forms of animal life for much of our knowledge of the function of similar organs and parts in the higher ; but there are differences of function in the different genera of animals, correlated with differences in structure and mode of life. Wherever it has been practicable, in the text, these differences have been pointed out.

August, 1898.

P. A. F.

TABLE OF CONTENTS.

EXERCISE	PAGE.
I. Preparation of Reagents	7
II. Albumins, Peptones, Globulins	8
III. Albuminates, Albuminoids	11
IV. Carbohydrates	13
V. Carbohydrates, Neutral Fats, Bone	15
VI. Test-Solutions for Albumins and Carbohydrates	19
VII. Salivary Digestion	20
VIII. Gastric Digestion	22
IX. Gastric Digestion	24
X. Pancreatic Digestion	25
XI. Pancreatic Digestion	28
XII. Bile	30
XIII. Milk	33
XIV. Extracts of Nervous, Muscular and Hepatic Tissues	36
XV. Blood	38
XVI. Blood	42
XVII. Examination of Urine, Reaction, Specific Gravity	44
XVIII. Examination of Urine, Chlorides, Sulphates, Phosphates	47
XIX. Examination of Urine, Urea, Uric Acid, Hippuric Acid	52
XX. Examination of Urine, Albumin	56
XXI. Examination of Urine, Sugar, Bile	59
XXII. Circulation, Heart, Cilia	63
XXIII. Circulation, Peripheral, Inflammation	65
XXIV. Nervous System, Reflex Action	67
XXV. Nervous System, Nerve-Muscle Preparation	69

I.

Students should provide themselves as soon as possible with the following: Labels suitable for test-tubes and bottles; notebooks for drawing and description. The notebooks are to be handed in occasionally for inspection and correction.

Each student will be furnished with the following articles and will be expected to report *promptly* any breakage or absence of material:

2 dozen test-tubes.	2 graduates.
1 test-tube brush.	1 watch-glass.
1 test-tube rack.	1 thermometer.
1 test-tube holder.	1 waste-jar.
2 cans for test-tubes.	1 burner.
2 flasks.	1 water-bath.
1 evaporating dish.	1 tripod.
1 funnel.	1 piece of wire gauze.
1 package of filter papers.	1 bottle of neutral litmus paper.
1 stirring-rod.	1 small roll of absorbent cotton.
6 pipettes.	

Before entering upon the work, the student should make a careful inventory of his equipment and report, at once, any deficiencies. As a beginning for the work to follow, each student will prepare and label the following reagents:

Sulphuric acid, concentrated, 2 oz. glass-stoppered bottle.

Nitric acid, concentrated, 2 oz. glass-stoppered bottle.

Hydrochloric acid, concentrated, 2 oz. glass-stoppered bottle.

Acetic acid, commercial, 2 oz. glass-stoppered bottle.

Ammonia, commercial, 2 oz. glass stoppered bottle.

Millon's Reagent, 2 oz. glass stoppered bottle. Made as follows: Weigh out 30 grams of mercury and 30 grams

of nitric acid. Dissolve the mercury in the nitric acid. Dilute the solution thus obtained with twice its volume of distilled water and decant the clear liquid.

Sulphuric ether, 2 oz. glass-stoppered bottle.

Strong alcohol, 2 oz. glass-stoppered bottle.

Hydrochloric acid, 0.2%. This is made by adding 3.25 cc. of the concentrated acid to one-half liter of distilled water.

Acetic acid, 2%. Made by adding 6 parts of the commercial acetic acid to 100 parts of water.

Caustic soda, 20%, 100 cc.

Caustic potash, 20%, 100 cc.

Caustic potash, 0.1%, 100 cc.

Sodium carbonate, 1%, 100 cc.

Barium chloride, saturated solution, 50 cc.

Copper sulphate, 1%, 100 cc.

Potassium ferrocyanide, 5%, 100 cc.

Ammonium sulphate, magnesium sulphate, and sodium chloride in dry form in bottles. Other reagents will be prepared as needed.

II.

ALBUMINS OR PROTEIDS.

1. Albumins are soluble in water and dilute saline solutions. They are coagulated by heat. Albumins may be classified as follows :

(a) Egg-albumin, a non-alkaline solution coagulable with ether ; when injected under the skin or introduced in large quantities into the stomach or rectum, it is given off by the urine. The undiluted albumin is coagulated by 40% formaldehyde.

(b) Serum-albumin is obtained from blood-serum. It is not coagulated by ether. When injected under the skin it does not appear in the urine. It is not coagulated by formaldehyde.

(c) Lact-albumin, the proteid found in milk.

2. Preparation of Egg-Albumin Solution. Break a small hole in the end of a fresh egg ; carefully pour out the white of the egg into a beaker. Let the yolk remain in the shell and reserve for later use. Add about twenty volumes of distilled water to the beaker containing the egg-white. Stir thoroughly with a glass rod to break up the membranes and thus liberate the albumin. Filter through a piece of muslin. Any opalescence is due to the precipitation of globulins. Egg-white contains about 11%-12% of egg-albumin, together with small quantities of globulins, grape-sugar, and mineral matter.

A good solution for laboratory use may also be prepared by dissolving 1 gram of dry albumin in 200 cc. of distilled water.

3. Heat 5 cc. of the albumin solution in a test-tube to boiling. Notice the coagulation. Add a little nitric acid, the coagulum may turn yellow but it does not dissolve.

4. Xanthoproteic reaction. To a little of the albumin solution in a test-tube, add some strong nitric acid; a precipitate is formed, white in color, which on being boiled, turns yellow. After cooling, add ammonia; the yellow color changes to orange. With weak solutions there may be no precipitate at all. If only traces of albumin are present, the yellow color with the nitric acid may fail to appear, but the addition of ammonia gives the final test with, perhaps, a yellow instead of an orange color.

5. To another portion of the solution add some of Millon's reagent; a white precipitate is formed, which on boiling, becomes brick-red in color.

6. Piowtrowski's reaction. Add excess of strong solution of caustic potash and then a drop or two of very dilute solution of cupric sulphate, when a violet color results. The reaction occurs more quickly if heat is applied, and the color deepens. (Peptones and albumoses give a pink color when only a trace of copper sulphate is used.)

7. Acidify another portion strongly with acetic acid and add a few drops of potassium ferrocyanide. A white precipitate is obtained. Peptones do not give this reaction. (Albumin is also precipitated by lead acetate, mercuric chloride ; picric acid ; strong acid, *e. g.*, nitric ; tannin ; and strong alcohol).

8. Make some of the albumin solution strongly acid with acetic acid, add some sodium sulphate and boil. All proteids except peptones are precipitated in this manner. The filtrate, after boiling, can be used for other tests as the acid and sulphate do not decompose the solution.

9. Indiffusibility of Albumin. Place some of the solution in a dialyzer. The salts (crystalloids) diffuse readily. Test for chlorides by adding a little silver nitrate solution to a portion of the diffusate. Apply to another portion of the diffusate any of the preceding tests for albumin. None will be found. Albumin belongs to the group of colloid bodies.

10. Peptones. Peptones are proteids soluble in water, but not coagulable by heat. They are diffusible through animal membranes. Albumoses or proteoses are substances intermediate in constitution between albumins and peptones.

11. Peptones differ from albumins as follows : They are not coagulated by heat ; they are not precipitated by adding sodium chloride ; they are not precipitated by acids or alkalis ; they are not precipitated by sodium sulphate ; they are not precipitated by potassium ferrocyanide ; they yield a pink color with Piowtrowski's test instead of a violet as given for albumin.

12. Like albumin they are precipitated by the addition of tannic acid ; they are also precipitated by alcohol.

13. Globulins.—Globulins are proteids insoluble in water, but are soluble in dilute saline solutions. They are coagulated by heat. Among the most important are : vitellin, crystallin (globulin), myosin, fibrinoplastin (paraglobulin), and fibrinogen (metaglobulin).

14. After washing it carefully, stir up the yolk that was saved (2). Put 2 or 3 cc. of the stirred yolk in a test-tube and fill it from $\frac{1}{2}$ to $\frac{2}{3}$ full of ether, and shake several times. The ether soon becomes yellow; transfer it to an evaporating dish. Let the ether evaporate at the room temperature, which will not take long, and note that a yellow oil remains. Pour a drop or two of the oil in some water and notice the globules of fat that are formed. To the remainder of the oil add a few drops of nitric acid. The mixture turns bluish green and finally becomes colorless. Add a few drops of a 1% solution of potassium sulphocyanide solution. A reddish color indicates the presence of iron.

15. The fatty portions of the yolk were dissolved in the ether, and impure vitellin remains in the test-tube. Remove some of this to a watch glass so that the ether may evaporate. When the substance is free from ether remove it to a test-tube, add some distilled water and note that the vitellin does not dissolve, now add a small pinch of salt and shake; a milky solution of vitellin is formed. Filter and to portions of the filtrate apply the xanthoproteic, Millon's and Piowtrowski's tests.

III.

DERIVED ALBUMINS.

16. Albuminates.—Action of acids and alkalis on albumin. Take three test-tubes and label them A, B, C. In each, place an equal amount of diluted egg-white, like that used at the last exercise. To A add a few drops of 0.1% solution of caustic potash. To B add the same amount of 0.1% solution of caustic potash. To C add a rather larger amount of 0.1% sulphuric acid, (1 part H_2SO_4 to 1000 of water.)

Put all three into a warm water bath at about the temperature of the body (36–40 C.).

17. After ten minutes remove test tube A and boil. The proteid is no longer coagulated by heat, having been converted into *alkali-albumin*. After cooling color with litmus solution, and neutralize with 0.1% acid. At the neutral point a precipitate is formed, which is soluble in excess of either acid or alkali.

18. Next remove B. This also now contains alkali-albumin. Add to it a few drops of a sodium phosphate solution, color with litmus, and neutralize as before. Note that the alkali-albumin now requires more acid for its precipitation than in A, the acid which is first added converting the sodium phosphate into acid sodium phosphate.

19. Now remove C from the bath. Boil it. Again there is no coagulation, the proteid having been converted into *acid-albumin* or *syntonin*. After cooling color with litmus and neutralize with 0.1% alkali. At the neutral point a precipitate is formed soluble in excess of acid or alkali. (Acid-albumin is formed more slowly than alkali-albumin, so it is well to take plenty of time).

20. Metallic albuminates. Add to separate tubes of albumin solution, a crystal each of copper sulphate, silver nitrate and a small amount of mercuric chloride. In each of the three tubes metallic albuminates will be precipitated.

21. Albuminoids.—Albuminoids consist of a number of bodies which, in their general characters and elementary composition resemble proteids, but differ from them in many respects. They are amorphous. Some of them contain sulphur, and others do not. The decomposition-products resemble the decomposition-products of proteids.

Gelatin is obtained by the prolonged boiling of connective tissues, for example, tendon, ligaments, bone and from the substance “Collagen” of which fibrous tissue is said to exist.

22. Make a watery solution of gelatin (5%) by allowing it first to swell up in the cold water, and then dissolving it with the aid of heat. It is insoluble, but swells up in about six times its volume of cold water.

23. After dissolving with the aid of heat, allow a small portion to cool ; it gelatinizes.

24. Apply the xanthoproteic test for proteids to some of the dissolved portion ; make notes of any differences as compared with proteids in this and in the following tests :

25. Use Millon's reagent.

26. Try Piowtrowski's reaction.

27. Is it precipitated by the acetic acid and potassium ferrocyanide test ?

28. Does it coagulate by heat ?

29. Is it precipitated by saturation with magnesium sulphate ?

30. What is the result of the addition of tannic acid ?

31. Add picric acid (saturated solution) ; if a precipitate appears apply heat and note any change that may occur upon cooling.

32. What is the effect of adding alcohol to the gelatin solution ?

IV.

33. Carbohydrates. The term *carbohydrates* includes an important group of substances, occurring especially in plants. Starch and sugar make up a large proportion of the parts of plants, while cellulose forms the chief material from which many parts of plants are constructed. Carbohydrates also occur to a less extent in animals, where they are represented chiefly by glycogen and some forms of sugars.

In elementary composition they are non-nitrogenous and consist of CH and O with the H and O in the same proportion as in water, that is, 2 atoms of H to 1 atom of O.

(This proportion is also obtained in other substances not belonging to the carbohydrate group).

Carbohydrates are indifferent bodies with a neutral reaction and form only loose combinations with other bodies, especially with bases.

34. Starch ($C_6H_{10}O_5$)_n is one of the most widely distributed substances in plants, and it may occur in all the organs of plants, either (a) as a direct or indirect product of the assimilation of CO_2 in the leaves of the plant, or (b) as reserve material in the roots, seeds or shoots for the later periods of generation or vegetation.

35. Squeeze some dry starch powder between the thumb and forefinger, and note the peculiar crepitation sound and feeling.

36. Place 1 gram of starch in a mortar, rub it up with a little cold water, and then add 50 cc. of boiling water. Transfer to an evaporating dish and boil over a water bath. Does the starch go into solution?

37. Add powdered dry starch to cold water. Is it insoluble? Filter and test the filtrate with a solution of iodine. A blue color denotes the presence of iodide of starch.

(Prepare the iodine solution as follows: Dissolve 2 grams of potassium iodide in 100 cc. of distilled water, add 1 gram of iodine and shake until dissolved).

38. To some of the boiled portion of starch, add solution of iodine. Heat and note any change that occurs. If not boiled too long another change may occur when cooled.

39. Render some of the starch mixture alkaline by adding caustic potash. Add iodine solution. What is the result?

40. Acidify with dilute sulphuric acid, then add iodine. What is the result?

41. Add some solution of tannic acid. Note result and then heat.

42. Place some strong starch solution in a parchment tube and the latter in distilled water. Allow it to stand for some time and test the water for starch.

43. Dextrin. ($C_6H_{10}O_5$) is an intermediate product in the hydration of starch.

44. Dissolve some dextrin in boiling water and cool. Add iodine solution—a reddish brown color, which disappears on heating and returns on cooling. (The student should take two test tubes placing the dextrin solution in one, and an equal volume of water in the other. Add to both an equal volume of iodine solution and thus compare the difference in color.)

45. Cellulose ($C_6H_{10}O_5$)_n occurs in every tissue of the higher plants, where it forms the walls of cells and the great mass of hard parts of wood. It is also found in the outer investment of the animals known as Tunicates. Purified absorbent cotton is a good example of cellulose.

46. Add some strong sulphuric acid to a little absorbent cotton in a test tube. Note any change and then add a drop of iodine solution.

V.

47. Glucose or Dextrose (Grape sugar) ($C_6H_{12}O_6$) exists in fruits and in small quantities in the blood and other fluids and organs. It is the form of sugar found in diabetic urine. It is readily soluble in water. Prepare 100 cc. of a 2% solution.

48. To a portion of this solution add a little iodine solution. Compare with starch.

49. Heat another portion of the solution with sulphuric acid;—it darkens slowly.

50. Trommer's test. To another part of the solution add a few drops of a dilute solution of copper sulphate, and afterwards add caustic potash in excess, that is, until the precipitate first formed is re-dissolved and a clear blue fluid is obtained. The hydrated oxide of copper precipitated from the copper sulphate is held in solution in presence of

glucose. Heat slowly turning the tube in the flame. A little below the boiling point, if glucose be present, the blue color disappears and a yellow (cuprous hydrate) or red (cuprous oxide) precipitate is obtained. If the upper surface of the fluid has been boiled, the yellow precipitate, when it occurs, contrasts sharply with the deep blue-colored stratum below. The precipitate is first yellow, then yellowish red, and finally red. It is better seen in reflected than transmitted light. If no sugar be present, only a black color may be obtained.

51. Fehling's solution. Solution A. 34.64 grams of pure crystalline copper sulphate are powdered and dissolved in 500 cc. of distilled water. Solution B. Sodio-potassium tartrate (Rochelle Salts) 173 grams. Pure caustic potash 125 grams. Add enough distilled water to make 500 cc. When needed for use take equal parts of solutions A and B.

The above stock solutions have been made and each student is to take 30 cc. of each solution. Keep in separate bottles and mix a few cc. of each when ready to make a test. A deep clear blue fluid is the result of the mixture, the Rochelle salt holding the cupric hydrate in solution. If kept too long it is apt to decompose. If in doubt as to the efficiency of the solution boil it, and if it remains blue it is good.

Add some of Fehling's solution to a portion of the glucose; boil, a yellowish (cuprous hydrate) or reddish (cuprous oxide) precipitate.

52. Add to a portion of the glucose solution some strong potassium hydrate solution and then a very small amount of the subnitrate of bismuth. Boil; a black precipitate results which sometimes forms a mirror on the walls of the test-tube.

53. The Phenyl-hydrazine Test. To about 10 cc. of the glucose solution in a test-tube add 0.2 gram of phenylhydrazine hydrochlorate, and 0.3 gram of sodium or potassium

acetate. Heat in the water-bath for 20–30 minutes; then cool the test-tube by allowing cold water to run upon it and set it aside. A yellow crystalline precipitate is formed which is known as phenyl-glucosazone. Examine some of this precipitate under a low power of the microscope and note the needle-like and feathery crystals sometimes arranged in the form of rosettes. Phenyl-glucosazone has a melting point of 204°C .

54. Conversion of starch into glucose. Boil some of the starch solution with a few drops of sulphuric acid until the fluid becomes clear. After neutralizing a small portion with sodium carbonate, test it for glucose by the iodine test.

55. Lactose. Milk sugar, ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$ and H_2O). Make 50 cc. of a 2% solution.

56. Heat a portion of this solution carefully with sulphuric acid,—it chars slowly.

57. Add to another portion excess of caustic soda and a few drops of copper sulphate solution and heat,—a yellow or red precipitate (like glucose).

58. Test another portion with Fehling's solution,—there is a reduction like glucose, but its reducing power is not so great as glucose. It requires 10 parts of lactose to reduce the amount of Fehling's solution that will be reduced by 7 of glucose.

59. Cane sugar ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$). Make 50 cc. of a 2% solution.

60. A portion of the solution should not reduce Fehling's solution. (Many of the commercial sugars, however, contain sufficient reducing sugar to do this.)

61. Trommer's test. Add excess of caustic potash and a drop of copper sulphate (it gives a clear blue fluid), and heat. With a pure sugar there should be no reduction.

62. Pour strong sulphuric acid on a little dry cane sugar in a test-tube. Add a few drops of water with a pipette, the whole mass is quickly charred.

63. Neutral fats. The neutral fats of the adipose tissue of the body generally consist of a mixture of the neutral fats, stearin, palmitin, and olein, the two former being solid at ordinary temperatures, while olein is fluid, and keeps the other two in solution at the temperature of the body. They are lighter than water : Sp. gr. 0.91-0.94.

64. Take a little lard or olive oil, and observe that fat is soluble in ether, also chloroform, but insoluble in water.

65. Take some of the ethereal solution of lard and let some of it fall upon some paper. The ether soon evaporates but a permanent greasy stain is left.

66. Add caustic potash to olive oil and boil. A glycerin-oleate is formed.

67. Heat some lard and caustic soda solution in an evaporating dish to form a soap, decompose the latter by heating it with dilute sulphuric acid (10%) and observe the liberated fatty acids floating on the top.

68. Shake a few drops of cod-liver oil with a small amount of dilute solution of sodium carbonate. The mass should become white—an emulsion. In an emulsion the particles of oil are broken up into innumerable finer particles which remain discrete, that is, do not run together. Milk is a typical emulsion. Examine some of the cod-liver oil emulsion under the microscope.

69. Bone. Organic basis obtained by decalcification. Place a small thin dry bone in dilute hydrochloric acid (1 part of the acid to 8 of water) for a few days. Its mineral matter is dissolved out, and the bone, although retaining its original form, loses its rigidity, and becomes pliable, and so soft as to be capable of being cut with a knife. What remains is the organic matrix of ossein.

70. Wash the decalcified bone thoroughly with water, in which it is insoluble, place it in a solution of sodium carbonate and wash again. Boil it in water, and from it gelatin will be obtained. Neutralize it with sodium carbonate. The solution gelatinizes. Test the solution for gelatin. (24-32.)

VI.

EXAMINATION OF A TEST SOLUTION FOR PROTEIDS AND CARBOHYDRATES.

71. Physical characters.

- a. Note color and transparency.
- b. Taste.
- c. Smell.

d. A persistent froth suggests an albuminous solution.

72. Divide the fluid into two portions, using one for the proteid and the other for the carbohydrate tests.

Test for proteids by xanthoproteic and Millon's tests. If present :

73. Test reaction to litmus paper. If acid or alkaline, test for acid or alkali-albumin, and if either be present, neutralize and filter off precipitate.

74. If original solution be neutral, acidulate faintly and boil. A coagulum may consist of native albumin or globulin, or both.

75. Distinguish between albumin and globulin by (a) dropping solution into water, precipitate indicates globulin ; (b) saturating solution with magnesium sulphate, precipitate indicates globulin. If precipitate obtained by (b), filter and boil filtrate, coagulum indicates native albumin. Distinguish between egg and serum-albumin by ether test (a non-alkaline solution of egg albumin is coagulated by ether, serum albumin is not).

76. Gelatin (albuminoid) gives xanthoproteic and Millon's reactions, also a violet color with caustic potash and copper sulphate. It is not coagulated by boiling and is not precipitated by acetic acid and potassium ferrocyanide.

77. Test for Carbohydrates.

First remove derived albumins by neutralizing and filtering and native albumin and globulin by boiling and filtering. Acidulate if necessary and add iodine.

78. Blue color disappearing on heating and returning on cooling indicates starch.

79. Mahogany-brown color disappearing on heating and returning on cooling indicates dextrin or glycogen. (The latter is precipitated by the addition of basic lead acetate).

80. Test for reducing sugar by Trommer's test.

81. Try the phenyl-hydrazine test. (53.)

VII.

82. Salivary Digestion. To obtain mixed saliva. Chew a small piece of paraffin or chewing gum, or inhale ether for a short time to stimulate the flow of the secretion. Collect it in a graduate until you have about 30 cc. Note that, in a short time, more or less of a sediment occurs due to the deposition of epithelial cells, débris of food, bacteria, etc. Numerous air bubbles are usually present upon the surface.

Filter. Is it translucent? Is there any great amount of viscosity? What is its reaction to litmus paper? The specific gravity is 1002—1006.

83. Add acetic acid. A precipitate indicates mucin. Not soluble in excess. Filter.

84. With the filtrate from 83, test for traces of proteids with the xanthoproteic reaction and Millon's test.

85. To a few drops of saliva in a porcelain evaporating dish add a few drops of dilute acidulated ferric chloride,—a red coloration indicates the presence of sulphocyanide of potassium, the color does not disappear on heating, nor on the addition of an acid, but is discharged by mercuric chloride. Meconic acid gives a similar color, but it is not discharged by mercuric chloride. The sulphocyanide is present only in the secretion from the parotid gland.

86. Test for chlorides by adding to the saliva a few drops of nitric acid followed by a few drops of silver nitrate. A

white precipitate indicates the combination of the chloride with the silver to form silver chloride.

87. Digestive action on starch. Prepare a mixture by placing 1 gram of starch in a mortar and adding a few cc. of cold water, and mix well with the starch. Add 200 cc. of boiling water, stirring all the while. Boil the fluid for a few minutes. This gives a 0.5% mixture.

88. Dilute the saliva with an equal volume of distilled water. Label four test tubes, A, B, C, and D. Into A place some saliva, boil it and add some starch mucilage. In B and C place starch mucilage and saliva, to B add a few drops of hydrochloric acid and to C some caustic potash. To D add merely the saliva to the starch mixture.

Place all four in a water bath not exceeding 40° C., and after a time test a small portion of them for sugar with Fehling's solution. Reserve a small amount of D. Why is no sugar formed in A? In B and C a strong acid and alkali arrest the action of ptyalin. Neutralize a portion of B and C and test again. Is there any result? In D the starch has been converted by the ptyalin into a reducing sugar.

89. Test a portion of D with iodine solution. The absence of any blue color indicates that the starch has disappeared, having been converted into a reducing sugar—maltose. Also test the remainder of A, B and C with the iodine solution.

90. Test another portion of D with phenyl-hydrazin, (53) crystals of phenyl-maltosazone should develop. Examine under the microscope.

91. Bread. Crumble up a small piece of bread in a test-tube and add some cold distilled water until it softens and with slight shaking disintegrates. Divide the mixture into two portions.

92. Apply a drop of iodine solution: blue color indicates starch.

93. Apply the xanthoproteic reaction to the other portion.

Any crumbs that may be in the solution if colored orange would indicate the presence of a proteid. The liquid portion may not show as deep a color, indicating a lesser amount in solution.

94. Try the above tests hurriedly by dropping a little iodine solution upon the bread. Similarly with the xanthoproteic test by letting a drop of nitric acid fall upon the bread and then a drop of ammonia upon the spot already covered by the nitric acid.

95. Potato. Boil a small piece of potato in water and let it cool. Divide the liquid into two parts.

Test one portion for starch with the iodine solution. Without boiling, the starch might give no reaction as the granules are enclosed in a coating of cellulose.

96. Apply the xanthoproteic test. Only a faint orange color appears, indicating that very little proteid is present.

VIII.

97. Gastric Digestion. For laboratory purposes a digestive extract is usually prepared by removing the gastric mucosa from the cardiac portion of the stomach of some domestic animal while the ferments are still active. The cardiac portion of the stomach of the pig is perhaps more commonly used and easily obtained from the slaughter house. The stomach of a cat or dog is equally useful and their deaths can be timed according to their digestive activity and the stomach obtained in a perfectly fresh condition. Extracts of considerable concentration may be made by any of the three following methods.

98. Formalin extract of pepsin. Remove the mucosa from the cardiac half of the stomach and mince it very finely with a chopping knife or meat grinder. Weigh the mucosa and add to it twenty-five times its weight of a 0.1%

solution of formalin. The solution will keep indefinitely, and remains active. For use take, after filtering through absorbent cotton, 1 cc. of this extract and add to it 6-8 cc. of 0.2% hydrochloric acid in a test-tube.

99. Glycerin extract of pepsin. Add to the minced mucosa, 2-3 times its weight of pure concentrated glycerine. Let it stand for a week or ten days, shaking it frequently. The pepsin is soluble in the glycerin and the extract thus prepared will keep for a long time. When ready to use strain through muslin and filter through absorbent cotton. Add hydrochloric acid as in 98.

100. Acidulated extract of pepsin. Mince the mucosa very finely. Add to it, in a flask, fifty times its weight of 0.2% hydrochloric acid and put in the incubator for 12 hours. This is a solution of pepsin in hydrochloric acid, and is quite powerful in its action. The solution also contains peptones, but these may be eliminated by dialysis.

101. For this exercise there have been prepared extracts of the gastric mucosa from the pig (omnivorous), and the cat or dog (carnivorous).

Label 5 test tubes, A, B, C, D, E, for the omnivorous extract, and for the carnivorous extract, A', B', C', D', E'. In A fill the tube half full of distilled water, and add 30 drops, or 2 cc. of the extract. Fill B half full of 0.2% hydrochloric acid. Treat C similarly to B, but add 30 drops of the extract. Fill D half full of a 1% solution of sodium carbonate and add 30 drops of the extract. Place 30 drops of the extract in E and add 2 or 3 cc. of distilled water and boil, then add enough 0.2% hydrochloric acid to make the tube half full. Treat A', B', C', D', E' in the same way with the carnivorous extract. In each of the 10 test tubes put a small thread of well-washed and boiled fibrin. Place all the tubes in a water bath at 40° C., and after an hour note any changes that may have occurred in any of the tubes. The rapidity of action will indicate the strength of the ferment. Explain in your

notes why no action has occurred in certain of the tubes. Test-tubes C and C' are to be plugged with cotton and reserved for later examination.

102. The following tubes are to be prepared exactly as C but omitting the fibrin. In the first tube a very small piece of cooked meat ; in the second a crumb of bread ; in the third a bit of boiled potato ; in the fourth a small piece of dried albumin ; in the fifth add a small piece of butter ; in the sixth, 1 cc. of milk diluted with 5 cc. of distilled water ; in the seventh test-tube, small amounts of all the above substances. These tubes, also, are to remain in the water bath at 40° C., and examined later for any changes.

IX.

103. The contents of tubes C and C' are to be divided, each tube, into 4 parts, 3 of which are to be used in the following tests, and the other part to be held in reserve.

104. Color one portion of the fluid with the litmus solution and neutralize with dilute caustic potash. At the neutral zone a precipitate will appear indicating acid-albumin (syntonin or parapeptone).

105. To another portion add sodium chloride and a few drops of nitric acid. A precipitate should appear which is dissolved on heating, but reappears on cooling, indicating the presence of albumose (proteose or propeptone). Albumose like peptone is soluble in water, and gives a biuret reaction.

106. Another test for albumose is to add to the solution enough neutral ammonium sulphate to saturate it. This brings down the albumoses in the form of a white precipitate.

107. Peptones behave differently from the native proteids in the copper sulphate and caustic potash test, if only a trace

of copper sulphate is used. They give a pink instead of a violet color. (Also true of albumoses.) The pink color is also given by the substance called biuret, hence the test is often called the *biuret reaction*. (Biuret is formed by heating urea; ammonia passes off and leaves biuret, thus: $2\text{CON}_2\text{H}_4$ (urea) $\rightarrow$ NH_3 (ammonia) + $\text{C}_2\text{O}_2\text{N}_3\text{H}_5$ (biuret).)

108. To the third portion add neutral ammonium sulphate to saturation. This precipitates all of the albumoses while the peptones remain in solution. Filter and test the filtrate for peptones by the biuret test as follows: Take another test-tube and put a few drops of 1% solution of copper sulphate in it; empty it out so that the merest trace of the copper sulphate is adherent to the wall of the tube, then add the filtrate and a few drops of strong caustic potash. A pink color (biuret reaction) should be produced.

109. If digestion has been quite complete the tests for acid-albumin and albumose may not be very satisfactory. The main fact, however, that an indiffusible proteid, before being converted into a diffusible peptone, must pass through intermediate forms—acid-albumin and albumose—is important, and must be kept in mind in this and succeeding experiments.

110. After filtering, treat the contents of the tubes containing meat, bread, potato, albumin, butter and mixed substances according to the above tests.

X.

111. Pancreatic Digestion. The pancreas of the pig, ox, or dog is perhaps the best adapted for class experimentation. Their various ferments are active and usually give clear and definite results. The steaptic or fat-splitting ferment is generally the most difficult one of the four to demonstrate,—the fresh pancreas being necessary for this purpose. The

treatment of the pancreatic tissue by strong alcohol does not, however, seem to interfere with the action of the other ferments. The digestive extracts may be prepared in different ways according to the ability of the extractive medium to dissolve the ferments. The ferments of the pancreas differ from those of the stomach, in that they can act only in an alkaline medium and not in the presence of acid. The pancreas should not be prepared immediately after death as the ferments are still in the form of zymogen granules. After remaining a few hours in a warm temperature the zymogen splits up into the various ferments. As the pancreatic juice is of an alkaline reaction, it affords an excellent medium for the growth of bacteria, and some antiseptic must be used if the extract is to be kept for any length of time. A few drops of alcoholic solution of thymol has proven very satisfactory.

112. Aqueous extract. Divest the pancreas of as much fat as possible. Mince it thoroughly or rub it well in a mortar with well-washed sand or bird gravel. Add cold water, stir and let the mixture stand for some hours. Strain through muslin and then filter through paper. A more powerful extract may be prepared by using a 1% or 2% solution of sodium carbonate in place of the water, digesting at 40° C. and adding a little 10% alcoholic solution of thymol to prevent putrefaction.

113. Glycerine extract. This may be made similarly to the glycerine extract of the gastric mucosa (99). After the pancreas has remained at the laboratory temperature for 24 hours, it is usually placed in strong alcohol for a day or two to remove the water and it is then put into glycerine for some days. Filter, and in experiments use 20-30 drops of the extract to 5 or 6 cc. of 1% sodium carbonate with the material to be digested. Gamgee states that all of the pancreatic enzymes are extracted from the gland by glycerine.

114. Brine extract. The fresh pancreatic tissue is minced

and placed in 5-10 volumes of a saturated solution of salt. The enzymes are dissolved and very active extracts are obtained. The fat-splitting ferment is not active in this extract. This method is highly recommended by Harris and Gow. (*Jour. of Physiology*, vol. XIII, 1892), but the solution putrefies after a time.

115. Dilute alcoholic extract. Roberts recommends dilute alcohol for obtaining an active solution of the pancreatic enzymes. The fresh pancreas is freed from fat and chopped up finely. Four times its weight of 25% alcohol is added and the mixture is allowed to digest 4-5 days with occasional agitation. It is then filtered through paper and is ready for use. The alcohol assists in the preservation of the solution.

116. There have been prepared for this exercise extracts from the pancreas of the pig, ox and dog. Proceed in the same manner with each extract and make notes of any differences in their activity. Prepare three series of tubes; one series for the pig, another for the ox and another for the dog.

117. When ready to use, each test-tube is to be half filled with 1% sodium carbonate and 30 drops of the pancreatic extract added. To tubes prepared as above add the following: 1, a bit of fibrin; 2, a small piece of dried albumin; 3, a piece of cooked meat; 4, a crumb of bread; 5, a bit of cooked potato; 6, a bit of cheese; 7, a small amount of each of the above substances. Keep these in the water bath at 40° C. Note particularly any changes that may occur in No. 1, and compare with the fibrin digested with the gastric juice. In those tubes which first show signs of digestive action, test the contents for alkali-albumin by neutralization. (Similar to the acid-albumin test the only difference being the reaction of the digestive fluid). Test also for albumoses. Place the tubes in the incubator until the next exercise and, after filtering again test them for alkali-albumin, albumose, and peptone, as with preceding tests. (104-107). Reserve

a portion of the contents of tubes No. 1 and 2 for indol and tyrosin tests later, (131-132).

118. Take three more tubes for each of the three series, add starch mucilage to each test-tube, about one-sixth full.

119. Half fill three test tubes with 1% sodium carbonate and add 30 drops of the pancreatic extract from the pig, ox and dog to their respective tubes.

120. Half fill one tube with 1% sodium carbonate and add 30 drops of bile.

121. Half fill three tubes with 1% sodium carbonate and add to each tube 30 drops of the respective extracts and 30 drops of bile.

Place these tubes in the water bath at 40° C. Test small portions of each every minute with iodine and note in which one the starch first disappears.

122. Test Nos. 119, 120 and 121 for sugar by Trommer's test, (50).

XI.

123. Prepare four test-tubes as follows, labeling them in order 1, 2, 3, 4, and adding a bit of fibrin to each tube. To No. 1 add 30 drops of the pancreatic extract from the pig, and some distilled water; to No. 2, the same amount of the pig's pancreatic extract and excess of 0.2% hydrochloric acid; to No. 3, some 1% sodium carbonate alone; for No. 4, put 30 drops of the pig's pancreatic extract into a separate test tube, add a little of the 1% sodium carbonate and boil, and then add the fibrin. Put all of the test tubes in the water-bath at 40°C. After a few hours examine them and explain the results.

124. Emulsion Experiment. Shake up a few drops of olive oil in a test-tube with some artificial pancreatic juice. Place the mixture for a few minutes in the bath at 40° C., and shake again, compare the results before and after warming.

125. In another tube with a little olive oil add some bile, shake and place in water-bath at 40° C. and compare the emulsive effect with 124.

126. Action on Fat. For this experiment it is necessary that the fat (olive or almond oil) should be perfectly neutral. Commercial oils usually contain free fatty acids. The following method has been recommended for neutralization by Krukenberg.

Place the oil in a porcelain capsule and mix it with not too much baryta solution, (Baryta mixture is prepared by mixing one volume of a solution of barium nitrate and two volumes of barium hydrate, both saturated in the cold), and boil for sometime. Allow it to cool. The unsaponified oil is extracted with ether. The ethereal extract is separated from the insoluble portion and the ether evaporated over warm water. (The flame *must not* be brought near the ether. Let the water come to a boil, put out the flame and then put the dish containing the ether upon the hot water). The oil should now be neutralized.

127. Take two test-tubes and fill each one-third full of neutral oil. Add some blue litmus to color. In the first tube place a small piece of fresh pig pancreas. Put both tubes in the incubator from $\frac{1}{2}$ to 1 hour or longer. Note if any change of color occurs in the one with the pancreas, due to the formation of fatty acids.

128. Another form of the experiment is to mix the oil with finely divided perfectly fresh pancreas in a mortar, and keep it for a time at 40° C. It soon becomes acid, owing to the formation of fatty acids. Test with litmus paper. These experiments often fail and the existence of a fat splitting ferment is totally denied by some.

129. Action on milk. Dilute 2 cc. of cow's milk with 10 cc. of distilled water in a test-tube and add 5-6 drops of pancreatic extract. Keep at 40° C. from $\frac{1}{2}$ to 1 hour. Note any change that has occurred.

130. Divide the above into two parts. To one part add a little dilute acetic acid, if there is no precipitate it indicates that the caseinogen has been converted into peptones. To the other part apply the biuret reaction for peptones.

131. With the reserved portion from the fibrin and albumin tubes (117), if digestion has been thorough, some further products may be found.

Dip the uncovered end of a wooden match in hydrochloric acid and then into the fluid from the fibrin tube. Repeat for the albumin. If the match is stained red from either fluid it indicates the presence of indol, one of the putrefactive products of pancreatic digestion.

132. Take equal parts of Millon's reagent and the respective fluids and boil together. A port wine color may appear. If so tyrosin is present. Tyrosin (and Leucin) represent digestive products beyond peptones. They do not occur in gastric digestion.

XII.

133. Bile. The bile used in these experiments has been obtained from an ox. Its color is greenish, that of man is brownish yellow. In pouring the bile from one vessel to another note that strings of so-called mucin are present. The mucin of bile is not formed in the liver but in the mucous glands of the gall-bladder and duct, the longer the bile has been in the gall-bladder the greater the precipitate which will be obtained. For general use the bile may be diluted 2-4 times with 0.1% formalin. The formalin will preserve the bile indefinitely.

134. Test the reaction of bile with litmus paper.

135. To a little bile in a test-tube add some strong alcohol. This produces a precipitate of mucin with some pigment entangled.

136. Mucin is also precipitated by the addition of acetic acid to bile. Perform this test. Filter off the mucin.

137. To a portion of the filtrate add a little hydrochloric acid and potassium ferrocyanide. A blue color indicates the presence of iron. The experiment may be modified by placing some thin sections of liver in a solution of potassium ferrocyanide for a few minutes and then in dilute hydrochloric acid. The sections turn bluish from the formation of prussian blue. With the microscope blue granules may be seen in some of the hepatic cells.

138. Pettenkofer's Test for Bile Acids. Take 2 cc. of clear diluted bile in a test tube and add 4 drops of a 10% solution of cane sugar. Add strong sulphuric acid, drop by drop, cooling the tube in a dish of cold water immediately after adding the acid. Not more than 2 cc. of the acid should be used. Too much heat causes carbonization of the sugar and the test is ruined. If bile acids are present, the fluid at first becomes opaque, then clear, and successively brown, red and purple. It may require an hour or more to accomplish this test. This reaction depends upon the production of furfural (C_4H_3OCHO) by the destruction of the sugar when the sulphuric acid is added. Furfural in turn combines with cholalic acid, formed by the action of the sulphuric acid on the bile acids, giving the color. Some other substances, as morphine, albumin, etc., give a very similar color, and the test must, therefore, be used with caution. In very dilute solutions of bile the reaction does not appear and cannot be used satisfactorily in testing urine for the presence of bile.

Pettenkofer's test may also be quite satisfactorily performed more quickly by putting a little of the bile in a porcelain capsule, adding a drop or two of a solution of cane sugar and then a few drops of strong sulphuric acid.

139. Gmelin's Test for Bile Pigments. Ox gall does not yield this test as readily as that from the omnivora or carnivora. To a small quantity of bile, in a test-tube, add, drop by drop, nitric acid, yellow with nitrous acid, (if the

acid is clear, add a single crystal of cane sugar, warm, and the acid becomes yellow from the development of a small amount of nitrous acid), shaking after each drop; the yellowish green color becomes first a dark green, then blue, then violet, then red, and finally a dirty yellow. The blue and violet colors are less obvious than the rest.

Repeat the test in the following way: place a drop of bile in a porcelain evaporating dish, and place a drop of yellow nitric acid so that it runs into the drop of bile; where the fluids mingle, zones of color, green, blue, violet, red and yellow, from the bile to the acid, are seen.

140. Add a little bile to some starch mucilage as in salivary digestion. Test for any reducing sugar. In this experiment fresh bile should be used as the formalin acts as a reducing agent.

141. To 5 cc. of undiluted bile add an equal volume of water and some alcohol to precipitate the mucin. Filter and divide the filtrate into two portions; to one portion add some hydrochloric acid which causes a precipitation of glycocholic acid; to the other portion add a little of a 1 % solution of neutral lead acetate which throws down lead glycocholate. Remove this by filtration, and to the filtrate add a little 1 % solution of *basic* lead acetate, which gives a further precipitation of lead taurocholate. [Long.]

142. Add a few drops of oleic acid to 5 cc. of bile in a test-tube, shake well and at once place a drop of the mixture on a slide and examine, under the microscope, the numerous fatty globules. Place the test-tube with the bile in a warm bath for an hour or so, shaking occasionally and then examine a drop with the microscope; comparatively few fatty globules will be seen. The oleic acid has combined with the base of the bile-salts to form a *soap*.

143. Prepare three test-tubes as follows: (1) In one test-tube put 5 cc. of bile and a drop of oleic acid. (2) In another 5 cc. of water. (3) In another 5 cc. of bile. To

each of the three add about 1 cc. of fresh melted butter. Shake well and place all three in a warm bath. Note in which tube the emulsion continues longer.

144. Free fatty acids have the power of decomposing the bile salts with liberation of their acids. The emulsifying power of bile is slight ; but in the presence of fatty acids it forms soaps, which have a much greater emulsifying power. Animal membranes moistened with bile permit the passage of fatty oils, while if they are moistened with water only the oil cannot pass through. This is important in connection with certain digestive phenomena.

XIII.

MILK.

145 Newly drawn milk is an opaque fluid of a white color. Its color and opacity are due to its being an emulsion, *i. e.*, consisting of little globules of fat suspended in a solution of albumin, sugar and salts. Each globule of fat is covered by a thin coating of casein. When the milk is allowed to stand, the fat globules, being lighter than the fluid in which they swim, rise in great part to the top and form cream, and part of the fluid often acquires a bluish tinge. It is said that a similar separation also takes place in the milk gland itself, so that the milk last drawn is richest in cream. The globules of fat are prevented from uniting by the thin albuminous coating which surrounds each, but when this is broken by agitation, they coalesce, forming butter. Changes also occur in the milk, sugar, casein and fats, more or less quickly, according to the higher or lower temperature to which the milk is exposed. The milk sugar becomes converted, apparently through the agency of a ferment, into lactic acid. This gives the milk an acid reaction, and precipitates the casein, causing the

milk to curdle. The coagulum or curd, incloses the fat globules. The liquid from which it is separated, a solution of milk, sugar and salts, is known as whey. The curd, when completely separated from the whey, is called cheese.

146. Test the reaction of milk. Fresh cow's milk may often be neutral or even acid. Sour milk is acid. The reaction of fresh human milk is always alkaline. Free lactic acid is present in the fresh milk of the carnivora.

147. Fill a cylindrical glass jar two-thirds full of unskimmed milk. Insert the hydrometer and take the specific gravity. Repeat the determination with the New York board of health lactometer. This instrument is so constructed that 100 at 60° F. represents a specific gravity 1.029, below which unadulterated milk is supposed never to fall. The laws of New York require milk to have a density of not less than 1.029, and total solids of not less than 12%, of which 3% must be fats.

148. Examine a drop of fresh cow's milk under the microscope. It consists of a clear fluid containing a large number of highly refractive fat globules. Let a drop of osmic acid solution run under the cover glass; in a short time the globules become stained brown-black.

149. Mix 5 cc. of fresh milk with 15 drops of *neutral* artificial gastric juice, and heat in the water bath to 40° C. In a short time the milk curdles so that the tube can be inverted without the *curd* falling out. By and by the *whey* is squeezed out of the clot. The curdling of milk by the rennet ferment present in the gastric juice is quite different from that produced by the "souring of milk," or by the precipitation of caseinogen by acids. Here the casein (carrying with it most of the fats) is precipitated in a neutral fluid.

150. To the same test-tube after the above process add 10 cc. of 0.2% hydrochloric acid, and put into the incubator until the next exercise. Note any changes when next examined.

151. Fill a test-tube one-third full of milk, add some rennet. Keep in the water bath at 40° C. for a few minutes and note the changes that occur.

152. Dilute 5 cc. of milk with 15 cc. of water, add a little dilute acetic acid and warm. A precipitate is formed. Filter and save both precipitate and filtrate. This precipitate is not the same as that obtained by rennet. The acid precipitate is caseinogen, and is freely soluble in dilute alkali, the rennet clot is "casein," and is much less soluble in dilute alkali. Cheese is made with rennet and cannot be made with acid.

153. The filtrate obtained from 152 is to be divided into two portions. To the first portion apply Trommer's test. A red precipitate indicates the presence of a reducing sugar—lactose.

154. To the second portion of the filtrate apply the xanthoproteic reaction. An orange color represents the presence of a proteid (lact-albumin).

155. To the precipitate obtained from 152 add a little ether in a test-tube and agitate for a few minutes. Pour off the ether upon some paper and note that it leaves a permanent greasy stain indicating the presence of fat.

156. To the residue left in 155 add a little dilute caustic potash (0.1%). A solution is effected. Apply the xanthoproteic reaction to this fluid. An orange color denotes the presence of a proteid—caseinogen.

157. To a test-tube half filled with 0.2% hydrochloric acid and 15 drops of gastric extract add a small piece of cheese. Put the tube in the incubator and examine at the next exercise for peptones and intermediate products.

158. The action of milk with pancreatic extract is somewhat complicated on account of the complexity of milk itself. The sugar, fat and proteids all undergo some change from the action of the different pancreatic ferments. Perhaps the most interesting of these changes is that produced

in the proteids, and is commonly called peptonization. The peptonization, or digestion, of milk is quite often practised in the preparation of food for the sick room, and is illustrated by the following experiment. Dilute about 10 cc. of milk with an equal volume of distilled water and add a half a gram of sodium bicarbonate. Then add a few drops of pancreatic extract, shake the mixture and keep at 40° C. on the water-bath for about a half an hour. Then filter and apply the biuret test for peptones. The pancreatic extract from beef acts more strongly upon the proteids; that from the pig is very active in converting starch into sugar.

159. Place a small quantity of milk in a warm place for one or two days; then test the reaction, it will be found to be acid; this is due to *fermentation*, in the process of which the milk sugar is converted into lactic acid.

XIV.

160. An examination of some of the more important tissues of the body.

161. Saline extract of Nervous Tissue. The solids of a saline extract of the brain are derived chiefly from the gray matter, (cinerea).

162. Apply the xanthoproteic test to a portion of the above extract.

163. Faintly acidify another portion with acetic acid and boil. If coagulated a proteid is present. This may be a native albumin or globulin or both.

164. An ethereal extract of the brain contains solids derived chiefly from the white matter (alba).

165. Pour out a little ethereal extract of brain upon paper and note that there is a permanent greasy stain, indicating the presence of fat. Remember that this is not wholly ordinary fat, but largely a nitrogenous, phosphorated fat—lecithin.

166. To another portion add, *very cautiously*, some strong sulphuric acid. If a cherry red color results at the junction of the fluids it denotes the presence of cholesterin. Make a control test by putting a little ether in a test-tube and adding some strong sulphuric acid. No red color should appear.

167. Saline extract of Liver. (Epithelial Cells.) Divide the fluid into three portions.

168. To one portion apply the xanthoproteic test.

169. Faintly acidify another portion with dilute acetic acid and boil. If coagulated it indicates native albumin or globulin or both.

170. Saturate the third portion with magnesium sulphate. A precipitate should occur of a proteid character. The proteid may be globulin and partly nucleo-albumin.

171. Filter the mixture obtained in 170. Boil the filtrate. Little or no coagulation may occur, indicating little or no albumin in the filtrate.

172. Mince a small piece of liver from an animal which has been dead for 24 hours. Boil the liver either in water or a saturated solution of sodium sulphate. Filter. The filtrate should not be opalescent.

173. Test the reaction of the filtrate to litmus paper.

174. Neutralize a portion of the filtrate with a little sodium carbonate and filter; then test with iodine for glycogen. If there is no deep brown mahogany color, glycogen is absent.

175. Test for grape sugar, 50-52. After death the glycogen is transformed into grape sugar, unless precautions be taken to prevent this transformation.

176. Saline extract of muscle. The reaction of perfectly fresh muscle to litmus is of an alkaline character. That of butchers' meat is acid due to the formation of sarcolactic acid. A muscle tetanized for a long time becomes acid.

177. Pour a few drops of the saline extract into a large

quantity of water. Observe the milky deposit of myosinogen. The precipitate is redissolved by adding a strong solution of common salt.

178. Test the coagulating point of another portion of the extract. Four proteids are coagulated by heat, each respectively at 47° , 56° , 63° , and 73° C. an albumose being left in solution. The fluid is acid in reaction. Filter off the coagula as they are formed.

179. Saturate the final filtrate with sodium chloride. The myosinogen is precipitated.

180. Collect some of the precipitate of 179 and dissolve it in a weak solution of sodium chloride and test for proteid reactions. Xanthoproteic and Millon's tests.

181. Make a solution of Liebig's extract of meat. Test a small portion of it for proteids.

182. Test another portion for glycogen by adding iodine solution; a red-brown or port wine color indicates glycogen. Make another test by adding a little basic lead acetate.

183. Test another portion for kreatinin by Weyl's test. Add a very dilute solution of sodium nitro-prusside, and very cautiously some caustic soda; an evanescent ruby-red color, passing into a straw color, indicates kreatinin.

XV.

184. Blood. Fresh blood may be obtained and defibrinated at a slaughter house, and a few drops of formalin added to it will prevent putrefaction for a long time. It is better, however, when possible, to obtain the blood by bleeding an animal. After the dog, or any other animal of convenient size, has been anesthetized, the carotid or femoral artery is exposed and isolated from surrounding parts for an inch or two of its length, and a clamp or ligature applied to the proximal portion of the artery, *i. e.*, as far as possible toward

the heart. A little distal to the clamp make an incision in the artery and insert a glass canula and tie it tightly in place. Remove the clamp or ligature and the blood will pass through the canula, and the animal allowed to bleed to death. When the animal is apparently dead, an interesting experiment may be performed by injecting into the artery some normal salt solution of the same temperature as the body and note the reviving effect.

185. The blood obtained as above directed is to be caught in four different vessels and each portion is to be treated as follows: One portion of the blood is to be defibrinated by immediate whipping with some broom straws tied in a small bundle and the fibrin as it collects on the straws is to be saved for future use. The defibrinated blood is also to be reserved for later study. Another portion of the blood is to be collected in a flask and the phenomenon of clotting or coagulation observed. Another portion of the fresh blood is to be mixed with a saturated solution of sodium sulphate. And still another portion into a solution of potassium oxalate.

186. Test the reaction of blood by pricking one of the fingers behind the nail. Put a drop of the blood on a piece of ordinary litmus paper which has been soaked in salt solution. The substances on which the alkaline reaction depend will diffuse out in a ring around the drop, while the hemoglobin remains in its original position.

187. Place a thin layer of defibrinated blood on a glass slide; try to read printed matter through it. The blood is too opaque and the print cannot be read, the light is reflected from the corpuscles in all directions, and but little passes through.

188. Place 1 cc. of defibrinated blood in a test tube and add 5 cc. of distilled water, and warm slightly. Note the change of color by reflected and transmitted light. By reflected light it is much darker—almost black, but by transmitted light it is transparent. This constitutes "laky"

blood due to the withdrawal of the hemoglobin from the red corpuscles into the water. Test the transparency by looking at some printed matter through this blood as in 187.

189. To 2 cc. of defibrinated blood in a test-tube add 5 volumes of a 10% solution of sodium chloride. It changes to a very bright, florid, brick-red color. Compare its color with No. 188.

190. Place a watery solution of defibrinated blood in a parchment tube, and suspend in a vessel of distilled water. After several hours note that no hemoglobin has passed into the water. Test the diffusate for chlorides with silver nitrate and nitric acid. Hemoglobin does not dialyze, although it is crystallizable.

191. Put a drop of blood on a slide. Heat it gently over a flame, so as to evaporate the water. Then add a small crystal of common salt and a drop of glacial acetic acid; put on a cover-glass, and again heat slowly till the liquid just begins to boil. Take the slide away from the flame for a few seconds, then heat it again for a moment, and repeat this process for two or three times. Now let the slide cool and examine with the microscope (high power). The small black or brownish-black crystals of hemin will be seen. This test is often important in some medico-legal cases where only a trace of blood is available for examination. If the blood stain be upon a piece of cloth, it may be soaked in a little distilled water and examined by the spectroscope or micro-spectroscope. The liquid may then be evaporated to dryness on the water bath and the hemin test made. Or perform the hemin test directly on the piece of cloth.

192. In the blood saved for clotting, note that in a few minutes the blood congeals, and when the vessel is tilted the blood no longer moves as a fluid, but as a solid. After an hour or so, pale yellow colored drops of fluid—the serum—are seen on the surface, having been squeezed out of the red mass, the latter being the clot and consisting of fibrin and red corpuscles.

Note in the clot of horse's blood the upper light colored layer of leucocytes—the buffy coat. Coagulation is slow in this animal and the red and white corpuscles on account of the difference in their specific gravity have time to separate.

193. Salted Plasma. Note that in the flask containing the mixture of blood and sodium sulphate, no coagulation has occurred. Place some of this fluid in the centrifuge to separate the corpuscles and plasma, the latter mixed with the saline solution is known as the salted plasma.

194. Oxalate Plasma. Note also that the potassium oxalate blood mixture does not coagulate. Centrifuge the mixture to obtain the plasma. The oxalate precipitates (as the oxalate of lime) the calcium which is necessary for coagulation.

195. To a portion of the oxalate plasma add a few drops of a 2% calcium chloride solution. Coagulation results (more quickly at 40°).

196. To another portion of the plasma add a little fibrin-ferment prepared by the demonstrator. The fibrin ferment is prepared as follows: Precipitate some blood serum with about ten times its volume of alcohol. Let it stand for several weeks, then extract the precipitate with water. The water dissolves out the fibrin-ferment, but not the other coagulated proteids.

197. Add a drop of freshly prepared tincture of guaiacum to a small amount of diluted defibrinated blood, and then some hydrogen peroxide. The color changes to blue. This is often used as a test for hemoglobin, but other substances (oxygen carriers) give a blue color under the same conditions.

198. Place some hydrogen peroxide over fibrin in a watch glass; bubbles of oxygen are given off.

199. Immerse a flake of fibrin in freshly prepared tincture of guaiacum, (5% of pure resin in alcohol) and then immerse the flake in hydrogen peroxide. A blue color is

developed, due to the ozone liberated by the fibrin and forming a blue color with the resin. Compare 197.

XVI.

200. Proteid reactions. Dilute 1 cc. of serum with 5 cc. of normal salt solution (0.65%). Add a little litmus solution to color and neutralize with 0.2% hydrochloric acid.

201. Heat another portion for the coagulation test. Use the same proportions as in 200, and

202. Apply the xanthoproteic reaction.

203. Acidify another portion strongly with acetic acid and add a few drops of a solution of ferrocyanide of potassium.

204. Apply Millon's reagent.

205. Apply Piowtrowski's test (6).

206. To another portion add a little alcohol.

207. Saturate another portion (proportions as in 200) with ammonium sulphate. This precipitates all of the proteids, globulin and albumin. Filter. The filtrate does not respond to any of the tests for proteids.

208. To another portion of the diluted serum add a little silver nitrate solution. A white, curdy precipitate forms, soluble in ammonia but not in nitric acid. Chlorides are present.

209. Add barium chloride. A white, heavy precipitate insoluble in nitric acid. Sulphates are present.

210. Add nitric acid and molybdate of ammonia and heat. A yellow precipitate indicates the presence of phosphates.

211. Test with Fehling's solution, and boil. Red, cuprous oxide indicates a reducing sugar—glucose.

212. To a little of the defibrinated blood in a test-tube add a few drops of sulphuric acid. Stir up the solution and note the peculiar odor of blood, intensified by the liberation of traces of volatile acids by the sulphuric acid.

213. Detection of paraglobulin (fibrinoplastin, or serum-globulin). Pass some CO_2 through a beaker of dilute serum for 20 minutes or more. (The CO_2 may be generated by the action of dilute hydrochloric acid upon small pieces of marble in a jar and the gas conveyed to the beaker.) Let the precipitate settle. It is paraglobulin. Decant and, after washing with water, dissolve some of it in a little dilute saline solution, use Piowtrowski's test and prove it a proteid.

214. Take equal quantities of blood and ether in a test-tube. Shake thoroughly and let the ether separate. Then pour the ether into a watch-glass or evaporating dish and when evaporated examine for globules of fat.

215. Evaporate a little blood to dryness in a crucible or evaporating dish. Raise the temperature to red heat to convert the blood to ash. When cool add a little nitric acid, heat, dilute with water and filter. Make the following tests with the filtrate :

216. To a small portion of the filtrate add a little sulphocyanide of potassium. A red color indicates iron.

217. To another portion add a little ammonium molybdate solution. A yellow precipitate, after allowing the mixture to stand for some time, indicates phosphates.

218. To another portion add a little silver nitrate solution. A white, cloudy precipitate indicates chlorides.

219. Examination of blood with a spectroscope. With a small direct vision spectroscope focus on the sky or bright light until the spectrum shows clearly. Narrow the slit until the spectrum is as distinct as it can be made. Hold the spectroscope so that the red is at the left of the field. Dip a wire into some water, and then into some salt or sodium carbonate, and hold it in a flame of a fish-tail burner. Note the change in the spectrum.

220. Arrange the apparatus with the aid of a demonstrator. so that the spectroscope, gas-flame, and substance to be

examined are in their proper relations. Half fill the vial or test-tube with defibrinated blood. Nothing can be seen until the blood is properly diluted. Continue diluting until two bands of oxy-hemoglobin appear in the spectrum. Note their position, and which one disappears first when the solution is diluted far enough.

221. Add a drop or two of ammonium sulphide solution to reduce the oxy-hemoglobin. Note the result.

222. Pass some illuminating gas through some blood for a considerable time. Examine with a spectroscope. Add a drop or two of ammonium sulphide. Compare this with 221.

XVII.

EXAMINATION OF URINE

223. Urine in man is a transparent, light straw or amber-colored watery excretion derived from the kidneys, containing nitrogenous matter, salts and gases. The most abundant constituents are water, urea, and sodium chloride. It has a peculiar odor, bitter saltish taste, and acid reaction. The normal quantity in an adult is about 1500 cc. ($2\frac{1}{2}$ pints), in twenty-four hours, although there may be considerable variation, dependent upon the pulmonary and cutaneous excretions.

The specific gravity of normal human urine ranges from 1015 to 1025, and is taken by means of the urinometer. Care must be taken that the instrument is perfectly dry; the urine must be clear and free from air bubbles on the surface; the urinometer must not touch the sides of the vessel. Urine constantly below 1015 is of low specific gravity and if constant may indicate diabetes insipidus or chronic Bright's disease. If above 1025 it is of high specific gravity, and if pale, diabetes mellitus or sugar in the urine may be suspected.

The amount of solids in the urine may be estimated from the specific gravity by Christison's formula ("Häser-Trapp's coefficient"). "Multiply the last two figures of a specific gravity expressed in four figures by 2.33. This gives the quantity of solid matter in every 1,000 parts." The number of grams in 1000 cc.

Example.—Suppose a patient passes 1400 cc of urine in twenty-four hours and the specific gravity is 1020, then, 20 times 2.33 equals 46.60 grams in 1000 cc. To ascertain the amount in 1400 cc. 1000 is to 1400 as 46.60 is to x. 1400 times 46.60 equals 65240.00, this divided by 1000 equals 65.24 grams, of solids in 1400 cc. of urine.

The formula is purely empirical, and is not applicable where the variations are very marked, as in saccharine diabetes and some cases of Bright's disease.

The normal or total solids or "solid urine" is about 70 grams (2 oz.) in twenty-four hours.

The reaction of human urine is usually slightly acid. The acidity being chiefly due to acid sodium phosphate, and acid urates and very slightly to free acids—lactic, acetic, oxalic etc. During digestion two or three hours after a meal, the urine becomes neutral or alkaline. With a vegetable diet the excess of alkali causes an alkaline urine. In herbivora it is alkaline; in carnivora it is very acid.

When urine is freely exposed to the air it undergoes two fermentations, acid and alkaline. The urine at first becomes slightly more acid, from the formation of lactic and acetic acids. (This is denied by some). Then it gradually becomes neutral, and finally alkaline from putrefaction. It becomes lighter in color, turbid, and a whitish heavy precipitate occurs. A pellicle forms on the surface, it swarms with bacteria and it has an ammoniacal odor, which is due to the splitting up of the urea. The urea is split up by a ferment said to be formed by the *Micrococcus ureae*.

224. Place some urine aside for some days in a warm

place. Observe it from day to day, noting its reaction, change of color, transparency, odor and any deposits that may form in it.

225. Dip some neutral litmus paper into some normal urine. It is turned red. Do the same with some stale urine. It is turned blue. Warm the paper just used, the blue may fade indicating the presence of a volatile alkali (ammonium carbonate). The blue color does not disappear with heating when a fixed alkali is present.

226. Inorganic constituents of urine. The ratio of inorganic to organic constituents is 1 to 1.2—1.7. The amount of salts excreted in twenty-four hours is 16 to 24 grams ($\frac{1}{2}$ to $\frac{3}{4}$ oz.).

Samples of omnivorous, carnivorous, and herbivorous urines are furnished. Apply the same tests to each at the same time and note any differences as to color, specific gravity, reaction and responsiveness to the various tests. In all cases the urine must be filtered and thoroughly clear before attempting the tests.

The herbivorous and carnivorous urines may be obtained with the aid of a catheter, when the bladder is full. The mare and the bitch are convenient animals for this purpose, as it is easier to pass the catheter in the female than the male. Human urine may be used as an example of the omnivorous excretion.

227. The urinometer may be a few degrees out of the way. Test it by filling the urinometer jar with distilled water at 15° C. (60° F.). Read the division of the scale corresponding with the surface of the fluid looking above or below the meniscus as is found to be the most correct for the zero reading. Always adhere to this method when using the same urinometer. Test the urines, making the necessary corrections. If the urine is warmer than 15° C. add 1° to the density for every 4° of extra C. temperature, or for every 7° of extra F. temperature.

228. Test the specific gravity with the specific gravity beads. This method has the advantage that but a small amount of urine is necessary for a test. The bead which floats midway in the urine indicates the specific gravity. If none float midway the specific gravity will lie between the figures on the bead that floats and the figures on the bead that sinks. This is ordinarily sufficient for an approximate result. To obtain the exact specific gravity the beads, marked respectively 5, 10, 15, 20, 25, 30 enclosed in a tube with an open bottom, are immersed in the urine. If three beads float we know that the specific gravity is between 1.015 and 1.020. To obtain the exact figure, add water, drop by drop, to the urine, until the third bead is in a state of equilibrium and only two float. If two fluid-drams (120 minims) of urine were taken to begin with, and 20 minims of water were required to bring the third bead to the point of sinking then the following ratio obtains $120:120+20::15:x$. The value of x is found to be 1.0175, the exact specific gravity of the urine. If the specific gravity is above 1.030 dilute the urine one-half with water and multiply the result by two.

The specific gravity of the urine of the horse ranges from 1.020—1.060; that of the ox from 1.010—1.030; that of the dog from 1.015—1.060.

229. Note and record the colors of the filtered and unfiltered urines, according to the chart of colors. Record their specific gravities by the urinometer and beads. Record also their reaction to litmus as acid, very acid or alkaline. Note the great amount of mucous present in the unfiltered herbivorous urine.

XVIII.

230. The water of the urine is derived from the food and drink, a small quantity being formed in the body.

231. Chlorides are chiefly those of sodium with a little potassium and ammonium, derived chiefly from the food and amount to an average of 12 grams daily.

Test a portion of each urine with a few drops of silver nitrate solution. A white, cheesy or curdy precipitate in lumps insoluble in nitric acid indicates the presence of silver chloride. The phosphate of silver may also be thrown down but this is soluble in nitric acid. In acute cases of pneumonia the chlorides may be absent from the urine, their reappearance in the urine is a good symptom. In normal urine the amount of sodium chloride remains quite constant, about 0.75%. A few drops of urine evaporated upon a slide will give octahedral or rhombic crystals, a compound of sodium chloride and urea.

232. Sulphates. The sulphates are chiefly those of sodium and potassium. The total quantity of sulphates is 3 to 4 grams daily. Only a small amount of them enters the body with the food, so that they are chiefly formed from the metabolism of proteids in the body. They have no clinical significance. Sulphuric acid, however, exists in the urine not only in combination with alkalis as stated above as "preformed sulphuric acid" but also with organic radicles, phenol, skatol, etc., forming "ethereal sulphates" or "combined sulphuric acid". The latter forming about 1/10 of the total sulphates, and, originate from putrefactive processes in the intestine.

After acidulating with hydrochloric acid to prevent the precipitation of phosphates, add to a small part of each urine, a little 2% barium chloride, a white heavy precipitate of barium sulphate is formed, insoluble in nitric acid.

233. To separate the combined (ethereal) sulphuric acid. Mix 50 cc. of omnivorous urine with an equal bulk of "baryta mixture". Stir and filter. This removes the ordinary sulphuric acid as sulphate of barium. Add 10 cc. of hydrochloric acid and keep in the water bath at 100° C.

for an hour and then allow the ethereal or combined sulphates to settle. "Baryta mixture is prepared by making saturated solutions in the cold of barium nitrate and barium hydrate, and adding 2 volumes of the hydrate to one volume of the nitrate."

234. The phosphates consist of *alkaline* and *earthy* salts in the proportion of 2 to 1. The latter are insoluble in an alkaline medium and are precipitated when acid urine becomes alkaline. They are insoluble in water, but soluble in acids; in urine they are held in solution by free CO_2 . The *alkaline* phosphates (sodium and potassium) are very soluble in water, and they *never* form urinary deposits.

The earthy phosphates are phosphates of calcium (Ca_3PO_4)₂ (abundant) and magnesium (MgHPO_4 plus $7\text{H}_2\text{O}$) (scanty). The quantity is 1 to 1.5 grammes. They are precipitated when the urine is alkaline, although not in the form in which they occur in the urine. In normal herbivorous urine the phosphates are said not to occur.

235. To portions of the three clear filtered urines add nitric acid, boil, and add barium chloride and boil again; a precipitate of barium sulphate may be formed. Filter and to the cool filtrate add ammonia; a precipitate of barium phosphate may appear. It is understood that the experiments are to be made upon samples of all three urines and any difference in their responsiveness to the tests is to be recorded.

236. The earthy phosphates have a clinical significance. They are *increased* in osteomalacia and rickets, in chronic rheumatoid arthritis after prolonged mental fatigue, and by food and drink; and they are *diminished* in renal diseases and phthisis.

The alkaline phosphates are chiefly acid sodium phosphate (NaH_2PO_4) with traces of acid potassium phosphate (KH_2PO_4). The quantity is from 2 to 4 grams and they are derived chiefly from the food and perhaps a small

amount from the oxidation of the phosphorus of nerve tissues.

237. To a small amount of urine add about half its volume of nitric acid, and then add a little ammonium molybdate solution and boil. A canary-yellow crystalline precipitate of ammonium phospho-molybdate may appear. The precipitate may appear slowly and if necessary, the tubes should be kept undisturbed for a few hours. In a very dark urine the precipitate may be of a brown instead of a yellow color. Make sure that the herbivorous urine is of an acid reaction before adding the ammonium molybdate.

238. To urine add half its volume of ammonia and allow it to stand, a precipitate of *earthy* phosphates is formed. Filter and test the filtrate with ammonium molybdate. This method separates the earthy from the alkaline phosphates.

239. To urine add half its volume of baryta mixture; a copious precipitate. Filter and test the filtrate with ammonium molybdate. No precipitate should occur as the baryta mixture precipitates the phosphates as well as the sulphates and carbonates.

240. Use a little *magnesia mixture* instead of the baryta mixture. Filter and test the filtrate with ammonium molybdate. The magnesia mixture is composed of magnesium sulphate and ammonium chloride, of each one part, ammonia water 1 part and distilled water 8 parts.

241. To urine add a few drops of acetic acid and then a few drops of uranium acetate, a yellow precipitate of uranium and ammonium double phosphate is formed.

242. Centrifugal analysis of urine. The chemical constituents of urine, both normal and abnormal, may be estimated by the centrifugal method. By the use of percentage tubes an approximate estimation of certain of the constituents may be obtained. These tubes are graduated in tenths of a cubic centimeter up to 10 cc. The lower portion of the tube is drawn out in a conical form, and fractions of the

first cubic centimeter may be more accurately read and measured. Above the graduated scale the tube possesses sufficient capacity for the reception of the reagents. After use, the tube must be thoroughly but carefully cleaned.

In operating the centrifuge the tubes are to be revolved for exactly three minutes and at as uniform rate as possible. The ungraduated tube may be filled with water or urine and the revolutions will cause a deposit to form at the bottom, in the case of the urine.

243. Chlorides. Fill the graduated tube to the 10 cc. mark with the urine to be tested. Add 15 drops of nitric acid to prevent precipitation of the phosphates; then fill to the 15 cc. mark with the silver nitrate solution. Close and invert the tube several times so that the reagents are thoroughly mingled. Revolve for three minutes at 1000 revolutions per minute when the quantity in bulk percentage may be read off from the graduated scale on the side of the tube. The percentage thus obtained ranges from 10% to 12% for normal human urine.

244. Sulphates. These are estimated as insoluble salts of barium. Fill the tube as before to the 10 cc. mark. Add 15 drops of hydrochloric acid to prevent precipitation of phosphates. Fill to the 10 cc. mark with barium chloride and after inverting the tube a few times, revolve for three minutes. The bulk percentage is obtained as before and is found to be about 0.8 % in normal human urine.

245. Phosphates. Fill with urine to the 10 cc. mark and add enough of the magnesium mixture to reach 15 cc. Invert the tube several times and revolve three minutes. Read off the bulk percentage which in normal human urine is found to be about 8%.

XIX.

246. Organic constituents of urine. Urea or carbamid (CON_2H_4) is the most important organic constituent in urine, and is the chief end-product of the oxidation of the nitrogenous constituents of the tissues and food. It has no effect on litmus; it is odorless, weak, cool, and has a bitter taste like saltpetre. It is very soluble in water, and in alcohol, and almost insoluble in ether. More than $\frac{9}{10}$ of all the nitrogen taken in is excreted in the form of urea.

247. Take 20 cc. of fresh, filtered omnivorous urine and add 20 cc. of baryta mixture to precipitate the phosphates. Filter, evaporate the filtrate to dryness and extract the residue with a little boiling alcohol over the water bath very carefully. Filter off the alcoholic solution, place some of it on a slide and allow the crystals of urea, usually long, fine, transparent needles, to separate out. Examine them under the microscope.

248. A simple method of detecting urea is to place a drop or two of the urine upon a glass slide and after adding a drop of nitric acid, gently warm over the flame. If urea be present, upon evaporation, the microscope will show the characteristic crystals of nitrate of urea, of rhombic or hexagonal form.

249. To a few drops of the suspected fluid in a test-tube add an equal quantity of solution of hypobromite of sodium, and a rapid evolution of bubbles of nitrogen takes place if urea be present. (Hypobromite of sodium is prepared by mixing 2 cc. of bromine to 23 cc. of a 40% solution of caustic soda.)

250. Heat some urea crystals in a test-tube. Biuret is formed, and ammonia comes off. Add a trace of copper sulphate solution and a few drops of 20% caustic potash. A rose-red color is produced—the biuret reaction.

251. Quantitative estimation of urea. Lyon's apparatus.

Place in the bottle 20 cc. of fresh hypobromite solution. Fill the little test-tube with urine to the 4 cc. mark and carefully lower it into the bottle so as not to spill any of its contents. Hook the bent overflow tube over a graduate and removing the little rubber cap, fill with water the graduated jar to slightly above zero. Put on the cap far enough to depress the surface of the water exactly to zero. See that all of the joints are tight, and tip the bottle so that the urine *slowly* flows out, and shake it occasionally. When the action appears to be at an end, pour into the graduate water enough to reach above the opening of the overflow tube, in order that cooling of the gas evolved, which is at first quite warm, may not draw air into the apparatus. Let it stand for a few minutes to cool. In half an hour lower the end of the overflow tube to the level of the water in the cylinder and read off the percentage of urea. This multiplied by 4.55 will give grains per fluid ounce, at a room temperature of 21° C. (70° F.).

252. Make another test with the Doremus ureometer. After filling the long arm and bend of the ureometer with the hypobromite solution and thoroughly washed the pipette, draw up exactly 1 cc. of urine and pass the pipette through the bulb of the ureometer as far as it will go in the bend. Compress the bulb of the pipette gently and steadily. The urine will rise through the hypobromite, and the urea instantly decompose, giving off nitrogen gas. Withdraw the pipette after the urine has been expelled, taking care not to press the bulb hard enough to drive the air out with the urine, and read the volume of gas, after allowing the froth to subside.

The CO_2 resulting from the decomposition of the urea is absorbed by the excess of soda in the hypobromite solution, and nitrogen is evolved, 0.1 gram of urea yields 35.4 cc. of nitrogen.

253. A man excretes 38 to 40 grams of urea daily; a

woman less, and children relatively more. Its amount varies with the nature of the food ; it is increased when the nitrogenous matters of the food are increased and is diminished by vegetable diet ; increased by copious draughts of water, salts. Saccharine diabetes and acute stages of fever increase its discharge. Bright's disease, anemia, cholera and the use of morphine decrease it. If retained in the body it gives rise to uremia, when it may be excreted by the skin or given off by the bowel.

254. Uric acid ($C_5H_4N_4O_3$) contains 33.33% of nitrogen and, next to urea, is the constituent of the urine whereby the largest quantity of nitrogen of the body is excreted, whilst in birds, reptiles and insects it forms the chief nitrogenous excretion. The proportion of urea to uric acid is 45 to 1. About 0.5 gram of uric acid is excreted daily in man. This quantity is diminished in gout, etc. Uric acid causes the brick-red deposit sometimes seen in urine after standing for a time.

255. In a conical glass, add 5 parts of hydrochloric acid to 20 parts of urine. Label and put in a cool place for 24 hours. Yellow or brownish colored crystals of uric acid are deposited on the sides of the glass, or form a pellicle on the surface of the fluid like fine grains of cayenne pepper. Examine some of the crystals microscopically.

256. Murexide test. Place a little uric acid in a porcelain capsule, add nitric acid and heat gently, not above $40^{\circ}C$. A yellow or reddish stain remains. Allow it to cool and bring a rod dipped in ammonia near the stain, and if this produces no effect, moisten the stain with strong ammonia ; a purple red color of murexide or purpurate of ammonia is formed. It turns bluer upon the addition of caustic potash.

257. Dissolve a little uric acid in 10% caustic soda or potash. Add a drop or two of Fehling's solution—or dilute cupric sulphate and caustic potash—a precipitate which at first may be white and after a time turning green or reddish.

258. Schiff's test. Dissolve a little uric acid in a small quantity of sodium carbonate. By means of a glass rod place a drop of silver nitrate solution on filter paper and then a drop of the uric acid solution so that the two drops partially overlap. A dark brown or black spot of reducing silver appears.

259. Hippuric acid ($C_9H_9NO_3$) occurs in large quantities in the urine of the horse and many herbivora. It is also found in man, from 0.5 to 1 gram being excreted daily. It dissolves readily in hot alcohol but is sparingly soluble in water. It is not present in the urine of carnivora. The amount in man is increased by eating pears, apples with their skins, cranberries and plums. It seems to be formed chiefly from the husks or cuticular structures. Nothing is known of its clinical significance.

260. Evaporate some of the herbivorous urine (50 cc.) with nitric acid (10 cc.) in a hood and heat the residue in a dry test tube. If hippuric acid be present an odor like that of oil of bitter almonds is plainly observable, due to the formation of nitrobenzol.

261. Kreatinin, ($C_4H_7N_3O$) is related to the kreatin of muscles. Add a very dilute solution of sodium nitro-prusside to some omnivorous urine, and very cautiously some caustic soda; a ruby red color develops. Boil, the color fades. While boiling add a little strong acetic acid, the color changes to blue.

262. Ferments in urine. It is said that urine contains pepsin. Some observers state that it also contains trypsin and a sugar-forming ferment, but the latter statement is denied.

The morning urine should be selected. Place in it for several hours a little well washed and boiled fibrin. The latter absorbs the ferment, and on placing it in 0.2% hydrochloric acid at 40° C. the fibrin is dissolved and peptones formed. Test for the peptones by the biuret reaction.

XX.

263. Albumin in urine. The presence of this substance in the urine is regarded as pathological. It must be borne in mind, however, that in the urine of a certain percentage of persons apparently enjoying perfect health, minute traces of albumin are sometimes present and, unless these traces persist, are not to be regarded as serious. If present in any considerable quantity, it must be regarded as distinctly abnormal. The most common cause of the appearance of the albumin is disease of the kidney (Bright's disease). Albuminuria is the term applied when albumin occurs in notable quantity in the urine. The chief form of albumin present is serum-albumin; in addition there may be serum-globulin, albumose, peptone, acid-albumin and fibrin.

264. In every case the urine must be clear before testing, by filtering it carefully.

Add a little acetic acid to the urine. Boil in a test-tube. Near the boiling point, if in small amount, the albumin will show a haziness; if in large amount, there will be a distinct coagulum.

Caution. If the urine be alkaline, boiling will not precipitate the albumin. Heat, by driving off the CO_2 , also precipitates the earthy phosphates, if they are present in large amount. Albumin, however, is coagulated before the boiling point is reached— 73°C .—while the phosphates are precipitated at the boiling point, 100°C . The phosphates are soluble in nitric acid, the albumin is not. When nitric acid is added, it is believed that some of the albumin is still kept in solution as an acid-albumin, so that the test is not regarded as the most accurate one. Nitric acid in excess will dissolve the albumin.

265. Heller's cold nitric acid test. Pour some of the urine gently upon the surface of some nitric acid in a test-tube. A ring of white coagulum occurs at the junction of

the two fluids. If the quantity of albumin is small, the coagulum may not occur for a few minutes.

Caution. A crystalline deposit of urea is sometimes obtained with a very concentrated urine. If the urine contain a large amount of urates, they may be deposited by the acid, but the deposit in this case occurs above the line of junction and disappears on heating.

266. The ferrocyanide test. Into the bottom of a clean test-tube is poured 15-30 drops of acetic acid, then 2-3 times that amount of 5% potassium ferrocyanide, and the two ingredients thoroughly mixed by shaking the tube. The urine is next added to the depth of two-thirds of the test-tube. If albumin be present it will be thrown down throughout the whole volume of the urine, in a more or less milk like form, or flocculent cloud.

As applied in this way it is said that this test acts upon nothing but albumin.

267. Picric acid test. (Johnson's.) Fill the test-tube half full of urine. Slightly incline the tube and gently pour down its side about 2 cc. of a saturated aqueous solution of picric acid, so that it may come in contact with the upper layer of the urine. Place the tube in an upright position. A layer of coagulated albumin will appear at the point of junction. The coagulation of albumin takes place at once and is thus not easily mistaken for precipitated urates, which require some time for their precipitation and disappear on the application of heat.

268. Potassio-mercuric Iodide. (Tanret's Reagent.) This solution is prepared according to the following formula: Potassium iodide, 3.32 grams; mercuric chloride, 1.35 grams; acetic acid, 20 cc.; and enough distilled water to make 100 cc. The potassium iodide and mercuric chloride should be dissolved separately, and the solutions mixed. Add a little of this reagent to the suspected fluid and heat; peptones, urates, alkaloids and mucin are also precipitated at first but

are dissolved by the heat. It is a delicate test and will detect one part of albumin in 20,000 of fluid. It has a value as a quick test, since if no precipitate is formed after its addition to the fluid, albumin is quite positively excluded.

269. Add 2 volumes of strong alcohol to the urine in a test-tube. The solution will become cloudy and a precipitate will form. Add a few drops of nitric acid and heat, if the precipitate disappears it was composed of phosphates, if it remains it is albumin. The test is a delicate one and will detect 1 part of albumin in 32,000 of the solution. If the albumin be very slight in amount it will require a number of hours for the precipitate to form. The alcohol also precipitates peptones and does not precipitate alkaloids.

270. Esbach's albuminometer method. Fill the tube with urine to the letter U, then add the reagent to the letter R. (The reagent is prepared by mixing 10 grams of picric acid and 20 grams of citric acid and adding enough water to make 1 liter. It is said that a 1% solution of acetic acid will give the same result). Invert the tube a number of times so that the contents may be thoroughly mixed. Close the tube tightly with the rubber stopper and set aside for 24 hours. After which time the amount of *dried* albumin, in one liter of urine can be read in grams on the tube. The percentage is obtained by dividing by ten. Thus if the coagulum stands at 3, the urine contains 3 parts of albumin per thousand or 0.3%. If the albumin is very abundant (above 4) the urine should be diluted to obtain an accurate result. Less than 0.5 per thousand of albumin cannot be accurately estimated by this method.

Caution. The coagulable substance found in the urine of Bright's disease is a mixture of "serum-albumin" and "serum-globulin" or "para-globulin". Saturation of the urine with crystallized magnesium sulphate precipitates the "serum-globulin", leaving the serum-albumin in solution.

271. Centrifugal method. The percentage tube is filled

to the 10 cc. mark with the urine to be tested, and $3\frac{1}{2}$ cc. of potassium ferrocyanide (1 to 10) are added; $1\frac{1}{4}$ cc. of acetic acid are next added and the reagents and urine are thoroughly mingled by inverting the tube several times. The tubes are placed in the centrifuge and revolved until all of the albumin has settled, leaving the fluid above perfectly clear. The bulk percentage of albumin is then read off, each $\frac{1}{10}$ cc. representing 1% bulk measure of albumin.

XXI.

SUGAR IN THE URINE. (GLYCOSURIA.)

272. It is maintained by many that the merest trace of glucose or grape sugar is normally present in urine, and that sugar in slightly larger quantities may appear transitorily without pathological significance. In diabetes mellitus it occurs in considerable amount.

Characters of diabetic urine. The quantity of urine passed is very large, even to 10,000 cc. in 24 hours; the specific gravity is high, 1030—1045; the color is usually a very pale straw, from the dilution—not diminution—of the urinary pigments; the urine is often somewhat turbid; it has a heavy sweet smell, and usually froths when poured from one vessel to another.

The presence of albumin interferes with tests for sugar, and the urine should first be tested for the presence of albumin.

273. Trommer's test. To about 3 cc. of urine in a test tube add just enough copper sulphate solution to tint the urine. Add about 3 cc. of 20% potassium hydrate, a greenish blue precipitate may at first be thrown down, but disappears on shaking. Heat the mixture to the boiling point. If sugar be present, the color changes to a yellowish or red-

dish tint, the sugar having reduced the cupric hydrate to cuprous oxide.

274. Fehling's solution. Place some Fehling's solution in a test-tube and boil it. If no yellow discoloration takes place it is in good condition. Add a few drops of the suspected urine and boil. If the mixture suddenly turns to an opaque yellow or red color, the presence of a reducing sugar is indicated.

275. The principle of the bismuth test is the same as the copper test. The glucose reduces the salt of bismuth in the presence of an alkali. Put equal quantities of urine and potassium hydrate in a test tube, and add a pinch of subnitrate of bismuth. Boil the mixture and if glucose be present the bismuth is reduced and turns black; if no glucose is present the powder remains white. Albumin and sulphur also reduce bismuth and must be removed from the urine, if the test is to be reliable.

276. Take 25 cc. of suspected urine and add 1 gram of phenyl-hydrazine hydrochlorate, and 1 gram of sodium acetate, and 10 cc. of distilled water. Keep the solution nearly to the boiling point for an hour. Even minute quantities of sugar yield a yellow precipitate, which under the microscope is seen to consist of fine, bright yellow crystals of phenyl-glucosazone. This is one of the most delicate and accurate tests known, and is said to be reliable in the presence of albumin, and the products of decomposing urine.

277. Picric acid test. To a small amount of the urine add an equal volume of a saturated aqueous solution of picric acid. Boil; an intensely deep red or reddish-brown color indicates the presence of a reducing sugar. The greater the amount of the sugar the greater the tint. The coloration is due to the formation of picramic acid.

278. Fermentation method. Robert's Differential Density Method. Take the specific gravity of the urine before and after adding the yeast and record it. Mix well 2 fluid

ounces (60 cc.) of urine with a quarter of a cake of compressed yeast in a bottle. Set aside for twenty-four hours in a moderately warm place. After the fermentation, take the specific gravity again and subtract from that taken before. Each degree of the remainder represents one grain of glucose to the fluid ounce. Multiply by 0.219 to get the percentage. Thus. Specific gravity before fermentation, 1035. Specific gravity after fermentation, 1015. 1035—1015 equals 20 degrees of density lost, or 20 grains of sugar to the fluid ounce. This test is conclusive as to the presence of sugar, though it is not absolutely accurate as to quantity.

279. Einhorn's Fermentation Saccharometer. Determine the specific gravity of the urine, which is diluted according to the specific gravity as follows :

Specific gravity,	1018—1022,	dilute it with 2 volumes of water.
“	“	1022—1028, “ “ “ 5 “ “ “
“	“	1028—1038, “ “ “ 10 “ “ “

Take one gram of compressed yeast. Shake it thoroughly in the test-tube with 10 cc. of the urine to be examined. Pour the mixture into the bulb of the saccharometer and by inclining the apparatus the fluid will displace the air in the cylinder and remain there by atmospheric pressure. Be sure that no air bubbles remain in the cylinder. It is always well to test a normal urine at the same time and in the same way as a control. The apparatus should remain in a moderately warm place for 24 hours. If the urine contains sugar, it is broken up by the fermentation into CO_2 and alcohol. The CO_2 gathers at the top of the cylinder, forcing the fluid back into the bulb. The changed level of the fluid in the cylinder shows that the reaction has taken place and indicates by the numbers the approximate quantity of sugar present. The scale on the tube is empirical and indicates directly the percentage of sugar in the urine.

280. Bile in urine. The biliary constituents appear in the urine in cases of jaundice and in poisoning with phosphorus.

Bile pigments. The urine has usually a yellow or yellowish green color and froths easily when shaken. Filter paper dipped into it gives a yellow stain on drying.

281. Gmelin's test (nitric acid containing nitrous acid). Place a few drops of the suspected urine in a white porcelain dish and near it a few drops of the impure nitric acid, and let the fluids run together and the usual play of colors is observed.

282. Take urine in a test-tube pour in the impure nitric acid until it forms a stratum at the bottom. If the bile pigments be present, at the line of junction of the fluids, a play of colors takes place—from above downwards—green, blue, violet or dirty red, and yellow. Nearly all urines give a play of colors, but *green* is the necessary and characteristic color to prove the presence of bile pigments.

283. Rosenbach's modification. Filter the urine several times through the same filter, dry the filter paper and to it apply the impure nitric acid, when the same play of colors is observed.

284. A solution of methyl violet poured on the urine by the contact method, gives a bright carmine ring at the point of contact.

285. Mix 1 cc. of urine with 2 cc. of ether and then agitate with five drops of tincture of iodine. After standing, the ether, holding in solution the iodine, will form the upper layer, while the urine beneath takes on a brilliant green coloration if biliverdin is present.

286. Bile Acids. (Glycocholic and Taurocholic acids). Pettenkofer's test. Add to the urine a few drops of syrup of cane sugar (10%), mix them, and pour strong sulphuric acid down the side of the tube until it forms a layer at the bottom. The temperature must not rise above 70° C. nor must the urine contain albumin. At the line of junction a cherry red or purple violet color indicates the presence of bile acids. Another method is to shake the urine and syrup

to get a froth, when the sulphuric acid is added the froth shows the color.

Blood in the urine (Hematuria), pus and other organic deposits in the urine require microscopic analysis.

XXII.

287. Place a frog on its belly and note the movements of the caudal lymph-hearts. They are situated between the hip-joint and the median line in a slight depression. The contractions of these hearts are usually visible through the skin, but are seen more distinctly if the skin is removed without injury to the hearts.

Later, note that the lymph-hearts cease to beat after the destruction of the caudal portion of the myel (spinal cord).

288. Pith the frog. This is accomplished by severing the brain from the myel with a thin bladed knife at the point where the cranium articulates with the atlas. A slight depression will be felt at this point, which will serve as a guide for the operation. The frog may be firmly held if wrapped in one corner of a towel.

289. After pithing, lay the frog on its back, and cut through the skin on the mid-line, and from the middle of this cut make lateral incisions through the skin. Raise up the end of the sternum and cut, a little to one side of the mid-line, through such parts as may be necessary to expose the heart. Pin the parts out, on the side and note the heart beating with some force and regularity. Pinch up the pericardium with a pair of fine forceps and remove it from the heart. Tilt up the apex of the ventricle and note a small band of connective tissue passing from its dorsal surface to the adjoining wall of the pericardium. Seize this band with the forceps and divide it between the forceps and the pericardial wall. Lift up the apex of the ventricle, by means of

this band, and with a sharp pair of scissors cut through the right and left aortae, the pre and post caval veins, and the surrounding tissue, taking care not to injure the sinus venosus. Place the heart in a watch-glass, moistening occasionally with normal saline solution.¹ The beats will not be interrupted at all, or for a very short time only.

290. Watch the beating of the heart. Do the auricles and ventricle contract simultaneously? What are the number of beats per minute?

291. Lift up the apex of the ventricle, and with the scissors cut off the apex at the upper third of the ventricle. Watch the separated portions. Is there any difference in the beating?

292. With the scissors separate the two auricles from each other, letting the attached portion of the ventricle remain to each auricle. Do they continue to beat?

293. The same frog, if it has been kept in a moist place, may be used for the following cilia experiment: Place the frog upon its back, and cut through the lower jaw, along the mid-line, continuing the incision down the oesophagus as far as the stomach. Pin the parts back and moisten the mucosa with normal salt solution, if it is at all dry. Place a small, thin piece of cork upon the mucosa just below the orbits, and note that the cork is carried toward the stomach by the cilia. Warm a little of the normal salt solution to 30° C., and repeat the experiment. Apply heavier bits of substance to the mucosa, and note if their positions are changed.

294. If the caudal lymph-hearts are still beating, pass a tracer or piece of wire down the spinal canal to destroy the myel. If thoroughly destroyed the lymph-hearts will cease to beat.

¹ Made by dissolving 6.5 grams of sodium chloride in one liter of distilled water.

XXIII.

295. The circulation of blood. This may be shown very nicely in the delicate external gill filaments of the *Necturus*, or in the tail of a tadpole, or in the web of a frog's foot which does not contain too much pigment. The animals should be injected with a drop or two of a 1% solution of curare, in order that they may not move, and arranged upon the stage of the microscope, so that the parts to be examined may come clearly into the field of vision. Precautions should be taken against drying, by keeping the animal well surrounded with moist cloth or absorbent cotton.

296. If the frog is more convenient, prepare it by destroying the brain and injecting the curare under the skin of the back. Place the frog on its belly on the frog board and pin out the digits so that the web will be slightly on the stretch. Keep the parts moist. Put a very *small* drop of water upon the web, and cover it with a triangular piece of cover-glass, being careful that it does not cut the digits and that no fluid flows over its surface. Examine first with a low power, and then, if possible with a high power.

297. Note the course of the blood from the arteries to the veins. Arteries may be distinguished from veins by the fact that the blood corpuscles scatter to enter the capillaries diverging from the artery, while in the veins the corpuscles accumulate from the capillaries converging to form the vein. A slight pulsation may sometimes be observed in the smaller arteries.

298. Note the greater velocity of blood in the arteries than in the veins; the individual corpuscles cannot, perhaps, be made out in either.

299. Note the axial and peripheral zones in the arteries and veins; the peripheral zone is small and under a low power appears free from corpuscles; under a high power a few leucocytes may be seen in the peripheral zone, if the current is not too rapid; in that of the veins a few leucocytes

and occasionally a red one will be seen moving along comparatively slowly.

300. Note the passage of the corpuscles usually in single file through the capillaries.

301. Note the elasticity of the red corpuscles, observing the way in which they bend and later regain their normal form.

302. Study of inflammatory conditions. Remove the cover-glass and absorb the fluid on the web; touch the middle of the web with the tip of a glass-rod that has been dipped in creosote (or a 2% solution of croton oil in olive oil) leaving a minute drop on the web. Put on a cover-glass as before and examine with the microscope.

303. Note the dilatation of the arteries, the more distinct appearance of the capillaries, and the enlargement of the veins, accompanied by a quickening of the current.

304. Note a little later, the slowing of the current, the vessels remaining dilated.

305. Note that the leucocytes increase in number in the peripheral zone of both arteries and veins; in the latter the leucocytes begin to cling to the sides, temporarily at first and then permanently. In the capillaries the leucocytes and, less frequently, the red corpuscles stick to the capillary walls, partially or completely blocking the way. Later stagnation may set in and there is then the appearance of the gradual obliteration of the outlines of the corpuscles.

306. Note the migration of the leucocytes from the capillaries and veins. This occurs when the circulation becomes slow. Watch, at intervals of 10 minutes, some particular leucocyte adhering to the wall of a capillary or vein.

307. Note the diapedesis of the red corpuscles from the capillaries, seen to the best advantage in those capillaries in which the current has almost ceased.

308. Note that the above effects are local, are of greatest intensity in the spot touched, they extend some distance

around the spot, but the circulation in the rest of the web is normal. If the injury has not been too severe, the circulation may become re-established in the stagnated spots, and the inflammatory appearances disappear.

309. Pin out the two horns of the tongue and observe that under the microscope. The tongue is at first pale but soon becomes reddened as the vessels become filled with blood. With a low power the peripheral zone in the arteries and veins may probably be seen better here than in the web.

310. Place the frog on its back, cut through the skin and muscles on one side and draw out the mesentery and pin out a loop of it under the field of the objective and observe the circulation. The inflammatory phenomena can be well seen in this preparation or that of the tongue. (309).

XXIV.

311. Experiments in reflex action. Pith a frog and place it on its belly. Note the position of its fore and hind limbs. Note the position of the head as compared with a normal frog. Are there any respiratory movements at the nostrils or throat?

312. Pull, very gently, one of the hind-limbs into an extended position and then let go. Does it return to its former location?

313. Gently tickle one flank with a feather or blunt needle. Is there any contraction of the muscles?

314. Pinch the same spot sharply with a pair of forceps. Is there any movement of the leg of the same or opposite side.

315. Pinch the skin around the anus with a pair of forceps. What is the effect upon the legs?

316. Place the frog on its back. Does it make an effort to get into a natural position? Does it show any sense of equilibrium?

317. Pass a hook through its lower jaw and hang it to the ring of a retort stand. How do the hind-limbs behave?

318. Pinch very gently the tip of one of the toes, what is the effect?

319. Fill two glasses, one with dilute sulphuric acid, the other with water. Raise the glass containing the acid, until the acid just touches the tip of the toes. Is the foot withdrawn? If so, raise the second glass and let the foot be immersed in it, to wash off the acid.

320. Cut a small piece of filter or blotting-paper, moisten it with strong acetic acid and place it on the flank of the animal. What is the effect upon the leg? Put the piece of paper upon the opposite flank and hold the leg so as to prevent it from moving. Is there any action of the opposite leg?

321. Place similar pieces of paper upon different portions of the body. Note any variety of movements and what seems to be their purpose.

322. Remove the frog from the hook and plunge it in a basin of water. This will wash off the acid. Does the frog make any movements in the water? Does it float?

323. Inject a small amount of strychnine solution under the skin of the frog's back. Let it remain for a few minutes and then note the effect of the slightest stimulus, such as jarring the table upon which it lies.

324. With a tracer or piece of wire destroy the myel, the convulsions cease. Try any of the preceding stimuli upon the frog now and note the result.

325. Make a nerve-muscle preparation of one of the hind limbs. Dissect away the skin and muscles upon the dorsal aspect of the leg, until the sciatic nerve is exposed, leaving it connected with the lumbar plexus. Denude the femur of its muscles, using the greatest care not to injure the sciatic nerve. Keep the nerve moist with normal salt solution. Pass a copper hook under the sciatic nerve and hang to a

tripod. Tilt the tripod so that the leg may come in contact with one of the iron supports. If the tripod has been painted, scrape the paint off. What happens when the contact is made? This is known as Galvani's experiment.

326. Make another nerve-muscle preparation of the other hind limb, but cut the sciatic nerve as near to the myel as possible and separate the leg from the body at the femoro-pelvic joint. Remove the skin as far as the foot. With the forceps crush the gastrocnemius muscle near the tendon of Achilles. See that the end of the nerve is cut off squarely. With a small brush or thin glass rod lift the nerve very carefully in such a way that its cross-section may fall upon the injured portion of the muscle. This stimulates the nerve and causes a contraction of the muscle due to the so-called demarcation currents.

XXV.

327. Each student is to have a frog, which is to be pithed and have its brain and myel destroyed by passing a tracer or seeker through the spinal canal. The legs are to be used for nerve muscle preparations. Dissect one leg for the first series of experiments, and reserve the other leg for the second series. Begin the dissection upon the dorsal aspect of the leg removing the skin and muscles very carefully until the sciatic nerve is exposed. Dissect out the nerve as long as possible, and moisten frequently with the normal salt solution. Remove all of the muscles as far as the knee, leaving the femur and nerve entirely isolated. Avoid all injury to the nerve during dissection, and apply the normal salt solution every few minutes with a camel's hair brush. Arrange the nerve muscle preparation by placing the femur in a clamp and allowing the nerve to hang freely. A small lever may be pinned to the foot to emphasize any movements that may occur. Apply the following stimuli :

328. *Mechanical.* Pinch the free end of the nerve sharply with a pair of forceps; the muscles contract and the foot is raised suddenly. Cut off the pinched portion. Contraction again occurs.

329. *Thermal.* To the same preparation apply at the free end of the nerve a wire or needle heated to a dull heat. Contraction again occurs. Cut off the dead part of the nerve.

330. *Chemical.* Place some saturated solution of sodium chloride in a watch glass and let the free end of the nerve dip in it. It requires a few moments for the salt to diffuse into the nerve on account of the difference in the specific gravity. Soon the joints of the toes twitch and by-and-by the whole limb is thrown into irregular, flickering spasms, which terminate in a more or less continuous contraction, constituting *tetanus*. Cut off the part of the nerve affected by the salt; the spasms cease.

(a) Finish the experiment by exposing the nerve to the vapor of strong ammonia in a test tube. The ammonia must not act directly upon the muscle, the tube should be raised slightly above the level of the muscle and the end of the nerve elevated to the mouth of the tube. There should be no contraction if the vapor has not come in contact with the muscle. The ammonia kills the nerve. Apply ammonia to the muscle. It contracts.

331. *Electrical.* For the following experiments use the other leg of the frog taking the same precautions in the dissection and application of the normal salt solution.

(a) Arrange the nerve-muscle preparation with the femur in a clamp and the foot of the frog connected with a recording lever. Arrange the Daniell Cell and DuBois Reymond Key with the induction coil. Connect the battery wires with the primary coil and remove the secondary coil to the lower end of the scale. Arrange the electrodes and a recording drum with smoked paper conveniently to the preparation.

When all is in readiness close the connection by means of the key by raising or lowering its lever. Or in other words "making" or "breaking" the current. Gradually move the secondary coil along the scale while making and breaking the current. The current is made when the connection is complete and broken when the connection is interrupted. Note at what point on the scale the first result appears and whether it be from "make" or "break". Make a table of your results as follows: In one column indicate the distance of the secondary coil from the primary. In another, Response at Make, and the last column, Response at Break. This is electrical stimulation in the form of *single induction shocks*.

(b) Remove the wires from the primary coil and connect them with the sockets leading to the vibrating hammer. On applying the electrodes to the nerve or muscle the latter is at once thrown into a state of rigid spasm or continuous contraction called tetanus. Compare this tracing with that of (a). This form of stimulation is known as the *interrupted current* or repeated shocks.

(c) Remove the induction coil and use only the battery with its wires and the key. Make and break the current as in (a). Notice that if the key be so arranged as to permit the current to flow continuously through the nerve, no contraction occurs provided there be no variation in the intensity of the current. Rapidly make and break the current by opening and closing the key; a more or less perfect tetanus is produced. This is the *constant current* form of stimulation. Scratch your name on the above tracings to identify them. The tracings may be made permanent by drawing the paper through a pan of shellac.